Molecular Pathology of Endocrine Diseases

Jennifer L. Hunt

Editor

Molecular Pathology Library

Series Editor: Philip T. Cagle

Springer
Molecular Pathology of Endocrine Diseases

Edited by

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Springer
The past two decades have seen an ever accelerating growth in knowledge about molecular pathology of human diseases, which received a large boost with the sequencing of the human genome in 2003. Molecular diagnostics, molecular targeted therapy, and genetic therapy are now routine in many medical centers. The molecular field now impacts every field in medicine, whether clinical research or routine patient care. There is a great need for basic researchers to understand the potential clinical implications of their research whereas private practice clinicians of all types (general internal medicine and internal medicine specialists, medical oncologists, radiation oncologists, surgeons, pediatricians, family practitioners), clinical investigators, pathologists and medical laboratory directors, and radiologists require a basic understanding of the fundamentals of molecular pathogenesis, diagnosis, and treatment for their patients.

Traditional textbooks in molecular biology deal with basic science and are not readily applicable to the medical setting. Most medical textbooks that include a mention of molecular pathology in the clinical setting are limited in scope and assume that the reader already has a working knowledge of the basic science of molecular biology. Other texts emphasize technology and testing procedures without integrating the clinical perspective. There is an urgent need for a text that fills the gap between basic science books and clinical practice.

In the Molecular Pathology Library series, the basic science and the technology is integrated with the medical perspective and clinical application. Each book in the series is divided according to neoplastic and non-neoplastic diseases for each of the organ systems traditionally associated with medical subspecialties.

Each book in the series is organized to provide specific application of molecular pathology to the pathogenesis, diagnosis, and treatment of neoplastic and non-neoplastic diseases specific to each organ system. These broad section topics are broken down into succinct chapters to cover a very specific disease entity. The chapters are written by established authorities on the specific topic from academic centers around the world. In one book, diverse subjects are included that the reader would have to pursue from multiple sources in order to have a clear understanding of the molecular pathogenesis, diagnosis, and treatment of specific diseases. Attempting to hunt for the full information from basic concept to specific applications for a disease from varied sources is time-consuming and frustrating. By providing this quick and user-friendly reference, understanding and application of this rapidly growing field is made more accessible to both expert and generalist alike.

As books that bridge the gap between basic science and clinical understanding and practice, the Molecular Pathology Library Series serves the basic scientist, the clinical researcher, and the practicing physician or other health care provider who require more understanding of the application of basic research to patient care, from “bench to bedside.” This series is unique and an invaluable resource to those who need to know about molecular pathology from a clinical, disease-oriented perspective. These books will be indispensable to physicians and health care providers in multiple disciplines as noted above, to residents and fellows in these multiple disciplines as well as their teaching institutions, and to researchers who increasingly must justify the clinical implications of their research.

Houston, TX

Philip T. Cagle
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Introduction

Oncogenesis in all organ systems is generally considered to be an extremely complex process, with poorly understood etiology and drivers. There is substantial evidence that much of carcinogenesis is driven by changes at the molecular level, at the DNA, RNA, or the protein expression level. New evidence also suggests that alterations in small RNA molecules (microRNA) can contribute to carcinogenesis, though this process is incompletely understood. Finally, environmental, nutritional, and external factors are almost certainly linked to carcinogenesis in some organ systems. It is precisely because of the complexity in all of these widely divergent drivers of carcinogenesis that we continue to search for the causes of cancer in most organ systems.

It is important to classify carcinomas based on one fundamental feature before any attempt at understanding the underlying etiology can be made. Tumors that are sporadic and tumors that are hereditary must be differentiated from one another. Though the genes involved may have overlapping profiles, in the sporadic case the alterations occur in terminally differentiated cells and in the hereditary case, the primary genetic events are inherited at the germline level. In many cases, carcinogenesis and the molecular pathways that lead to tumors are more clearly delineated in hereditary tumors. In sporadic tumors, the pathways tend to be highly complex and involve many different molecular events.

In nonhereditary, sporadic tumors and hyperplasias, a number of factors can be causally related to the pathology. The genetic causes will be extensively discussed in the following chapters for each endocrine organ system and individual tumor types. But, it is also important to consider other factors, such as environmental, nutritional, and external factors. For example, an important driver of thyroid disease in certain unique patient populations is exposure to radiation. The link between tumorigenesis and exposure to radiation link has been best studied in the population of individuals who were exposed to nuclear fallout after the Chernobyl disaster, which occurred in northern Ukraine in 1986. In most studies, a significant increase in thyroid cancer was identified in the years following the exposure. This included thyroid cancers occurring in young children, and thyroid cancers with a much more aggressive clinical course. The molecular alterations in these patients are different from sporadic thyroid tumors.

Though the source of radiation and the mechanisms are different from those seen in nuclear disasters, external beam radiation has also been linked to thyroid cancer. In past decades, radiation was used to treat banal disease, such as acne, thymus enlargement, and tinea. Patients who were exposed to this radiation were found to be at risk for future development of thyroid cancer, particularly when dosages were relatively low, and the radiation occurred during childhood and early adolescence. Interestingly, the molecular analysis of these tumors indicates that they are more similar to the tumors in Chernobyl patients than to sporadic tumors.

Despite all these potential contributors to carcinogenesis, one of the most important drivers of tumors remains alterations in tumor associated genes, in particular tumor suppressor genes (TSGs) and oncogenes. These are discussed in detail in this chapter, along with some of the more typical assays that are used to detect mutations and alterations in these tumor genes.

Tumor Suppressor Genes

Tumorigenesis through the inactivation of tumor suppressor genes was first postulated by Dr. Alfred George Knudson in the early 1970s, in seminal work with retinoblastoma. Knudson elegantly showed that hereditary retinoblastoma and sporadic tumors had similar molecular mechanisms and involved the retinoblastoma gene. His theory, which is now known as Knudson’s Hypothesis, predicted that both copies of the retinoblastoma gene needed to harbor mutations for tumorigenesis to occur.

Normal cells have two functional copies of each wild-type (nonmutated) tumor suppressor gene, one inherited from each parent. In the process of tumorigenesis, one copy of the
TSG develops an inactivating mutation. This first mutation is often a point mutation or small deletion mutation, and this is termed the first genetic hit. This first hit can occur spontaneously, or it can be inherited in cancer syndromes that make patients susceptible to specific tumors. Alone, the first mutation is not tumorigenic and usually has no ramifications for cell function, because the second copy of the TSG is still functioning. This is why tumor suppressor genes are also referred to as “recessive genes” – they require both copies to be mutated in order for loss of function. The second genetic hit affects the second copy of the TSG, and this may be due to point mutations or more commonly, larger deletion mutations. It is uncommon for cells to have two large deletion mutations (biallelic deletion), because these would make patients susceptible to specific tumors. In this classic example, germline mutations in the APC gene (chromosome 5q21) occur in patients affected by Familial Adenomatous Polyposis as the first hit, and somatic mutations (usually deletions) in the second copy of the APC gene represent the second hit. With both copies of the gene functionally lost, colon cancer progression occurs. The colon adenoma to carcinoma pathway is probably one of the best studied examples of tumorigenesis via the tumor suppressor pathway. Some of the most common tumor suppressor genes are listed in Table 1.1.

### Table 1.1. Common tumor suppressor genes, their chromosomal location, function, and any associated inherited syndromes.

<table>
<thead>
<tr>
<th>Tumor suppressor gene</th>
<th>Chromosomal location</th>
<th>Function</th>
<th>Inherited syndromes</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTEN</td>
<td>10q23</td>
<td>Regulated cell survival</td>
<td>Cowden syndrome</td>
</tr>
<tr>
<td>DCC</td>
<td>18q21.3</td>
<td>Transmembrane receptor</td>
<td></td>
</tr>
<tr>
<td>APC</td>
<td>5q21</td>
<td>Signaling through adhesion molecules</td>
<td></td>
</tr>
<tr>
<td>NF2</td>
<td>22q12.2</td>
<td>Linkage of cell membrane to cytoskeleton</td>
<td>Neurofibromatosis type 2</td>
</tr>
<tr>
<td>NF1</td>
<td>17q11.2</td>
<td>Catalysis of RAS inactivation</td>
<td>Neurofibromatosis type 1</td>
</tr>
<tr>
<td>WT1</td>
<td>11p13</td>
<td>Transcriptional regulation</td>
<td>Wilm’s tumor</td>
</tr>
<tr>
<td>RB1</td>
<td>13q13</td>
<td>Cell cycle regulation</td>
<td>Retinoblastoma</td>
</tr>
<tr>
<td>P53</td>
<td>17p13</td>
<td>Cell cycle regulation, apoptosis</td>
<td>Li–Fraumeni syndrome</td>
</tr>
</tbody>
</table>

Assays Used to Detect Tumor Suppressor Gene Mutations

**DNA Polymorphisms**

A large percentage of the genetic code is redundant, with only a small amount of DNA being unique from person to person. The differences in the genetic code are predominantly due to variable regions, called “polymorphisms.” There are several different types of variable areas within the genetic code. The most common and most utilized for genetic testing are the short tandem repeats (STR) and the single nucleotide polymorphisms (SNP). STRs are short redundant nucleotide sequences that vary from 2 to 7 base pairs in length that are repeated for a variable number of times. Polymorphisms have different levels of variability that are highly population dependent. When an individual has inherited two copies of the polymorphism that are different from one another, the two copies of the polymorphism can be discriminated from each other by PCR-based assays.

Being able to test for polymorphisms has great value for determining identify, which is used particularly in the area of forensic testing and paternity testing. But, polymorphisms are also used in diagnostic testing in anatomic pathology as well, especially for loss of heterozygosity (LOH) and microsatellite instability testing.

**Detecting Loss of Heterozygosity**

Loss of heterozygosity (LOH) analysis relies upon our ability to discriminate between the two inherited copies of a particular gene. Unfortunately, the coding regions of our genes are usually highly conserved through evolution and are therefore identical in sequence on each of the two alleles that we have inherited (one maternal allele and one paternal allele). To do this type of assessment, molecular techniques utilize the polymorphisms in the genome that are in close proximity to genes of interest.

PCR is used in an LOH analysis, with primers that flank the polymorphism. In an informative person at that locus, the PCR amplicons will have different lengths, since they have two copies of the gene that have different numbers of repeat units. PCR products of different sizes will migrate at different speeds during electrophoresis. In normal cells, if we analyze an STR locus in an informative patient, we expect that there will be two differently sized PCR products of approximately the same amount, one from each chromosomal copy of the gene. In tumor cells, there may be deletion mutations present, which will alter the ratio of the PCR product amount.
from the two different PCR products. When one copy of the STR is lost, there will be only one PCR product, or the genotype will appear to be homozygous at that locus. Because the normal was originally heterozygous, we label this situation as “loss of heterozygosity.”

A ratio of the amount of PCR product present for the two alleles can be obtained from capillary electrophoresis electropherograms as the ratio of the peak heights for the two products. If one is using gel based electrophoresis, this assessment is by visual inspection – if one band is less than 50% the intensity of the second band, this is indicative of loss. The calculation to determine if there is true loss of genetic material should include a comparison of the tumor allele ratio to that of the normal allele ratio. This will help to account for variability in PCR for the two alleles that might be secondary to the size of the PCR products or other variables that affect amplification efficiency.32

Oncogenes

Proto-oncogenes are nonmutated genes that, when mutated, stimulate carcinogenesis through activation. When mutated, these genes are designated as oncogenes. Oncogenes are referred to as dominant genes, because only one copy of the gene needs to be mutated to lead to overexpression or activation. Examples of some oncogenes are given in Table 1.2.

A variety of mutational events can transform a proto-oncogene to an oncogene, including point mutations, translocations, amplifications, and deletions. Because most of these mutations will be activating mutations, the protein product of the oncogene is often overexpressed because of the mutation. This protein product may be detectable by immunohistochemistry (IHC) or overabundant mRNA may be able to be detected by molecular techniques as well. However, overexpression of an oncogene protein product by IHC alone does not imply that there is a genomic mutation, since epigenetic mechanisms can also be responsible for protein overexpression.

In surgical pathology, some of the most common oncogene tests are those for translocations, amplifications, and point mutations. Though translocations were first described in sarcomas and hematologic malignancies, solid tumors are now being described with novel translocations as well. These translocations, for the most part, involve an oncogene partner that is juxtaposed next to an activating gene. The oncogene is then aberrantly activated in the tumor cells. Some common translocations in different types of tumors are listed in Table 1.3.

Amplifications are also now becoming common targets for assessment at the molecular level. There are several genes that have amplifications in specific tumor types that have prognostic or even therapeutic value, with HER2/neu in breast cancer and EGFR in several tumor types being the classic examples.30,35,38,39

Finally, assays for point mutations are increasing in clinical importance because of the association between some oncogene point mutations and potential drug targets. CKIT mutations in gastrointestinal stromal tumors and EGFR mutations in lung cancer were among the first tumor mutational assays that were proposed to have real significance in selecting targeted therapies.1,11,14,15,20 As targeted therapies are introduced into the market place, it is likely that molecular analysis will continue to increase in importance. These targeted drugs are expensive and can have significant side effects, making the demand for companion diagnostic testing even higher to avoid the risks of uninformed treatment of patients who may not even respond to a particular drug. Assessing for additional acquired point mutations during tumor treatment will also likely become an important application of molecular technology. Many of the tumors being treated with targeted therapies are at risk for developing secondary mutations that will confer a resistance to the drug treatment.7,14,26

Assays Used to Detect Oncogene Mutations

Detecting Translocations

Reverse-transcription and polymerase chain reaction (RT-PCR) has long been considered to be the gold standard as the most powerful assay to detect translocations. In this assay, mRNA is extracted from the tissue and subjected to reverse transcription to yield cDNA. The cDNA is then amplified using PCR. The primers are carefully selected to amplify
In fusion probe detection, dual color probes, or chromosome specific paints, are used to localize each chromosome involved in the translocation. After hybridization, abnormal apposition of the two probes, which are normally distant to one another, indicates the presence of a translocation. Because the cells can be fragmented or cut in several planes or overlapping in paraffin embedded tissues, the signals for the two gene partners can be entirely or partially absent or can also be artificially overlapping each other. Therefore, a minimum number of cells must be examined before a definitive call is made about the presence of the translocation; this cutoff should be established in each laboratory that does the assay as part of the validation process.

In break-apart probe systems, the two probes are localized to the same gene, but flank the area that is typically a known break point. This system is preferable for translocations that have one consistent gene partner and a variety of other potential partners. In normal cells, the two signals are next to one another and overlapping. In cells that harbor a translocation, the two signals are separated, as they are now located on two different chromosomes secondary to the translocation.

### Detecting Amplifications

FISH can also be used very effectively to detect gene amplifications. In this type of assay, a region specific probe is used in combination with a second probe for the chromosome in general. For example, to assess for EGFR amplification, there will be a probe for the EGFR gene and a second probe for chromosome 7 to be used as a comparison. By assessing both the copy number of the gene and the number of chromosomes present, one is able to resolve whether there is true gene amplification as opposed to polyploidy.

### Detecting Point Mutations

The detection of point mutations in specific genes in the practice of anatomic pathology has been long complicated by several key issues. First, the DNA that can be obtained from paraffin embedded tissues is degraded, as compared to that obtained from fresh tissues. Even though the DNA is degraded, most assays can be successfully implemented with careful planning. PCR-based assays should be designed with short PCR products (ideally under 200 basepairs) to account for the DNA fragmentation that is caused by formaldehyde.

Second, samples from tumors are notoriously heterogeneous. They contain not only tumor cells, but stromal cells, inflammatory cells, areas of potential necrosis. These tissue factors will also affect the sensitivity of any given assay. To address the heterogeneity of tissues, many tumor assays utilize microdissection to first obtain a relatively pure tumor sample.

There are a number of excellent approaches that can be used to detect point mutations in oncogenes. The most traditional approach is conventional gene sequencing. Most assays of this type combine an initial PCR reaction for

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### Table 1.3. Examples of tumors that harbor translocations.

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Molecular mutations</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>MLL–AF-4</td>
<td>t(4;11)</td>
</tr>
<tr>
<td></td>
<td>BCR–ABL</td>
<td>t(9;22)</td>
</tr>
<tr>
<td></td>
<td>ELA–PBX</td>
<td>t(1;19)</td>
</tr>
<tr>
<td></td>
<td>TEL–AML1</td>
<td>t(12;21)</td>
</tr>
<tr>
<td></td>
<td>MYC–IgH</td>
<td>t(8;14)</td>
</tr>
<tr>
<td>Alveolar rhabdomyosarcoma</td>
<td>PX3–FKHR</td>
<td>t(2;13)</td>
</tr>
<tr>
<td></td>
<td>PX7–FKHR</td>
<td>t(1;13)</td>
</tr>
<tr>
<td>AML-M2</td>
<td>ETO–AML1</td>
<td>t(8;21)</td>
</tr>
<tr>
<td>AML-M3</td>
<td>PML–RARA</td>
<td>t(15;17)</td>
</tr>
<tr>
<td>AML-M4eo</td>
<td>CFB–MYH11</td>
<td>t(16;16)</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
<td>BCL1–IgH</td>
<td>t(11;14)</td>
</tr>
<tr>
<td>Burkitt’s lymphoma</td>
<td>IgH–MYC</td>
<td>t(8;14)</td>
</tr>
<tr>
<td></td>
<td>IgK–MYC</td>
<td>t(2;8)</td>
</tr>
<tr>
<td></td>
<td>IgL–MYC</td>
<td>t(8;22)</td>
</tr>
<tr>
<td>Clear cell sarcoma</td>
<td>EWS–ATF1</td>
<td>t(12;22)</td>
</tr>
<tr>
<td>CML</td>
<td>BCR–ABL</td>
<td>t(9;22)</td>
</tr>
<tr>
<td>Dermatofibrosarcoma protuberans</td>
<td>COL1A1–PDGF</td>
<td>t(17;22)</td>
</tr>
<tr>
<td>Desmoplastic small round cell tumor</td>
<td>EWS–WT1</td>
<td>t(11;22)</td>
</tr>
<tr>
<td>Ewing’s sarcoma</td>
<td>EWS–FLI1</td>
<td>t(11;22)</td>
</tr>
<tr>
<td></td>
<td>EWS–ERG</td>
<td>t(21;22)</td>
</tr>
<tr>
<td></td>
<td>EWS–ETV1</td>
<td>t(7;22)</td>
</tr>
<tr>
<td>Extraskeletal myxoid</td>
<td>EWS–CHN</td>
<td>t(9;22)</td>
</tr>
<tr>
<td>chondrosarcoma</td>
<td>RBP56</td>
<td>t(9;17)</td>
</tr>
<tr>
<td>Fibrosarcoma</td>
<td>ETV6–NTRK3</td>
<td>t(12;15)</td>
</tr>
<tr>
<td>Follicular lymphoma</td>
<td>BCL2–IGH</td>
<td>t(14;18)</td>
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<tr>
<td>Follicular thyroid carcinoma</td>
<td>PPARY–PAX8</td>
<td>t(2;3)</td>
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<tr>
<td>Large cell lymphoma</td>
<td>BCL6–IgH</td>
<td>t(3;14)</td>
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<tr>
<td></td>
<td>IgH–BCL8</td>
<td>t(14;15)</td>
</tr>
<tr>
<td>Marginal zone lymphoma</td>
<td>API2–MALT1</td>
<td>t(11;18)</td>
</tr>
<tr>
<td>Myxoid/round cell</td>
<td>TLS–CHOP</td>
<td>t(12;16)</td>
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<tr>
<td>liposarcoma</td>
<td>EWS–CHOP</td>
<td>t(2;22)</td>
</tr>
<tr>
<td></td>
<td>EWS–CHOP</td>
<td>t(12;22;20)</td>
</tr>
<tr>
<td>Papillary thyroid carcinoma</td>
<td>RET–PTC</td>
<td>Rearrangement on 10q11.2</td>
</tr>
<tr>
<td>Synovial sarcoma</td>
<td>SYT–SSX1 or SYT–SSX2</td>
<td>t(x;18)</td>
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</tbody>
</table>
the specific area of interest (i.e., the exons that most commonly harbor mutations) with subsequent sequencing reactions. Although sequencing used to use radioactivity for the analysis, advanced sequencers are now available that are rapid, cost-effective, and use other detection technology that does not harbor the same risks as radioactivity. One typical approach is to use capillary electrophoresis machines to analyze the products from the sequencing reaction. Abnormal peaks represent mutant basepairs, and these are usually easily recognized in tumor samples.

The most significant problem that arises with gene sequencing is one of sensitivity. The assay will only detect a mutant allele when the cells that harbor it represent approximately 20% or more of the initial tumor sample. Therefore, tumor purity becomes a very important issue to address before using traditional gene sequencing. Other approaches, including pyrosequencing and allele specific PCR, have better sensitivities and are able to detect mutant that is present in the 1–2% range.

References


Introduction

Autoimmunity is defined as an immune response directed against a self-antigen – an antigen of host origin, within host tissue. Autoimmunity encompasses nonpathologic, naturally occurring immune responses, such as cold autoantibodies to red blood cell antigens, as well as pathologic conditions caused by the autoimmune response: i.e., autoimmune disease. Any component of the immune system may be involved in autoimmunity, including the innate immune system, B-cell responses, and T-cell responses.

Autoimmune diseases may be classified by the scope of organ involvement as either localized or systemic. Localized autoimmune diseases are characterized by an immune attack restricted to a specific organ or tissue, for example type I diabetes mellitus, Hashimoto’s thyroiditis, Addison’s disease, and lymphocytic hypophysitis. In some forms of localized autoimmune disease, although the targeted self-antigen is limited, the consequences of the tissue damage may be systemic, for example, type I diabetes mellitus. By contrast, the targeted self-antigen in systemic autoimmune diseases, tends to be more widely distributed, leading to widespread tissue damage: for example, systemic lupus erythematosus (SLE) and vasculitis. It thus follows that endocrine organ involvement by autoimmune disease may be localized (Hashimoto’s, Grave’s, Addison’s, etc.) or can be part of a systemic process when the organ contains the targeted self-antigen.

Understanding of the etiologic factors of autoimmune disease, both genetic and environmental, is increasing. The genetic component of autoimmune diseases tends to be multigenic. In addition to genes linked to specific diseases (to be discussed in the sections that follow), several genes have been implicated as general risk factors for autoimmunity (Table 2.1). There is accumulating evidence that vitamin D deficiency may be an environmental factor predisposing to autoimmune disease; vitamin D normally participates in the immunoregulation as well; prolactin promotes B-cell proliferation and immunoglobulin production in response to antigen, and inhibits apoptosis and negative selection of autoreactive B-cells. Hyperprolactinemia may confer a general risk for the development of autoimmune disease. An additional possible risk factor for autoimmune disease is microchimerism – the presence of non-self stem cells or their progeny within an individual, most commonly derived from transplacental trafficking of stem cells during pregnancy. Persistent microchimerism over years may elevate the risk for autoimmune disease.

This chapter will review systemic autoimmune disease focusing on the molecular/genetic aspects (when known), and endocrine organ manifestations.

Systemic Lupus Erythematous

Systemic lupus erythematous (SLE) is the quintessential systemic autoimmune disease, notable for the heterogeneity in clinical presentation and course. The annual incidence of SLE ranges from 1.9 to 5.6 per 100,000, and, as is true for most autoimmune diseases, predominantly affects females of reproductive age. While any organ may be involved, the most common clinical scenario includes constitutional symptoms, and skin, musculoskeletal, and hematologic manifestations.

The spectrum of disease in SLE is an expression of the ubiquity of the target antigens: DNA/protein complexes, RNA/protein complexes, and cell membrane and intracellular molecules. Tissue damage is mediated primarily by pathogenic autoantibodies and immune complex deposition, although a role for direct tissue toxicity by T-cells may also contribute.

Both environmental and genetic factors are implicated in the etiology of SLE. Environmental factors include UV light, infection, drugs, and (female) gender.
Clinical Features

The diagnosis of SLE is based on a combination of clinical and laboratory features. Clinical features are manifold; the following represent the more common manifestations and are used to identify patients for clinical studies:

- Skin disorder: malar rash, discoid rash, photosensitivity
- Oral ulcers
- Arthritis
- Serositis: pleuritis, pericarditis, effusions
- Renal disorder: proteinuria, cellular casts
- Neurologic disorder: seizures, psychosis
- Hematologic disorder: cytopenias
- Immunologic disorder: false positive serologic test for syphilis, positive lupus erythematosus cell preparation, anti-Smith (Sm) nuclear antigen
- Antinuclear antibody

Vasculitis, presumably due to antiphospholipid antibodies, anti-endothelial cell antibodies, and anti-double stranded (ds)-DNA antibodies, may also occur.

Laboratory studies useful in the diagnosis of SLE include cell counts to assess for cytopenias, urinalysis to detect glomerular disease, and serology. Autoantibodies assayed, with a range of sensitivity and specificity, are directed against nuclear antigens (ANA), ds-DNA, Sm, ribonucleoprotein (RNP), Ro, La, phospholipids, and ribosomal P.

Pathogenesis

The key initiating event in SLE appears to be the release of self-antigen in a susceptible individual. As suggested by murine studies, the pathogenesis can be summarized by the following “antinucleosome to anti-ds-DNA hypothesis.”

External factors such as UV light, infection, drugs, and dietary factors may cause the release of self-antigen either by direct cellular injury or by indirect injury mediated by an immune response to exogenous antigens. Cellular injury culminating in apoptosis releases nuclear antigens within nucleosomes, triggering the formation of anti-DNA/protein antibodies. With maturation and somatic mutation some of these initial anti-DNA/protein antibodies evolve into autoantibodies with anti-single stranded (ss)-DNA and anti-ds-DNA specificity.

As noted, these events must occur in the background of a susceptible individual; “susceptibility” encompasses genetic and hormonal factors, which result in multiple derangements of the immune system:

- Hyperactivated B-cells
  - Increased numbers of B-cells
  - Abnormal B-cell responses to activating signals
  - Impaired apoptosis of autoreactive B-cells
  - Increased levels of B-cell promoting cytokines: interleukin (IL)-10, IL-6
- Hyperactivated T-cells
  - Change in ratio of helper to suppressor/natural killer (NK) cells (skewed towards helper T-cells)
  - Abnormal activation
  - Accelerated apoptosis
  - Increased levels of T-cell promoting cytokines: IL-2, sIL-2R, interferon (IFN)-γ
- Abnormalities of monocytes and macrophages
  - Defective processing of immune complexes
  - Increased secretion of IFN-γ
- Abnormalities of immunoregulation
  - Possible defective immune tolerance
  - Inadequate clearing of immune complexes

These alterations create an environment with increased release of autoantigens, increased responsiveness to autoantigens with autoantibody production, decreased down-regulation of the immune response, and impaired clearance of antigen–antibody complexes.

Genetics

A genetic predisposition for SLE is evidenced by a 58% concordance rate of disease in monozygotic twins (a tenfold increase compared to dizygotic twins), as well as an eightfold increased risk for SLE in first degree relatives of an affected individual. Several susceptibility genes have been identified (Table 2.1).

Table 2.1. Systemic autoimmune disease: general susceptibility genes for autoimmune disease.1–4

<table>
<thead>
<tr>
<th>Gene</th>
<th>Name</th>
<th>Location</th>
<th>Function</th>
<th>Disease associations</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA region</td>
<td>Human leukocyte antigen</td>
<td>6p21.3</td>
<td>Antigen presentation; immune regulation</td>
<td>Specific haplotypes associated with different ADs (see text)</td>
</tr>
<tr>
<td>CTLA-4</td>
<td>Cytotoxic T-lymphocyte associated antigen-4</td>
<td>2q33</td>
<td>Expressed on activated T-cells; down regulates T-cell function</td>
<td>Animal models: SLE, MS, IDDM</td>
</tr>
<tr>
<td>FCRL3</td>
<td>Fc receptor-like 3</td>
<td>1q22–q22</td>
<td>Role in early B-cell maturation, activation</td>
<td>SLE, RA, AITD</td>
</tr>
<tr>
<td>PTPN22</td>
<td>Protein tyrosine phosphatase, non-receptor, type 22</td>
<td>1p13</td>
<td>Lymphoid tyrosine phosphatase</td>
<td>SLE, IDDM, RA, AITD</td>
</tr>
<tr>
<td>PDCD1</td>
<td>Programmed cell death 1</td>
<td>2q37</td>
<td>Inhibition of T-cell proliferation and cytokine production</td>
<td>SLE, RA</td>
</tr>
</tbody>
</table>

AD autoimmune disease; SLE systemic lupus erythematosus; MS multiple sclerosis; IDDM insulin dependent diabetes mellitus; RA rheumatoid arthritis; AITD autoimmune thyroid disease.

As noted, these events must occur in the background of a susceptible individual; “susceptibility” encompasses genetic and hormonal factors, which result in multiple derangements of the immune system:

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Genetics

A genetic predisposition for SLE is evidenced by a 58% concordance rate of disease in monozygotic twins (a tenfold increase compared to dizygotic twins), as well as an eightfold increased risk for SLE in first degree relatives of an affected individual. Several susceptibility genes have been identified (Table 2.2).

HLA genes, particularly class II (DR, DQ, DP) and class III (complement components C2 and C4), have been found to be associated with SLE. HLA-DR2 and DR3 confer a relative risk of developing SLE of 2–3 across diverse ethnic groups, while different extended HLA haplotypes predispose
to disease in different ethnic groups, and to different spectra of clinical manifestations. Amino acid sequences in disease associated class II proteins appear to enhance autoantibody production. Deficiencies of HLA class III complement components C2 and C4, as well as of non-HLA complement components C1q, C1r, and C1-inhibitor (INH) impart a high risk for SLE. Such deficiencies impair clearance of apoptotic bodies, increasing exposure to self-antigens.¹⁰

Clearance of immune complexes is impaired by certain polymorphisms of white blood cell surface Fc-receptors; these include polymorphisms of genes encoding the FcγRIIA and FcγRIIA receptors (FCGR2A, FCGR3A).¹⁰

Certain promoter polymorphisms in the IL-10 gene have also been linked to SLE. As noted previously, serum levels of IL-10, a cytokine that stimulates B-cell activation, proliferation, differentiation, and immunoglobulin (Ig) production are elevated in SLE. These promoter polymorphisms appear to enhance IL-10 production.¹⁰

Linkage analysis studies have identified three additional chromosomal regions linked to SLE: 1q41–43, 4p16–15, and 16q12–13. The gene poly(ADP-ribose) polymerase, encoding an enzyme involved in apoptosis-associated DNA repair is a candidate gene within the chromosome 1 locus. On chromosome 16 is the candidate gene nucleotide-binding oligomerization domain containing 2 (NOD2), encoding a protein involved in monocyte interaction with bacteria. Variants of NOD2 are associated with Crohn’s disease.¹⁰

### Endocrine Organ Involvement

Autoantibodies may arise in SLE that react with antigens within endocrine organs. The thyroid is the endocrine organ most commonly affected; antithyroid antibodies, including anti-thyroglobulin and anti-microsomal antibodies, are detectable in up to 35% of SLE patients. Hypothyroidism, usually clinically silent, is present in 6–15%,⁹,¹³–¹⁸ a rate higher than the normal population. Euthyroid sick syndrome is seen in up to 15%.¹⁵

Abnormalities in pituitary hormone levels have been identified in SLE, including elevated follicle stimulating hormone, luteinizing hormone, and prolactin, compared to normal controls.¹⁹,²⁰ It is unclear whether this finding represents a hormonal milieu that predisposes to SLE or results from the disease.

Endocrine organs may also theoretically be affected by the vasculitis that occurs in SLE.

### Sarcoidosis

Sarcoidosis is a systemic inflammatory disease featuring noncaseating granulomas in multiple organ systems, most commonly in the lungs and intrathoracic lymph nodes. The etiology is unknown, but evidence points to an exuberant T-cell mediated immune response to an antigenic stimulus. As in SLE, exogenous triggers including infectious agents and occupational exposures are suspected to initiate the inflammatory cycle in genetically predisposed individuals.¹¹,²¹

### Clinical Features

Sarcoidosis may affect almost any organ, with presentation depending on the pattern of involvement. It is not unusual for the diagnosis to result from work up of incidental chest radiographic abnormalities in an asymptomatic patient. Commonly involved organs include the lungs, lymph nodes, skin, heart, eye, and nervous system. Biopsy, most commonly

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</tr>
<tr>
<td>C2, C4</td>
<td>Complement components 2 and 4</td>
<td>6p21.3</td>
<td>Complement cascade; deficiency impairs clearance of apoptotic bodies</td>
<td>Deficiency associated with high risk for SLE</td>
</tr>
<tr>
<td>C1q</td>
<td>Complement component 1, q subcomponent</td>
<td>1p36.3–p34.1</td>
<td>Complement cascade; deficiency impairs clearance of apoptotic bodies</td>
<td>Deficiency associated with high risk for SLE</td>
</tr>
<tr>
<td>C1r</td>
<td>Complement component 1, r subcomponent</td>
<td>12p13</td>
<td>Complement cascade; deficiency impairs clearance of apoptotic bodies</td>
<td>Deficiency associated with high risk for SLE</td>
</tr>
<tr>
<td>C1-INH</td>
<td>Complement component 1 inhibitor</td>
<td>11q11–q13.1</td>
<td>Complement cascade; deficiency impairs clearance of apoptotic bodies</td>
<td>Deficiency associated with high risk for SLE</td>
</tr>
<tr>
<td>FcγRIIA</td>
<td>Cell surface Fc-receptor</td>
<td>1q21–q23</td>
<td>Binds Fc portion of Ig; clearance of immune complexes</td>
<td>Certain polymorphisms predispose to SLE</td>
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</tr>
<tr>
<td>IL-10</td>
<td>Interleukin-10</td>
<td>1q31–q32</td>
<td>Stimulates B-cell activation, proliferation, differentiation, and Ig production</td>
<td>Promoter polymorphisms enhance IL-10 production</td>
</tr>
<tr>
<td>MBL</td>
<td>Mannose binding lectin</td>
<td>10q11.2–q21</td>
<td>Activates complement cascade</td>
<td>Polymorphisms in promoter and exon 1 associated with SLE</td>
</tr>
<tr>
<td>PDCD1</td>
<td>Programmed cell death 1</td>
<td>2q37</td>
<td>Inhibition of T-cell proliferation and cytokine production</td>
<td>Intronic SNP associated with SLE in Europeans and Mexicans</td>
</tr>
</tbody>
</table>

Ig immunoglobulin; SNP single nucleotide polymorphism.
of the lung, intrathoracic lymph nodes, and skin, with demonstration of noncaseating granulomas and exclusion of other causes of granulomata, remains the gold standard for the diagnosis of sarcoidosis.11 Angiotensin converting enzyme (ACE) levels and T lymphocyte subsets in bronchoalveolar lavage fluid are adjunctive studies. The extent and course of disease may be monitored by serial imaging.

Pathogenesis

The proximate cause of sarcoidosis is unknown. Granulomas in tissue result from a predominantly T helper cell mediated immune response leading to release of cytokines such as IFN-γ, IL-2, and tumor necrosis factor (TNF)-α, and recruitment of macrophages. The antigen triggering the initial response is unknown, but a variety of infectious agents and occupational/environmental exposures have been implicated.11,21 An autoimmune component may be operational in the maintenance of the immune response.

Genetics

The existence of a “genetically predisposed” state is suggested by familial clustering of sarcoidosis as well as by increased incidence of disease in monozygotic compared to dizygotic twins. Weak associations have been identified with several genes. HLA-DRB1*15 and HLA-DQB1*0602 are linked to more chronic forms of disease, while HLA-DRB1*03 and HLA-DQB1*0201 are associated with a milder form of disease.21 Polymorphisms in transporter associated with antigen-processing (TAP) genes 1 and 2, encoding transporters essential in antigen processing for MHC-I antigen presentation, have been identified in British and Polish sarcoidosis patients. Disease associated polymorphisms have also been found in genes encoding inflammatory mediators such as the TNF-α promoter, ACE gene, IL-1α, complement receptor 1, and chemokine receptor genes.21

Endocrine Organ Involvement

Endocrine organ involvement in sarcoidosis may result from granulomatous infiltration or autoimmune mechanisms.18 Granulomatous infiltration is the cause of hypothyroid–pituitary sarcoid, with diabetes insipidus representing the most frequent clinical manifestation. Other manifestations include syndrome of inappropriate antidiuretic hormone (SIADH), hyperprolactinemia, hypothyroidism, hypoadrenalinism, growth hormone deficiency, and morbid obesity.22–25 Sarcoid granulomas may rarely involve the thyroid, resulting in hypothyroidism, cold nodules, and, uncommonly, hyperthyroidism.22,26–28 The adrenal is also a rare site for granulomatous infiltration, which may result in primary adrenal insufficiency.22

Endocrine involvement in sarcoidosis associated with autoimmunity has been reported in 19.2% of patients, based on serologic evidence,18,22,29 with autoimmune thyroid disease (Graves and autoimmune thyroiditis), Addison’s disease, and polyglanular autoimmune syndrome type II occurring more frequently than in the general population.22,30

Additionally, suppression of parathyroid function may follow the hypercalcemia seen in 5–10% of sarcoidosis patients. Hypercalcemia appears to be caused by elevated levels of 1,25-dihydroxyvitamin D3 and parathyroid-hormone related protein generated by sarcoid granulomas.22

Sjogren’s Syndrome

Sjogren’s syndrome is a common systemic disorder characterized by xerostomia and xerophthalmia secondary to autoimmune injury to exocrine glands. Half of Sjogren’s syndrome cases are primary, while the remainder are secondary: associated with another autoimmune connective tissue disorder. The prevalence has been reported as 0.5–5%, with 90% of cases occurring in women in midlife.10

Clinical Features

Autoimmune destruction of salivary and lacrimal glands is responsible for the predominant clinical manifestations of xerostomia and xerophthalmia with associated sequelae such as photophobia, redness, ocular fatigue/pain, infection, dental caries, and intraoral candidiasis.10 Additional xeroses include dry sinonasal mucosa, vaginal dryness, dry skin, and decreased sweat volume. Sjogren’s syndrome is associated with systemic symptoms such as musculoskeletal (arthritis, myalgias), cutaneous (dry skin, purpura, vasculitis), gastrointestinal (esophageal dysmotility, pancreatitis, hepatitis), renal (renal tubular acidosis, interstitial nephritis), neurologic (neuropathy, central nervous system disease), and hematologic (leukopenia, anemia, lymphoma). Diagnosis hinges on the presence of xeroses accompanied by either autoantibodies or a positive minor salivary gland biopsy demonstrating a quantitatively appropriate mononuclear inflammatory infiltrate with glandular damage.10

Pathogenesis

The autoimmune attack on exocrine glandular epithelium is hypothesized to be triggered in a genetically susceptible individual by an emotional or physiologic stress or an epitheliotropic virus, such as Epstein–Barr virus (EBV), that activates the epithelium. Because epithelial cells express MHC class I and II molecules as well as CD80 and CD81, they are capable of acting as antigen presenting cells and activating T-cells. Lymphocytes may thus be recruited and activated, launching a cycle of epithelial damage via apoptosis, release/presentation of autoantigens, and further immune attack. This process is mediated by proinflammatory cytokines IL-1β, TNF-α, IL-2, and IL-6.10,31
Within salivary glands, the lymphocytic infiltrate is predominantly T-cell; however, a B-cell contribution exists as well: foci of predominantly IgA expressing plasma cells and occasional germinal centers may be noted. Several different autoantibodies may be identified in Sjogren’s syndrome patients, and may contribute to epithelial destruction. Antibodies to ribonuclear proteins Ro (SS-A) and La (SS-B) are present in 75 and 40% of patients respectively. Additional autoantibodies may target fodrin (a cytoskeletal protein) and muscarinic M3 receptor.10

An additional mechanism of glandular damage is suggested by the presence of matrix degrading metalloproteinases within salivary glands in Sjogren’s syndrome patients.10

Genetics
Familial clustering and twin studies suggest a genetic component to Sjogren’s syndrome. Polymorphisms with the HLA DRB1/DQA1/DQB1 haplotype correlate with susceptibility to Sjogren’s syndrome in different ethnic groups, with clinical features, and with production of autoantibodies. Also linked to Sjogren’s syndrome are TAPs and TNF alleles on chromosome 6, as well as the IL-10 promoter region and glutathione transferase M1 on chromosome 1.10

Endocrine Organ Involvement
Sjogren’s syndrome is strongly associated with autoimmune thyroid disease. Thyroid disease, is more common in Sjogren’s syndrome patients than controls (30% versus 4%),32,33 and may precede or follow the diagnosis of Sjogren’s syndrome.10,31,32 Antibodies to thyroid antigens – thyroid peroxidase, thyroglobulin, T3, T4 – are frequently identified.18 Clinically, such patients usually present with Hashimoto’s thyroiditis, although Graves disease may occasionally result, with concordant histologic findings.

Systemic Sclerosis
Systemic sclerosis (scleroderma) is a connective tissue disorder of unknown etiology manifesting as thickening and fibrosis of the skin, internal organs, and blood vessels. The incidence is 18–20 per million per year, affecting predominantly women, with onset typically between 30 and 50 years. Systemic sclerosis occurs worldwide and affects all racial groups.10

Clinical Features
Approximately 70% of patients initially present with symptoms of systemic sclerosis related vasculopathy: Raynaud’s phenomenon – changes in skin color (blue, white, red) in fingers, toes, ears, and nose due to vasospasm/relaxation triggered by cold or emotional stress. With disease progression, the skin becomes thickened, taut, and indurated. The extent of skin involvement distinguishes clinical subgroups with varying prognosis. Involvement of the heart, lungs, gastrointestinal tract, and kidney by fibrosis or injury secondary to vasculopathy contributes most prominently to morbidity and mortality in systemic sclerosis.10

Pathogenesis
Alterations in fibroblasts and extracellular matrix are the immediate cause of disease. Fibroblasts are hyperproliferative, hypersecretory, and resistant to apoptosis. Both the structure of the collagen produced and its degradation are normal. Evidence strongly supports that the generalized fibrosis of systemic sclerosis is a secondary phenomenon; however, the proximate cause is unknown. Autoantibodies, including nonspecific ANAs, anticentromere antibodies (seen in 50–96% of patients with limited disease), and anti-DNA topoisomerase I (Scl70-70) antibodies (seen in 20–40% of patients), have been identified in systemic sclerosis patients. There is also evidence of T-cell activation and turnover. The significance of these immune abnormalities, the role of proinflammatory and fibroblast stimulating cytokines, and whether they are a cause or an effect of the disease are unclear.10

Histopathologic findings in affected skin include an increase in hyalinized collagen within the reticular dermis and subcutis and a mononuclear perivascular and interstitial inflammatory infiltrate. Small arteries and microvasculature exhibit lumen reduction by a collagenous hyperplasia of the intima.10

Genetics
Based on rare reports of familial clustering and disease concordance in identical twins higher than accounted for by chance, a genetic component of the disease appears likely. Linkage with HLA-A1, -B8, and -DR3 haplotypes has been established.10

Endocrine Involvement
Hypothyroidism is identified in up to 25% of systemic sclerosis patients; it is often occult. Fibrosis of the thyroid gland, found in 14% of unselected systemic sclerosis patients at autopsy, is the most likely mechanism of thyroid hypofunction. Lymphocytic infiltration of the gland and antithyroid antibodies are uncommon.10,34–37 A case of hypoparathyroidism due to parathyroid fibrosis has been reported.38

Rheumatoid Arthritis
Rheumatoid arthritis (RA) is a systemic autoimmune disorder in which synovial tissue is the target, although constitutional symptoms, and multiorgan manifestations are also common. Disease prevalence is 0.5–1% of adults, affecting women
twice as often as men and increasing in prevalence with age. Distribution is worldwide. Smoking, coffee consumption, and silica exposure are risk factors for disease, while estrogen use is protective.\textsuperscript{11}

**Clinical Findings**

Most patients with RA present with complaints of joint pain, stiffness, and/or swelling of gradual onset and involving multiple joints – typically the small joints of the hands and toes. Involvement of the proximal interphalangeal and metacarpophalangeal hand joints and metatarsophalangeal toe joints, with sparing of the distal interphalangeal joints is characteristic. Constitutional symptoms are often present. With disease progression, larger joints may be affected, and loss of function and deformity result. On exam the involved joints are warm and swollen.\textsuperscript{11}

Extra-articular RA may have numerous manifestations. In the skin, rheumatoid nodules and pyoderma gangrenosum may be seen. Cardiac involvement may present as pericarditis, valvular disease, and premature atherosclerosis. Interstitial lung disease, pleural effusions, and bronchiolitis obliterans may occur. Neurologic involvement may take the form of entrapment neuropathy, cervical myelopathy, and mononeuritis simplex. Anemia, thrombocytosis, lymphadenopathy, and Felty’s syndrome may be present. The kidney may develop amyloidosis. Ocular disease may occur. RA associated vasculitis may be identified in any organ system.\textsuperscript{11}

Autoantibodies are frequently present in RA. Approximately 80% have rheumatoid factor (RF), an antibody specific to IgG, while 70% have anti-cyclic citrullinated peptide (CCP) antibodies. ANAs and anti-neutrophil cytoplasm antibodies may be detected in up to 30% of patients.\textsuperscript{11}

**Pathogenesis**

The etiology of RA is unknown. Similar to other systemic autoimmune diseases, it is postulated that in a genetically susceptible individual, external triggers set into a motion a cycle of cellular injury, autoantigen exposure, and immune response. Proposed triggers are many, including microbial antigens and smoking. Both cellular and humoral immune responses appear critical in perpetuating the cycle. Activated T helper cells are prominent in synovial tissue. The role of autoantibodies such as RF and anti-CCP is unclear; however, their presence is correlated with more aggressive disease.\textsuperscript{11}

The attack on the synovium eventuates in proliferative synovitis with an infiltrate of lymphocytes and plasma cells, forming a pannus that covers the articular surface and erodes and infiltrates the underlying cartilage and bone.

**Genetics**

The contribution of genetic factors to development of RA is significant. Concordance rates in twins are 15–20% for monozygotic and 5% for dizygotic. HLA-D4, specifically polymorphisms of the amino acid sequence of the third hypervariable region on the DR\(\beta\)1 chain, are strongly associated with RA. This amino acid sequence has been termed the “shared epitope” or “at-risk allele” and correlates with more aggressive disease. Another susceptibility gene has identified as a polymorphism in the intracellular protein tyrosine phosphatase nonreceptor 22 (PTPN22).\textsuperscript{11}

**Endocrine Organ Involvement**

Thyroid gland involvement is common in RA patients, manifesting as hypothyroidism, hyperthyroidism, or nodular goiter in up to 34% of patients.\textsuperscript{39} Thyroid disease is primarily autoimmune in pathogenesis; antithyroid antibodies including anti-thyroperoxidase and anti-thyroglobulin, occur with greater frequency in patients than in controls.\textsuperscript{18,37,39–43}

**Idiopathic Inflammatory Myopathies**

The idiopathic inflammatory myopathies encompasses a diverse group of muscle diseases characterized by proximal muscle weakness and nonsuppurative inflammation of skeletal muscle. Included under this rubric are polymyositis (PM) and dermatomyositis (DM), as well as myositis associated with malignancy or collagen vascular disease, and inclusion body myositis.\textsuperscript{10}

**Clinical Features**

The prevalence of idiopathic inflammatory myopathies ranges from 0.5 to 9.3 cases per million. Women are affected more frequently than men. In the United States, African-Americans have the highest rate of disease. Onset of muscle weakness is typically insidious, with a bimodal age of onset: 10–15 years old in children, and 45–60 years old in adults.\textsuperscript{10}

Criteria for the diagnosis of PM are proximal muscle weakness, elevated serum levels of skeletal muscle derived enzymes (creatine kinase, aldolase, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase), electromyographic evidence of myopathy, and skeletal muscle inflammation on biopsy. Weakness most commonly first presents in the shoulder and pelvic muscle girdles. The addition of a skin rash to these criteria allows for the diagnosis of DM; skin involvement is variable between patients and over the disease course. Constitutional symptoms and involvement of other organ systems (cardiac: conduction abnormalities, cardiomyopathy, congestive heart failure; pulmonary: interstitial fibrosis) may occur.\textsuperscript{10}

Numerous autoantibodies are associated with PM and DM, some of which are specific for myositis, while other may be present but are nonspecific. Among the specific antigens are several aminoacyl-transfer RNA (tRNA) synthetases, the most common of which is anti-histidyl-tRNA (anti-Jo-1). Individual patients express only one myositis specific autoantibody, and autoantibodies correlate with clinical subsets/prognosis.\textsuperscript{10}
Pathogenesis

Evidence suggests that the idiopathic inflammatory myopathies are autoimmune processes initiated by exogenous triggers in a genetically predisposed individual. Seasonal variation in disease onset, and animal models lend support to the hypothesis that viruses are the exogenous triggers.

The histologic changes seen on muscle biopsy are nonspecific. In PM, fiber necrosis and regeneration with an endomysial mononuclear inflammatory infiltrate is classic. In DM, perivascular inflammation is prominent. These morphologic differences correlate with differences in pathogenesis. Although autoantibodies are present in PM and DM, PM appears to result from antigen-directed cell-mediated cytotoxicity. The lymphocytes surrounding and invading muscle fibers in PM are predominantly oligoclonal CD8+ cytotoxic T-cells, and muscle fibers exhibit increased surface expression of HLA class I molecules. T-cell-muscle fiber adhesion is enhanced by intercellular adhesion molecule-1 (ICAM-1) on fibers and complementary adhesion molecule, lymphocyte function associated antigen-1, on lymphocytes. Cell death results by an unknown mechanism; apoptosis has not been demonstrated.10

Humoral immune mechanisms appear to be operational in DM, with CD4+ T-cells mediating B-cell production of autoantibodies, which, with complement, cause damage to perimysial vasculature.10

Genetics

Familial clustering suggests a genetic component to the idiopathic inflammatory myopathies, although no specific genetic marker has been uncovered. HLA-B8, HLA-DR3, AND HLA-DRW52 are strongly associated with adult and pediatric PM and DM. Greater prevalence of the TNF-α-308A allele is seen in Caucasian PM/DM patients.10

Endocrine Organ Involvement

Endocrine organ involvement in PM/DM has not been systematically documented, although several case reports have identified an association of PM or DM with autoimmune thyroid disease, manifesting as hypo- or hyperthyroidism.18,44–48

Mixed Connective Tissue Disease

Mixed connective tissue disease (MCTD) is a systemic autoimmune disease that can be viewed as an overlap syndrome encompassing features of SLE, scleroderma, and PM/DM. Whether MCTD represents a distinct clinical entity remains an unresolved debate. Epidemiology is similar to other autoimmune connective tissue diseases.

Clinical Features

Features of SLE, scleroderma, and PM/DM often present metachronously, over the course of several years. Diverse organ involvement is typical, notably involving the skin and mucous membranes, joints, muscle, lungs, heart, GI tract, blood vessels, hematologic system, and kidney. Constitutional symptoms are common as well.10

Diagnosis rests on the symptom profile in the presence of high titer autoantibody against a ribonuclease-sensitive extractable nuclear antigen, U1 RNP, corresponding to a speckled pattern ANA. Anti-endothelial antibodies are also frequently identified, and correlate with pulmonary disease and spontaneous abortion.10

Pathogenesis

Pathogenic models revolve around the mechanism of exposure of U1 RNP to the immune system. U1 RNP is a uridine rich RNA particle that participates in messenger RNA splicing.49,50 As such, it is a nuclear antigen, normally sequestered from the immune system. The central pathogenic role of U1 RNP is supported by development of MCTD-like lung disease in mice immunized with portions of the U1 RNP antigen.49 In human disease, viral infection has been suggested as a trigger of apoptotic release of nuclear antigens; molecular mimicry with viral antigens may also contribute to the autoimmune response.50,51

Genetics

A subset of MCTD patients with erosive arthritis exhibits an increased frequency of HLA-DR4.52 An increased frequency of immunoglobulin allotypes Gm1.3 and 3 has been reported in patients.53

Endocrine Organ Involvement

Autoimmune thyroid disease is particularly common in MCTD patients: Hashimoto’s thyroiditis is 556-fold and Graves disease is 76-fold more common in MCTD than in the general population.17,54

Vasculitis

Systemic vasculitis encompasses approximately 20 types of primary vasculitis (classified by the caliber of vessel affected), as well as several types of secondary vasculitis (associated with other disease processes such as connective tissue disease, malignancy, and infection). All are united by the presence of destructive inflammation of blood vessels. A detailed discussion of each type of vasculitis is beyond the scope of this chapter; this section will focus on common themes in clinical features, pathogenesis (when known), and histologic findings of the primary vasculitides (Table 2.3).10,11,55
<table>
<thead>
<tr>
<th>Type of vasculitis</th>
<th>Predominant vessel size</th>
<th>Sites involved</th>
<th>Pathogenesis</th>
<th>Histologic features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Takayasu’s arteritis</td>
<td>Large</td>
<td>Aorta and major branches; pulmonary arteries</td>
<td>Cytotoxic T-cell attack on smooth muscle cells</td>
<td>Granulomatous vasculitis with GC; fibrosis</td>
</tr>
<tr>
<td>Giant cell arteritis</td>
<td>Large</td>
<td>Branches of aorta and carotids</td>
<td>CD4+ T-cell response to UK antigen; granuloma formation</td>
<td>Granulomatous vasculitis with GC</td>
</tr>
<tr>
<td>Cogan’s syndrome</td>
<td>Large</td>
<td>Eyes, audiovestibular system</td>
<td>UK; aberrant T-cell response?</td>
<td>Mixed inflammation; occasional granulomas</td>
</tr>
<tr>
<td>Behçet's disease</td>
<td>Large</td>
<td>Orogenital mucosa, eye, skin</td>
<td>Inflammatory cascade initiated by UK trigger; immune complex mediated?</td>
<td>LCV</td>
</tr>
<tr>
<td>Polyarteritis nodosa</td>
<td>Medium</td>
<td>Skin, peripheral nerves, GI tract, other viscera</td>
<td>Immune complex mediated (HBV infection)</td>
<td>Necrotizing vasculitis with mixed inflammation</td>
</tr>
<tr>
<td>Buerger’s disease</td>
<td>Medium</td>
<td>Arteries and veins in distal extremities</td>
<td>UK; anti-endothelial Abs and ANCA's have been identified</td>
<td>Neutrophilic, lymphocytic vasculitis with thromboses and microabcesses</td>
</tr>
<tr>
<td>Kawasaki’s disease</td>
<td>Medium</td>
<td>Oral mucosa, skin, lymphadenopathy, heart</td>
<td>UK; superantigen causing generalized immune system activation?</td>
<td>Segmental necrosis with prominent neutrophilic infiltrate and karyorrhexis</td>
</tr>
<tr>
<td>Cutaneous leukocytoclastic vasculitis</td>
<td>Small</td>
<td>Skin, joints</td>
<td>Immune complex mediated</td>
<td>LCV with occasional eosinophils</td>
</tr>
<tr>
<td>Henoch–Schönlein purpura</td>
<td>Small</td>
<td>Skin, joints, GI tract, kidneys</td>
<td>Immune complex mediated (IgA); commonly follows upper respiratory infection</td>
<td>LCV with lymphocytes and eosinophils</td>
</tr>
<tr>
<td>Essential cryoglobulinemia</td>
<td>Small</td>
<td>Skin, joints, kidneys, lungs, peripheral nerves</td>
<td>Immune complex mediated (HCV infection)</td>
<td>LCV</td>
</tr>
<tr>
<td>Wegener's granulomatosis</td>
<td>Small</td>
<td>Upper and lower respiratory tract, kidneys, skin, eyes</td>
<td>ANCA mediated</td>
<td>Necrotizing, granulomatous vasculitis; mixed inflammation</td>
</tr>
<tr>
<td>Microscopic polyangiitis</td>
<td>Small</td>
<td>Kidneys, lungs, joints, skin</td>
<td>ANCA mediated</td>
<td>Necrotizing glomerulonephritis, LCV</td>
</tr>
<tr>
<td>Churg–Strauss syndrome</td>
<td>Small</td>
<td>Upper and lower respiratory tract, heart, peripheral nerves</td>
<td>ANCA mediated; associated with atopy</td>
<td>Necrotizing, granulomatous vasculitis; mixed inflammation with prominent eosinophils</td>
</tr>
</tbody>
</table>

*GC* giant cells; *UK* unknown; *LCV* leukocytoclastic vasculitis; *ANCA* anti-neutrophil cytoplasm antibody; *Abs* antibodies.
Clinical Features

There is considerable variation in the epidemiologic profiles of the different vasculitides, including age predominantly affected, and geographic prevalence. Apart from a link between smoking and Buerger’s disease, and smoking and giant cell arteritis in women, no environmental or occupational risk factors have been identified.10,11 Although most vasculitides have a predilection to involve specific organs, and the constellation of presenting features correlates with the profile of organ involvement, vasculitides share certain features. These include constitutional symptoms such as fever, weight loss, rash, and elevated acute phase reactants. Lesions of vasculitis are typically painful, and patients will report pain corresponding to the involved organs. Multiorgan involvement is the rule.10,11

The differential diagnosis is extensive, and diagnosis of vasculitis rests on biopsy of affected tissue.

Pathogenesis

The etiology of most forms of vasculitis is unknown. While united by the histologic finding of destructive inflammation of blood vessels, the destruction is not in all cases due to an autoimmune attack on blood vessel antigens.

Varying pathogenic models have been postulated in several types of vasculitis. In large vessel vasculitides, the adventitial layer appears to be the initial target of the pathologic process, and becomes inundated with activated T-cells. For example, in Takayasu’s arteritis, cytotoxic CD8+ T-cells contribute to the damage of smooth muscle cells via perforin and granzyme B. By contrast, the T-cells in giant cell arteritis appear to be predominantly CD4+ cells, which are attracted by an as yet unknown adventitial antigen; a neoantigen generated by an infectious agent or by aging has been suggested. IFN-γ secretion promotes granuloma formation, with subsequent necrosis and elastic lamina destruction.10

Medium and small vessel vasculitides present additional pathogenic mechanisms:10,11

• Superantigens, which are microbial derived proteins that can stimulate large populations of T-cells via binding of class II MHC molecules outside the antigen-binding groove, are implicated in Kawasaki’s disease.

• Viral infection may be associated with immune complex-mediated vascular injury due to the deposition of antibody–viral antigen complexes in vessels. Such a mechanism appears to underly most cases of polyarteritis nodosa, associated with hepatitis B (HBV) infection, and mixed cryoglobulinemia, associated with hepatitis C (HCV) infection.

• Three types of vasculitis, Wegener’s granulomatosis, microscopic polyangitis, and Churg–Strauss syndrome, are associated with anti-neutrophil cytoplasm antibodies (ANCAs) – antibodies directed against antigens within the primary granules of neutrophils and monocytes. The targets of the most common ANCAs are proteinase 3 (PR3) and myeloperoxidase (MPO). Surface expression of these antigens is abnormally elevated early in the disease process, and binding of ANCAs triggers granulocyte activation and release of reactive oxygen species, granule contents, and lysosomal enzymes, all of which effect endothelial injury.56 This process is promoted by cytokines and CD4+ T-cells.

• A variety of infectious agents have been proposed to be triggers of vasculitis. Possible pathogenic mechanisms include molecular mimicry–microbes sharing epitopes with vessel walls; bystander injury due to widespread inflammation leading to exposure of autoantigens; and generation of neoantigens via the interaction between microbe and vessel molecules.57

• Antiendothelial antibodies have been identified within the sera of vasculitis patients in in vitro assays. While in vitro endothelial injury has been demonstrated, the significance of these autoantibodies in vivo is unknown.

On biopsy, there may be histologic overlap among the varying subtypes of vasculitis, depending on the type and the stage of disease. Most commonly a mixed inflammatory infiltrate is seen, although specific morphologic features may dominate the picture (see Table 2.3).

Genetics

Behçet’s disease has been linked to HLA-B51, which is present in 80% of Asian patients.66 Familial occurrence of giant cell arteritis has been reported, and 60% of patients have HLA-DRB1*04 variants that share a common sequence in the B1 molecule.10

Endocrine Organ Involvement

While none of the vasculitides appear to target endocrine organs, these organs may occasionally be affected. An increased rate of hypothyroidism and higher frequency of antithyroid antibodies have been reported in patients with giant cell arteritis,58 however a multicenter case-control study failed to confirm a significant difference from the control population.59 Rare cases of multinodular goiter associated with giant cell arteritis of the thyroid arteries have been reported.60 Rare cases of pituitary involvement have been reported in patients with vasculitis including Wegener’s granulomatosis, Takayasu’s disease, Behçet’s disease, and Cogan’s syndrome.61–64

Isolated occurrences of pheochromocytoma in Behçet’s disease have been noted.55,66

Summary

Systemic autoimmune diseases share a proximate etiology of an immune attack on self-antigens. In most cases, the trigger of this attack is unknown. Mounting evidence suggests that
autoimmune diseases result from a complex interaction of environmental and other unknown factors in a genetically predisposed individual. Genetic predisposition is complex as well, with polymorphisms in multiple genes, most commonly related to immune function, imparting variably increased susceptibility to disease.

References

2. Systematic Autoimmune Diseases


Introduction

MicroRNAs are noncoding single-stranded 18- to 24-nt long RNAs that regulate diverse cellular processes, including cell death and proliferation.\(^1\) Production and function of microRNA requires a set of proteins.\(^2\)

Less than 2% of the human genome is translated into protein, yet more than 40% of the genome is transcribed into RNA, that is into noncoding RNA.\(^3\) Transporting RNA (t-RNA) is probably the best-known example of a noncoding RNA. A more recently described species of noncoding RNAs, such as short interfering RNA (siRNA) and microRNA, are involved in regulation of gene expression through complementary interaction with 3’ untranslated region (UTR) of target messenger RNA (mRNA). MicroRNA and siRNA are quite distinct (Table 3.1) and no connection between microRNAs and siRNAs was made until 2001, when Dicer, the enzyme that converts long double stranded RNA into siRNAs, was discovered to convert precursor microRNAs into mature microRNAs.\(^4\)

Many microRNA genes are mapped to intergenic areas and are expressed independently.\(^5\) About two thirds of microRNAs are encoded in clusters of two to three microRNA genes and are transcribed as polycistrons showing similar expression patterns.\(^2,6\) High sequence homology and concordant expression pattern of microRNA-221 and microRNA-222 in papillary thyroid carcinoma (PTC) (see below) are most likely due to their clustering on X chromosome.\(^5\) Finally, about a quarter of microRNA genes are located within introns. Some of the latter microRNAs follow a recently described alternative pathway of microRNA maturation, in which a subset of debranched introns have structure of precursor microRNA allowing them to enter microRNA-processing pathway without Drosha-mediated cleavage.\(^7,8\)

Differential expression of microRNA is described in several types of human endocrine neoplasms, including thyroid carcinomas (PTC,\(^6,11\) anaplastic thyroid carcinoma, ATC,\(^12\) follicular thyroid carcinoma, FTC,\(^13\)) and pituitary adenoma, PA.\(^14,15\)

The list of miR with known cancer gene targets continues to grow: let-7 negatively regulates Ras,\(^16\) microRNA-221 and microRNA-222 down-regulate kit receptor\(^17\) and p27(Kip1) protein, a key player in cell cycle control,\(^18\) and microRNA-16-1 and microRNA-15a repress Bcl-2.\(^19\)

MicroRNA and Neoplasms of Thyroid Gland

Currently, most of the effort is directed to more common sporadic thyroid neoplasms derived from follicular epithelial cells (i.e., PTC, follicular carcinomas, follicular adenomas, and ATC).

Our present knowledge of microRNA abnormalities in follicular cell derived thyroid carcinomas is based on several studies of 70 benign thyroid tissue samples and benign fine needle aspiration biopsies (FNAB) and about 180 samples of thyroid neoplasms (including eight FNA samples “suspicious for carcinoma”) (Tables 3.2 and 3.3). When clinical information is available, it appears that a very heterogeneous group of thyroid carcinomas was studied. For instance, analyses of PTC routinely include various histological variants, pathological stages T1 through T4a, cases with and without metastases to lymph nodes, etc.\(^9,11\) The set of PTC examined by Tetzlaff et al, is somewhat more uniform/homogenous, with classic variants only being included.\(^11\) In that study, the microRNA signatures was apparently unique to PTC. However, it might be too early to assume that all PTCs will exhibit the same microRNA profile independently of patients’ age, gender, or mutational status.

On a more technical note, although most of microRNA profiling and validation was done on fresh frozen tissue, the utility of formalin-fixed paraffin embedded tissue has also been convincingly demonstrated.\(^11,12\) All studies used total RNA and arrays with probes up to 245 human microRNAs. For a summary of differentially expressed and validated microRNAs see Table 3.3.
The following issues are raised by currently available results and practical necessities of diagnostic pathology (Tables 3.2 and 3.3):

1. At present, no single study directly and comprehensively compared microRNA expression across all major types of thyroid carcinomas. Only limited comparative analysis of follicular carcinomas to follicular adenomas and anaplastic thyroid carcinomas to papillary thyroid carcinomas was performed as part of validation panels (Table 3.3). At first glance, “unique microRNA signatures” characterize PTC, follicular carcinomas, and anaplastic thyroid carcinomas. However, there remain several caveats. For instance, microRNA downregulation as a general trend initially seemed to be restricted to anaplastic thyroid carcinoma. Now, it is described in histologically benign tissue of multinodular goiter (MNG). Ironically, it is the same microRNA-30a-5p, among others, that now appears to be downregulated in both anaplastic carcinoma and multinodular goiter.11,12 Furthermore, all three studies of microRNA expression in PTC, highlighted microRNA-222 as microRNA that is consistently upregulated, when compared to normal thyroid tissue and hyperplastic/colloid nodules of MNG. However, microRNA-222 appears to be upregulated 1.98-fold \((p=0.0212)\) in ATC.12 Since microRNA-222 upregulation of 1.98-fold was less than arbitrary cutoff of twofold change used by authors, it was not included in validation experiments. Of note, microRNA-222 and microRNA-221, the two microRNAs most consistently upregulated in PTC (Table 3.3), are both clustered on chromosome X and show concordant expression patterns.4 In light of this fact, the mechanism of isolated microRNA-221 upregulation in histologically benign thyroid tissue warrants further investigation before it can be reliably included in a clinical test.5,11

2. In everyday practice, hyperplastic/colloid nodules are being sampled by FNAB to rule out thyroid malignancy. These nodules may be diagnostically challenging when they are solitary. When they are multiple, meaningful clinical surveillance is very difficult. Evaluation of microRNA

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**Table 3.1. Distinctive features of MicroRNA and siRNA.**

<table>
<thead>
<tr>
<th>MicroRNA</th>
<th>siRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target</td>
<td>Regulate hundreds or more of endogenous genes per microRNA</td>
</tr>
<tr>
<td>Effect</td>
<td>Number of microRNAs that are bound to the target mRNA and determines the degree of translational inhibition</td>
</tr>
<tr>
<td>Processing and products</td>
<td>MicroRNA is generated from one arm of the stem-loop to yield a single-stranded RNAa</td>
</tr>
<tr>
<td>Origin</td>
<td>Endogenous gene</td>
</tr>
<tr>
<td>Potential application</td>
<td>Diagnostic and therapeutic</td>
</tr>
</tbody>
</table>

aMature microRNA entering RNA Induced Silencing Complex (RISC) is single-stranded.

---

**Table 3.2. Thyroid samples and microRNA profiling.**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Tissue type</th>
<th>Normal thyroid samples, (n)</th>
<th>Thyroid cancer types (n)</th>
<th>Neoplastic samples (including samples used for validation), (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Frozen</td>
<td>26 PTC</td>
<td>20</td>
<td>PTC 8, FA 8, FNA “suspicious for cancer,” 8</td>
</tr>
<tr>
<td>10</td>
<td>Frozen</td>
<td>10 PTC</td>
<td>69; FNA “suspicious for cancer,” 8</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>FFPE</td>
<td>20 PTC</td>
<td>20</td>
<td>PTC 17, FA 12</td>
</tr>
<tr>
<td>13</td>
<td>Frozen</td>
<td>4 FTC</td>
<td>17</td>
<td>PTC 12</td>
</tr>
<tr>
<td>12</td>
<td>Frozen/FFPE</td>
<td>10 ATC</td>
<td>30</td>
<td>PTC 30; ATC 30; FA 12</td>
</tr>
</tbody>
</table>

**FTC** follicular thyroid carcinoma; **FA** follicular adenoma; **PTC** papillary thyroid carcinoma; **ATC** anaplastic thyroid carcinoma; **MNG** multinodular goiter; **FNAB** fine needle aspiration biopsy; **FFPE** formalin fixed paraffin embedded tissue;

---

**Table 3.3. Quantitative MicroRNA abnormalities and thyroid carcinomas.**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Thyroid cancer type</th>
<th>MicroRNA deregulation</th>
<th>Successfully validated microRNA and method</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>PTC</td>
<td>221, 222, 213, 220, and 181b</td>
<td>None; NB: 221, 222, 181b; RT-PCR: pre-miR-221, -222, -181b (up-regulated in 7/8 FNABs)</td>
</tr>
<tr>
<td>9</td>
<td>PTC</td>
<td>221, 222, 146, 21, 181a, 220, 155</td>
<td>None; NB: 221, 146b; RT-PCR: 221, 222, 146b</td>
</tr>
<tr>
<td>11</td>
<td>PTC vs. MNG</td>
<td>221, 222, 21, 31, 172, 213, 223, 181b, 34a</td>
<td>345, 292as, 300, 218; RT-PCR: 21, 31, 221, 222</td>
</tr>
<tr>
<td>13</td>
<td>FTC vs. FA ATC</td>
<td>192, 197, 328, 346</td>
<td>None; RT-PCR: 197 and 346; NB and RT-PCR: 30d, 125b, 26a, 30a-5p; ISH: 30d</td>
</tr>
</tbody>
</table>

**FTC** follicular thyroid carcinoma; **FA** follicular adenoma; **PTC** papillary thyroid carcinoma; **ATC** anaplastic thyroid carcinoma; **FNAB** fine needle aspiration biopsy; **MNG** multinodular goiter; **FFPE** formalin fixed paraffin embedded tissue; **NB** northern blot; **ISH** in situ hybridization; **pre-miR** precursor microRNA.
profiles in thyroid carcinomas and their benign histological mimics appears to be a potentially practical approach. Currently, with the exception of work by Tetzelaff et al, the general trend is to compare thyroid neoplasms to grossly unremarkable thyroid tissue – situation where routine cytological or histological evaluation remains a “gold standard” diagnostic approach.11

3. Finally, microRNA profiling still needs additional validation, where the profiling is done on a well-characterized set of thyroid neoplasms controlled for time-proven histological and genetic prognosticators (e.g., pathologic stage, age, gender, BRAF, RAS, and ret/PTC1 and ret/PTC3 mutational status).

Papillary Thyroid Carcinoma, C-KIT, p27Kip1, and MicroRNA-221/222 Cluster

C-Kit is a tyrosine kinase receptor involved in cell differentiation and growth. Reduced C-kit levels in PTCs were reported more than a decade ago.20 It is apparent now, that C-kit mRNA is targeted by microRNA-221 and -222.17 Furthermore, He et al showed that downregulation of Kit protein correlated with strong overexpression of microRNA-221, microRNA-222, and microRNA-146b. Sequencing of microRNA-221 and microRNA-222 binding domains in KIT 3'-UTR revealed 3169G→A single nucleotide polymorphism (SNP) within microRNA-221 and microRNA-222 recognition sites. Heterozygosity for 3169G→A leads to modification in microRNA:target messenger RNA duplex conformation and decrease in efficiency of microRNA mediated C-kit translational inhibition. These results illustrate how changes in microRNA target genes influence its responses to microRNA.9 Of note, microRNA-221 overexpression in PTC cell line (NPA) boosted cell proliferation with more than twofold increase in number of colonies. When microRNA-221 was blocked in NPA cells, approximately 20% decrease in cell numbers was noticed 96 h after microRNA-221 antisense oligonucleotide transfection.10

Another direct consequence of microRNA-221/222 cluster upregulation is reduced level of p27(Kip1) protein and progression to the S phase of cell cycle.18

MicroRNA and Papillary Thyroid Carcinoma Cell Lines with RET/PTC and BRAFV600E Mutation

Genetically, PTCs feature abnormalities in RET/PTC-RAS-BRAF pathway.21 Limited studies of microRNA profile in cell lines harboring ret/PTC1 rearrangement [Nthy-ori 3-1 transfected with ret/PTC 1 and TPC-1] and normal thyroid cell lines revealed significant upregulation of four microRNAs (128a, 128b, 139, and 200a) and downregulation of another four microRNAs (154, 181a, 302b, 302c).22

Two cell lines containing BRAFV600E point mutation [Nthy-oriBRAF and KAT-10] featured dramatic upregulation of microRNA-200a, microRNA-200b, and microRNA-141 with downregulation of microRNA-127, microRNA-130a, and microRNA-144 when compared to normal thyroid cell lines N-thy-ori 3-1.23

Since none of the studies summarized in Tables 3.2 and 3.3, characterized mutational status of their PTC cases, it is difficult to extrapolate/compare microRNA abnormalities described in these three cell lines to clinical specimens. None of the microRNAs highlighted by studies of clinical samples was differentially expressed in cell lines with known mutations. However, one could still suggest the possibility of correlation between microRNA profiles and ret/PTC and/or BRAF mutations.

MicroRNA and Pituitary Adenoma

Comprehensive microRNA profiling has revealed a set of 24 microRNAs capable to differentiating 15 functioning and 17 nonfunctioning pituitary adenomas from five normal pituitary glands.14 Furthermore, microRNA signatures appeared to suggest the hormone-secreting profile of PA.

Moreover, microRNA-15a and microRNA-16-1 expression in pituitary adenomas secreting growth hormone and prolactin is about 70% lower than in noneoplastic pituitary tissue. The downregulation of microRNA-15a and microRNA-16-1 correlates with increase of RARS mRNA (arginyl-tRNA synthetase), which leads to decrease in p43 secretion, a cytokine involved in angiogenesis and inflammation.24

References

Section 2
Thyroid Diseases
Introduction

While thyroid cancer is relatively rare overall, accounting for less than 1% of all human malignancies, it is the most common endocrine malignancy, comprising nearly 90% of all endocrine cancers. The vast majority are well-differentiated cancers derived from follicular epithelium and most of these are papillary carcinomas (PTC). Prognosis is generally favorable, with only 1,500 thyroid cancer-related deaths per year, and a 10-year survival of greater than 90%. However, the incidence of PTC has been on the rise during recent years, perhaps due to increased rates of detection with the almost ubiquitous use of imaging studies with ever-increasing sensitivity.

The traditional diagnosis of thyroid cancer relies on the use of ultrasound-guided fine needle aspiration biopsy (FNA) of thyroid nodules, which in most situations yields a sensitivity of approximately 94%. Treatment then includes surgical resection followed by radioactive iodine ablation therapy, and subsequent TSH suppressive therapy for confirmed cases of thyroid cancer. Despite the excellent outcomes for most patients using this regimen, intense research continues to be conducted into novel forms of therapy for thyroid cancer. These studies are focused on several clinical situations, where traditional therapy has failed. For example, up to 20% of patients with well-differentiated thyroid cancer will experience a recurrence of their disease following traditional therapy. As many as 5% of these patients will have tumors that undergo high grade transformation resulting in tumors with more aggressive growth, metastatic spread, and loss of the ability to take up iodine. These features render the tumors unresponsive to traditional therapy. Poorly differentiated and anaplastic thyroid cancers also do not respond to conventional chemotherapy or external beam radiation. Novel therapies to treat these patients are needed.

Medullary thyroid cancers (MTC) which are derived from parafollicular C-cells, also tend to be more aggressive than well-differentiated follicular cancers and do not take up radioactive iodine. There are few therapeutic options for patients with medullary carcinoma who have advanced disease or distant metastases.

A final area of intense research concerning thyroid cancer relates to improving the ability to diagnose cancer preoperatively. FNA is often able to identify papillary thyroid cancers pre-operatively, at rates approaching 95% in most situations. However, when cytologic analysis reveals a hypercellular follicular lesion, FNA cannot distinguish between benign follicular lesions and follicular carcinoma since capsular or vascular invasion can only be confirmed by histologic analysis. As the risk of malignancy in this setting is 20%, many patients with a pre-operative diagnosis of follicular lesion will undergo surgery and have histologically confirmed benign disease.

With the completion of the human genome project, a powerful new set of tools is emerging for researchers to tackle such problems. With advancing insights into the molecular pathogenesis of thyroid malignancies, potential targets for the development of novel therapies and diagnostic modalities are being revealed (Figure 4.1). This chapter focuses on how these new molecular techniques are being utilized in the management of thyroid cancer.

Follicular-Derived Carcinoma

There have been two approaches in utilizing molecular techniques to improve the accuracy of preoperative diagnosis of follicular thyroid cancer. The first is to examine known genetic alterations (discussed in Chap. 7) and gene expression patterns associated with thyroid cancer in FNA specimens. The other is to use a peripheral blood assay to predict and then follow the progression of thyroid carcinoma.
Differential Diagnosis Through Molecular Testing

**Gene Profiling for Diagnosis**

While the above studies have focused on investigating the ability of molecular markers known to be associated with thyroid cancer to distinguish between benign and malignant lesions through RT-PCR or immunocytochemical analysis of cytology specimens, another group of researchers have attempted to tackle the same problem using a different approach. As mentioned previously, the completion of the human genome project in 2003 provided researchers with a powerful new tool with which to answer these questions. New technologies emerged to allow researchers to utilize the wealth of information waiting to be discovered in the 3,000,000,000 base pairs and 25,000 genes contained in the human genome. One important technology to emerge has been the use cDNA microarrays. This is a powerful method for quantitative analysis of cancer-specific gene expression associated with the pathology or altered biology of cancer cells, where tissue samples are applied to a gene chip containing every gene in the human genome. Sophisticated software allows quantification of the expression of each of these genes, allowing comparison of expression patterns of thousands of genes in hundreds of tumors in a relatively short time-period.

Several authors have published reports showing that sets of genes of various sizes can accurately separate thyroid lesions into predictable categories. Weber and colleagues identified three genes, cyclin D2, prostate differentiation factor, and protein convertase 2, which classified follicular lesions into benign and malignant groups with 100% sensitivity and 95% specificity.11 Rosen et al showed that a six-gene combination accurately distinguished between benign and malignant thyroid lesions with 75% sensitivity and 100% specificity.12 Other groups use all differentially expressed genes determined using microarray analysis to separate lesions into benign and malignant groups, such as Barden and colleagues who used 105 differentially expressed genes in follicular adenomas and carcinomas to cluster unknown thyroid lesions into benign and malignant groups with 100% accuracy.13 Several other groups have reported similarly promising results suggesting that this technology will likely have a role in the management of thyroid lesions in the future.14,56 Several obstacles remain, however, such as standardizing the assays, as there

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**Fig. 4.1.** Molecular targets involved in thyroid cancer pathogenesis.
Peripheral Blood Assays for Diagnosis

In addition to analyzing molecular markers in FNA specimens, researchers have looked at thyroid-specific markers in the peripheral blood as a means of diagnosing thyroid cancer. Using RT-PCR to detect and amplify thyroid-specific genes in the peripheral circulation, several targets have been identified to detect thyroid carcinoma, including thyroglobulin (Tg), thyroid peroxidase, thyrotropin (TSH) receptor, and the sodium/iodide symporter. The most extensively studied gene has been Tg, and this has been studied primarily for its use in the follow up of differentiated thyroid cancer following thyroidectomy and T4 suppressive therapy, particularly in the setting of present anti-Tg antibodies. However, there have been conflicting results concerning its ability to detect thyroid cancer, with some studies detecting this marker in healthy subjects, and others failing to detect it in patients with known metastatic disease, raising doubts about its clinical utility.

A peripheral blood marker which has shown promise in the preoperative diagnosis of thyroid cancer has been TSHR-mRNA. Chia and colleagues showed that this marker had potential utility in distinguishing benign from malignant follicular neoplasms, correctly predicting 71% of follicular lesions diagnosed by cytology. They additionally showed a direct relationship between peripheral TSH-mRNA levels and extent of disease. While studies are ongoing, this marker has promise as an adjunct to the preoperative diagnosis of follicular thyroid lesions as well as in the follow up of differentiated thyroid cancer.

Treatment and Molecular Targets

In addition to advances in the diagnosis of thyroid lesions, there has been a great deal of research into providing new and better therapies for thyroid cancers that do not respond to traditional treatment options. Up to 30% of differentiated thyroid cancers do not respond to traditional radioactive iodine or standard chemo-radiotherapy regimens. Similarly, advanced medullary thyroid cancers with distant metastases is a challenge to treat, as they do not respond to the traditional adjuvant therapies available, and they are obviously not responsive to radio-active iodine. Consequently, there has been much research directed at seeking alternative therapies using molecular markers as targets. Such therapeutic strategies include the use of kinase inhibitors, redifferentiation therapy, and gene therapy. Table 4.2 summarizes potential targets for novel therapeutic agents in the treatment of thyroid cancer.

### Table 4.2. Potential molecular targets for therapy in thyroid cancer.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kinase inhibitors</strong></td>
<td></td>
</tr>
<tr>
<td>Sorafenib (BAY-43-9006)</td>
<td>BRAF, VEGFR, RET, PDGFR</td>
</tr>
<tr>
<td>Axitinib (AG-013736)</td>
<td>VEGFR, c-KIT, PDGFR</td>
</tr>
<tr>
<td>Motesanib (AMG-706)</td>
<td>VEGFR, PDGFR, FLT-3</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>VGFR, EGFR</td>
</tr>
<tr>
<td>NVP-AEE788</td>
<td>VEGFR, c-MET</td>
</tr>
<tr>
<td>XL-184</td>
<td>EGFR</td>
</tr>
<tr>
<td>Gefitinib (Iressa)</td>
<td>VEGFR, EGFR, RET</td>
</tr>
<tr>
<td>Vandetanib (ZD6474, Zactima)</td>
<td>c-KIT, PDGF, Bcr-Abl, RET</td>
</tr>
<tr>
<td>Imatinib (Gleevec)</td>
<td></td>
</tr>
<tr>
<td><strong>Other therapeutic agents</strong></td>
<td></td>
</tr>
<tr>
<td>Celecoxib</td>
<td>COX-2</td>
</tr>
<tr>
<td>Irinotecan</td>
<td>Topoisomerase</td>
</tr>
<tr>
<td>Bortezomib (PS-341)</td>
<td>26s proteasome, NF-κB</td>
</tr>
<tr>
<td>17-AAG</td>
<td>Hsp90</td>
</tr>
<tr>
<td>Combrestatin A4 (CA4P)</td>
<td>Tabulin</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td>PPAR-γ agonist</td>
</tr>
<tr>
<td>KP372-1</td>
<td>Akt</td>
</tr>
<tr>
<td>SAHA</td>
<td>Histone deacetylase</td>
</tr>
<tr>
<td>Depsipeptide (FK288)</td>
<td>Histone deacetylase</td>
</tr>
<tr>
<td>Sodium butyrate</td>
<td>DNA methyltransferase</td>
</tr>
<tr>
<td>Retinoic Acids</td>
<td>Nuclear receptors</td>
</tr>
</tbody>
</table>

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**Table 4.1. Molecular targets being investigated to differentiate benign and malignant thyroid lesions pre-operatively.**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Method of analysis</th>
</tr>
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<tbody>
<tr>
<td>Galectin-3</td>
<td>Immunocytochemistry</td>
</tr>
<tr>
<td>CD44</td>
<td>Immunocytochemistry, RT-PCR</td>
</tr>
<tr>
<td>Telomerase</td>
<td>TRAP assay, RT-PCR</td>
</tr>
<tr>
<td>HMG1(Y) protein</td>
<td>RT-PCR, immunocytochemistry</td>
</tr>
<tr>
<td>COX-2</td>
<td>RT-PCR</td>
</tr>
<tr>
<td>HBME-1</td>
<td>Immunocytochemistry</td>
</tr>
<tr>
<td>TSHR mRNA</td>
<td>RT-PCR of peripheral blood</td>
</tr>
<tr>
<td>Genetic profiling</td>
<td>Microarray analysis</td>
</tr>
</tbody>
</table>

RT-PCR reverse transcriptase-polymerase chain reaction; TRAP telomerase repeat amplification protocol; HMG1(Y) high mobility group 1 (Y); COX-2 cyclooxygenase-2; HBME-1 Hector Battifora mesothelial cell; TSHR mRNA thyrotropin receptor messenger ribonucleic acid.
Gene Therapy

The strategy of most gene therapy approaches is to introduce an exogenous gene designed specifically to manipulate tumor cells to render them more susceptible to localization and destruction. Several areas of study employing gene therapy are underway, but most are in early stages using in vitro and in vivo animal models. The following section will summarize the areas of gene therapy undergoing active investigation.

Corrective Gene Therapy

Corrective gene therapy aims to restore normal function to a gene which has been deleted or harbors a mutation; typically, these targets are tumor suppressor genes. p53, which is involved in DNA repair and apoptosis, has been shown to be involved in thyroid dedifferentiation in a high percentage of poorly differentiated thyroid cancers. Early studies have shown that introduction of wild-type p53 into thyroid cells harboring a mutated p53 gene results in re-emergence of a more differentiated phenotype, measured primarily by the presence of thyroid peroxidases, thyroglobulin, and the sodium/iodide symporter.

Immunomodulatory Gene Therapy

This method of gene therapy introduces genes that enhance or induce the host immune system to respond to the presence of malignant cells. While many tumor cells express tumor-specific antigens, they develop mechanisms to evade the host immune system. The cytokine interleukin 2 (IL-2) activates natural killer (NK) cells and other effector cells, thereby upregulating both specific and nonspecific immunity. Several published reports have shown this approach to be feasible. Using a BALB/c mouse model of medullary thyroid cancer, the authors showed tumors engineered to secrete high levels of IL-2 exhibited significant inhibition of growth. Additionally, tumor cells containing the IL-2 gene were injected into tumor-free mice without any subsequent growth, suggesting long-term immunity.

Cytoreductive Gene Therapy

Cytoreductive gene therapy employs the strategy of introducing novel genes which cause tumor cell death, or facilitate the use of selective cytotoxic agents against tumor cells. Typically, a gene which encodes for an enzyme is introduced into tumor cells. This enzyme activates a non-toxic prodrug, which will then attack only those cells with the enzyme and spare healthy cells. One study showed that the herpes simplex virus thymidine kinase and the prodrug gancyclovir resulted in dose-dependent death in two thyroid cancer cell lines. Gancyclovir, a nucleotide analog that is preferentially phosphorylated by the HSV thymidine kinase, competes with normal nucleotides during replication and consequently inhibits cell propagation.

Gene Silencing

This technique employs the use of antisense RNA segments that bind to specific genes, usually oncogenes, and inhibit their expression. C-myc is a proto-oncogene that encodes for a nuclear protein involved in cell proliferation. It has been shown to be constitutively expressed in thyroid cancers, and that the use of antisense RNA to silence this gene may lead to tumor growth inhibition.

Redifferentiation Therapy

Dedifferentiated thyroid cancers lose the ability to concentrate iodine and cease to express TSH receptors, thus rendering them impervious to standard radioiodine and TSH suppressive therapy. Redifferentiation therapy attempts to induce the reversal of these changes in tumor cells to make them susceptible once again to standard therapy.

Retinoic Acids

Retinoic acids are the biologically active metabolites of vitamin A. These have been used in several human cancers, with the greatest effects being seen with certain types of leukemia. Several in vitro studies showed retinoic acid inhibited growth and the ability of thyroid cells to metastasize. They also stimulated thyroid specific functions, such as expression of the sodium-iodide symporter and increased iodine uptake. These results led to clinical trials in patients who had unresectable dedifferentiated thyroid cancer with no iodine uptake. Radiiodine uptake increased in 40% of patients; there was reduction of tumor size in 11%, and 31% showed no further growth.

Deoxyribonucleic Acid Methyltransferase Inhibitors

Promoter methylation is a common mechanism of regulation of gene expression. Methylation prevents transcription from occurring, in effect turning the gene off until required by the cell. Many thyroid cancers have been shown to contain a high degree of methylation. Methylation is likely to be involved in the loss of the sodium-iodide symporter function in carcinomas, which is responsible for preventing iodide uptake by cancer cells. Published reports have shown using in vitro and in vivo models of thyroid cancer that the use of the demethylating agents sodium butyrate and 5-azacytidine inhibit tumor growth and cause dedifferentiated thyroid cancer cells to regain the ability to take up iodide.

Histone Deacetylase Inhibitors

Histones are nuclear proteins that are involved in the condensing of DNA into chromatin. Histone deacetylation is required to unfold DNA, allowing access of promoter regions for gene transcription. Inhibitors of histone deacetylation, such as depsipeptide (FK288), have been shown to increase the expression of the sodium-iodide symporter and iodide uptake in poorly differentiated and undifferentiated thyroid cancer cells.
There are phase II human trials of recurrent or metastatic thyroid cancer currently underway for desipeptide.

Suberoylanalide hydroxamic acid (SAHA) is another histone deacetylase that has been studied in the treatment of thyroid cancer. Preclinical studies with anaplastic and poorly differentiated thyroid cancers showed growth arrest and induction of apoptosis with this agent. Phase I study results of 73 patients, six of whom had thyroid cancer, showed one patient with a partial response, two patients with disease stabilization, and one patient with increased uptake of radioiodide in metastases.40

Tyrosine Kinase Inhibitors

An area of intense research in the treatment of thyroid cancer is in the use of tyrosine kinase inhibitors. Receptor tyrosine kinases are the most commonly overexpressed receptors in thyroid cancers. Consequently, these have become natural targets for novel directed therapies in these cancers. The following section will summarize the status of this area of research.

Angiogenesis Inhibitors

Angiogenesis is the development of new blood vessels which, in part, allows tumors to grow. Angiogenesis is a complex process with multiple factors that are recently beginning to be elucidated. One protein which has become the focus of research in many types of cancer is vascular endothelial growth factor (VEGF). This protein is a tyrosine kinase which stimulates neovascularization by binding to VEGF receptors on endothelial cells.41 VEGF has been shown to be upregulated in thyroid cancers. Several inhibitors of VEGF have been developed, and many of these have been studied in the treatment of thyroid cancer.

Sorafenib

Sorafenib is a multitarget inhibitor which binds to both the VEGF and BRAF tyrosine kinase receptors. This has been evaluated in the treatment of thyroid cancer in a phase II trial, where 19 patients with metastatic, iodine-resistant papillary thyroid cancer were treated. Five patients manifested a partial response to therapy, and eight others had no disease progression. Median duration of response was 14.2 months with a median time to progression of greater than 4 months (4 days to 14 months). Evaluation of tumor tissues showed treatment with sorafenib caused reduction of pERK, downstream target of VEGFR and BRAF, and pAKT, downstream of VEGFR.42 This drug currently is approved for the treatment of renal cell cancer and hepatocellular carcinoma.

Axitinib

Axitinib (AG-013736) is an inhibitor of VEGFR-1, 2 & 3. Sixty patients with metastatic or unresectable thyroid cancer were evaluated with this drug in a single arm multicenter clinical trial. All patients had cancers refractory to radioiodine therapy, or were not suitable candidates for this treatment for other reasons. Most patients had differentiated thyroid cancer, with two having anaplastic cancer. 18 patients had partial responses and 30 had stable disease, with a median progression free survival that had not been reached at 273 days of follow up. Therapy with this drug was generally well tolerated at a dose of 5 mg twice a day.

Motesanib

AMG 706 (Motesanib) is another drug that inhibits several tyrosine kinases, including VEGFR, PDGFR, c-Kit, and RET. This was evaluated in a phase II trial of patients with advanced differentiated or medullary thyroid cancer, with patients receiving daily doses until disease progression occurred or toxicity became unacceptable. Of the 93 patients with advanced differentiated disease, 12% had a partial response and 69% had stable disease. Of the 83 patients with sporadic or hereditary MTC, two had partial responses and 43 had stable disease for longer than 6 months. Toxicity included diarrhea and hypertension, and was generally well tolerated.

Sunitinib

Sunitinib is an orally administered tyrosine kinase inhibitor with activity against VEGFR, platelet derived growth factor receptor (PDGFR), and fms-related tyrosine kinase 3 (FLT-3). There is a phase II trials of this drug currently underway. Other VEGF receptors such as NVP-AEE788 and PTK787/ZK222584, have shown promise in animal models or in vitro models, but have yet to undergo human trials.43 XL-184, which inhibits multiple tyrosine kinases such as VEGFR and c-MET is currently undergoing phase I/II trials of several tumor types, including thyroid cancer. Results of phase I trials showed partial responses in three of seven patients with MTC.

Other Targets of Signaling Pathways

Gefitinib

This is another tyrosine kinase inhibitor which targets epidermal growth factor receptor (EGFR). This has been shown to be expressed on malignant thyroid cells and has been associated with a worse prognosis in differentiated thyroid cancers. A phase II trial of patients with radioiodine resistant thyroid cancer showed no objective response using RECIST criteria, but there was reduction in tumor size in 32% of patients, and 12% of patients showed no progression of disease after 1 year. Overall survival was 17.5 months.

Celecoxib

COX-2 has been shown to be upregulated by two oncogenes associated with thyroid cancer, RET/PTC1 and 2. Celecoxib, a COX-2 inhibitor, seemed a possible target for directed therapy, but showed poor results in a phase II study of patients with metastatic differentiated thyroid cancer.44
The PI3K/Akt signaling pathway is involved in cell proliferation and growth and has been recognized as being involved in cancer pathogenesis. Inhibiting this signaling pathway with the Akt inhibitor KP372-1 has been shown to induce apoptosis in thyroid cancer cells in preclinical studies.

**Medullary Thyroid Cancer**

Medullary thyroid cancer has been associated with rearrangements in the RET protooncogene, another receptor tyrosine kinase. Inhibitors of this kinase have been studied as new therapies for advanced cases of MTC unable to be cured surgically.

**Vandetanib**

ZD6474 (Vandetanib, Zactima), is an anilinoquinazoline compound which inhibits VEGFR2 & 3, EGFR, and RET tyrosine kinases. A single arm phase II trial of 30 patients showed a 17% partial response rate, and a 50% stable disease rate. Calcitonin levels were decreased by at least 50% in 23 patients. These results have led to an international phase II trial comparing this drug with placebo. Additionally, sorafenib, motesanib, sunitinib and axitinib have all been evaluated in the treatment of MTC with varying results.

**Imatinib**

Imatinib (Gleevec) is a tyrosine kinase inhibitor with activity against several kinases, including c-KIT, PDGF, Ber-Abl, and RET. More commonly known for its treatment of GI stromal tumors, it has also undergone phase II trials for the treatment of MTC. Unfortunately, 15 patients with disseminated disease showed no objective responses, although four patients had stable disease over 24 months. Three patients had to discontinue treatment because of side effects. There is also a phase II trial ongoing for the treatment of anaplastic thyroid cancer, although early results show limited efficacy. Several of these tyrosine kinase inhibitors with activity against multiple kinases are being looked at as possible therapeutic options for both follicular cancers and MTC.

**Anti-CEA Monoclonal Antibodies**

In addition to calcitonin, 70–90% of MTC’s express carcinoembryonic antigen (CEA) on their surface. Researchers have tried to exploit this as a means of directing targeted therapy at advanced MTC. A group of researchers published results of a study examining the use of 90Y-labeled human anti-CEA monoclonal antibodies to treat advanced MTC in a phase I trial. Of 15 patients treated with this in combination with doxorubicin, partial responses were seen in three, with one patient exhibiting a 68% reduction in local and hepatic metastatic disease. While the overall therapeutic response was modest, the authors concluded that this may be a therapeutic option in selected patients, particularly those with more limited, earlier stage disease.

**Topoisomerase Inhibitors**

The topoisomerase inhibitor irinotecan has been shown to have inhibitory effects on MTC alone or in combination with other agents such as cetuximab and CEP-751 in preclinical studies. There is a phase II clinical trial of irinotecan for the treatment of MTC ongoing.

**Proteasome Inhibitors**

The proteasome is a 26S protein involved in proteolysis, and has been shown to represent the primary degradation pathway for proteins involved in the regulation of cell proliferation, survival, and apoptosis. Proteins targeted for degradation undergo modification by the covalent addition of the low molecular weight polypeptide ubiquitin, after which the proteasome complex recognizes the protein and degrades it. Regulation of the proteasome has been found to be involved in many pathologic processes, including the pathogenesis of malignant transformation.
Bortezomib (PS-341) is a proteasome inhibitor which has been approved for use in the treatment of progressive multiple myeloma. A large scale phase II trial is currently underway examining its efficacy in the treatment of a variety of other tumors. Early studies have also been published examining its potential role in the treatment of MTC and anaplastic thyroid cancer. One group has shown that treatment with bortezomib induced apoptosis of MTC cells in vitro. Another published study reports the induction of significant apoptosis of anaplastic thyroid cancer cells at doses achieved in the clinical setting.

NF-κB

NF-κB is a transcription factor known to be a central activator of antiapoptotic cascades, and felt to play a key role in cancer pathogenesis. Inhibitory factors called IκB prevent NF-κB action, but phosphorylation of these inhibitory factors leads to ubiquitination and degradation by the 26S proteasome. Early in vitro and in vivo experiments have shown that treatment with nonspecific NF-κB inhibitors results in massive apoptosis of thyroid cancer cells, and deserves additional investigation.

Other Targets in Thyroid Cancer

Hsp90 Inhibitors

Hsp90 is a chaperone that stabilizes growth factor receptors and molecules involved in cell signaling. Inhibition of this protein leads to downregulation of signaling pathways involved in cell proliferation and growth, such as MAPK. 17-Allylamino-17-demethoxygeldanamycin (17-AAG) is the first Hsp90 inhibitor to enter into clinical studies. Early studies have shown that treatment with 17-AAG increased radioiodide accumulation in thyroid cancer cells. Currently, there is a phase II trial of 17-AAG in advanced thyroid cancer.

Combrestatin A4

Combrestatin A4 (CA4P) is a tubulin binding protein that has been shown to have direct antineoplastic activity against thyroid cancer cells. Studies in mouse models of anaplastic thyroid cancer (ATC) showed significantly lower tumor sizes in treated mice. A phase I clinical trial showed a durable response in one patient with ATC, and a phase II trial is underway currently.

PPAR-γ Agonists

This protein is involved in the regulation of cellular growth and differentiation, and is thought to be involved in thyroid cancer pathogenesis. Preclinical studies have shown growth inhibition and induction of apoptosis in papillary thyroid cancer cell lines after treatment with the PPAR-γ agonists rosiglitazone and troglitazone. A clinical trial of rosiglitazone in the treatment of advanced or metastatic thyroid cancer is currently underway. Table 4.3 summarizes the status of current clinical trials for novel treatment methods for thyroid cancer.

The wealth of ongoing research has yielded promising results in the development of new diagnostic and treatment modalities in the management of thyroid cancer. It is a very exciting time in the field of thyroid oncology, and the possibility that some of these new modalities could soon be added to our armamentarium in the management of thyroid cancer seems tantalizingly close. The dedication of the many talented scientists involved in the ongoing studies in this area will hopefully transform these promising results into clinical reality in the near future.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Target</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorafenib</td>
<td>RET, BRAF, VEGFR</td>
<td>Recruiting for phase II trial in patients with advanced ATC and metastatic MTC</td>
</tr>
<tr>
<td>Imatinib</td>
<td>c-KIT, PDGF, Bcr-Abl, RET</td>
<td>Recruiting for phase II trial of patients with ATC, and phase I/II trial in combination with other chemotherapeutic agents of patients with MTC</td>
</tr>
<tr>
<td>Axitinib</td>
<td>VEGFR, c-KIT, PDGF</td>
<td>Recruiting for phase II trial of patients with 131I-refractory metastatic or locally unresectable differentiated thyroid cancer</td>
</tr>
<tr>
<td>Motesanib</td>
<td>VEGFR, PDGF, RET</td>
<td>Recruiting for phase II trial in patients with advanced differentiated or MTC</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>VEGFR, PDGF, FLT-3</td>
<td>Recruiting for phase II trial in patients with 131I-refractory advanced differentiated or MTC</td>
</tr>
<tr>
<td>Gefitinib</td>
<td>EGFR</td>
<td>Recruiting for phase II trial of patients with 131I-resistant thyroid cancers</td>
</tr>
<tr>
<td>Vandetanib</td>
<td>VEGFR, EGFR, RET</td>
<td>Recruiting for phase II trial of patients with metastatic or locally advanced MTC</td>
</tr>
<tr>
<td>Irinotecan</td>
<td>Topoisomerase</td>
<td>Recruiting for phase II trial of patients with metastatic MTC</td>
</tr>
<tr>
<td>Bortezomib</td>
<td>26S proteasome</td>
<td>Recruiting for phase II trial of patients with metastatic papillary or follicular carcinoma</td>
</tr>
<tr>
<td>Combrestatin A4</td>
<td>Tubulin</td>
<td>Recruiting for phase II trial of patients with advanced ATC</td>
</tr>
<tr>
<td>17-AAG</td>
<td>Hsp90</td>
<td>Recruiting for phase II trial of patients with advanced MTC or differentiated thyroid cancer</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td>PPAR-γ</td>
<td>Recruiting for pilot study of patients with advanced differentiated thyroid cancer</td>
</tr>
<tr>
<td>FK228</td>
<td>Histone deacetylase</td>
<td>Recruiting for phase II study of patients with 131I-refractory metastatic non-medullary thyroid cancer</td>
</tr>
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</table>
References


Introduction

The discovery in 1956 that Hashimoto’s thyroiditis is an autoimmune disease spelled the end of the concept of horror autotoxicus and introduced the reality of human autoimmunity. Soon after, Graves’ disease was discovered to be directly caused by a serum factor, subsequently identified as an autoantibody. These diseases have served as invaluable models for the understanding of the organ specific autoimmunity. While Graves’ disease and Hashimoto’s thyroiditis are the two most common autoimmune thyroid diseases, autoimmune etiology has been also implicated in other forms of thyroiditides like Riedel’s, postpartum, silent and focal lymphocytic thyroiditis.

Graves’ Disease

Graves’ disease is characterized by diffuse hyperplasia of the thyroid gland, thyrotoxicosis due to excessive thyroid hormone synthesis, and the presence of thyroid-associated autoantibodies in the serum.

Historical Background

As early as the fifth century BC, the ancient Greeks described the combination of goiter, exophthalmos, and palpitations which is the known constellation of symptoms in patients with Graves’ disease.1 In 1786, Parry2 was the first to recognize and describe a group of patients with a combination of symptoms including rapid heart beat, goiter, and sometimes exophthalmos. These findings were, however, published only in 1825 after Parry’s death by his son in an obscure book.3 Later Robert Graves, in 1835, and Carl Basedow, in 1840, also described a group of patients with symptoms similar to those described by Parry.4 The continental European doctors being unaware of Graves’ description, called it Basedow’s disease, and this term is still used by some in Europe.4 Both Parry and Graves thought that these symptoms were a result of a cardiac disease, and even after Basedow’s report the goiter was not considered of much importance.4 In the 1860s after the description of cases with nervousness by Charcot, the cardiac origin gave way to a neurologic etiology, a belief that was dominant for the rest of the nineteenth century.4 By the late nineteenth century, with the introduction of thyroidectomies, it was observed that the removal of goiters improved nervousness in patients who survived surgery. This fact was supported by other observations in the 1890s, when too much thyroid extract led to nervousness and weight loss, symptoms similar to Graves’ disease. This discovery gave way to the thyroid origin of what is now universally known as Graves’ disease.4 The autoimmune origin was established only in 1957.

Clinical Features

Graves’ disease is seen mostly in women, with a female-to-male ratio of 8:1. The most common age of presentation is in the third or the fourth decade.5 Patients usually present with a characteristic clinical triad of diffuse enlargement of the thyroid (goiter), thyrotoxicosis with associated cardiovascular symptoms and ocular changes. The third component, localized infiltrative dermopathy (pretibial myxedema) is seen only in selected cases.

The most common clinical symptoms and signs include nervousness, excessive sweating, heat intolerance, palpitations, fatigue, tachycardia, muscle wasting, weight loss, diffuse goiter, tremors, and eye changes.5

Pathogenesis

The hallmark of GD is the production of TSH receptor stimulating autoantibodies. Other secondary autoantigens include thyroid peroxidase, thyroglobulin, and the sodium/iodide cotransporter6,7 can be also detected. The TSH receptor-stimulating antibody is specific to Graves’ disease.8 It functions like TSH, activates the adenyl cyclase-cAMP and protein kinase C-phosphoinositide signal transduction pathways,9 leading to increased synthesis and release of thyroid hormone and hyperplasia of thyroid follicular cells. T cells,
predominantly T helper (CD4) cells of both H1 and H2 subtypes, constitute the majority of the intrathyroidal lymphocytes in Graves’ disease. Other minor cell populations include T suppressor (CD8) lymphocytes, B lymphocytes, and plasma cells.

What initiates the autoimmune reaction in Graves’ disease is not entirely clear. Possible mechanisms include molecular mimicry or cross-reactivity between infectious agents and thyroid proteins. Such similarity was detected between Yersinia enterocolitica antigens and the TSH receptor. There is no evidence however, that either Yersinia or viral infection causes Graves’ disease. Other mechanisms suggested for the initiation of the immune reaction in Graves’ disease include direct induction of major histocompatibility complex (MHC) class II molecule expression such as HLA-DR on the surface of thyrocytes as a result of viral infection. Thus, the thyroid follicular cells may act themselves as antigen-presenting cells, presenting their own antigens, generating production of thyroid autoantibodies. Risk factors of Graves’ disease include genetic susceptibility such as HLA-B8, HLA-DR3 in Caucasians; HLA-DR5 in Japanese; HLA-DR9 in Chinese; HLA-b13 and DR5/8 in Koreans; polymorphism of CD40 and CTLA-4 genes affecting the T helper cell function (reviewed in ref. 13) infections, stress, sex steroids, pregnancy and the postpartum period, cigarette smoking, and iodine.

Pathology of Graves’s Disease

The gross and microscopic appearance of the thyroid gland in Graves’ disease varies with preoperative treatment. In general, grossly the thyroid gland shows mild to moderate diffuse symmetrical enlargement with a smooth surface (Figure 5.1). The gland has a softer consistency than the normal thyroid and sectioning reveals a fleshy red-brown cut surface. Histological examination (Figure 5.2a–d) shows markedly hyperplastic follicles with sometimes irregular jagged contours, lined by tall columnar cells. The decreased amount of colloid may appear pale and depleted, with scalloping observed at the periphery. The thyrocytes may show epithelial hyperplasia with papillary and pseudopapillary formations. This latter feature together with the presence nuclear chromatin clearing and rare foci of calcifications mimicking psammoma bodies should not be mistaken for papillary carcinoma. The important distinguishing feature in Graves’ disease is the diffuse presence of these changes throughout the gland, along with the presence of round, basally placed nuclei, and the absence of other nuclear changes of papillary carcinoma. Additional histological features in Graves’ disease include a variable degree of lymphocytic infiltrate of the stroma. The interstitial lymphoid cells are predominantly of T-helper (CD4) subtype that aid in the production of antibodies of the B cells. Preoperative medical treatment may alter the characteristic histologic picture in Graves’ disease: larger follicles filled with colloid or atrophied follicles with Hürthle cell metaplasia can be found mimicking Hashimoto’s thyroiditis. Iodine therapy can diminish hyperplasia resulting in almost normal appearing gland. Radioactive iodine may cause follicular disruption and atrophy with associated Hürthle cell metaplasia, fibrosis, and cellular and nuclear pleomorphism. Propylthiouracil therapy may increase hyperplasia.

Thyroid Nodules and Cancer in Graves’ Disease

Thyroid nodules are a common clinical problem in the general population, but the prevalence of palpable thyroid nodules in Graves’ disease is increased by more than threefold when compared to the general population. Whereas the prevalence of palpable thyroid nodule is 3.2–4.7% in iodine-
sufficient areas, in one large multi-institution study the prevalence of palpable thyroid nodules in patients with hyperthyroidism was 15.8%. Other studies have reported similar results. The prevalence of cancer in Graves’ disease has been reported to range from 0 to 9.8%, while in Graves’ disease patients with palpable nodules the prevalence increased, ranging from 5.8 to 45.8%. In a study that included 325 patients with Graves’ disease, Stocker et al found papillary thyroid carcinoma in 1.85% of all these patients, in 15.2% of these patients with cold nodules on scintiscan; in 25% of these patients with palpable nodules, and in 27.3% of those undergoing surgery. Thus, it seems that thyroid scintigraphy is an important preliminary investigation in the evaluation of Graves’ disease patients, and the high prevalence of thyroid cancer in those patients with cold nodules and palpable nodules warrants further diagnostic evaluation including a fine needle aspiration biopsy. Thyroid cancer arising in the background of Graves’ disease is thought to be of a more aggressive phenotype when compared to that in euthyroid patients. The patients may present with nodal and distant metastases, bilateral and multicentric tumors with invasive growth. This, however, has not been supported by some other studies. It is interesting to hypothesize if the anti-TSH receptor-stimulating antibodies may stimulate the thyroid cancer cells for an early metastatic growth in the same way as TSH stimulates growth of tumor cells expressing TSH receptor.

### Hashimoto’s Thyroiditis

**Historical Background**

In 1912, H. Hashimoto described four cases goiter for which he called “struma lymphomatosa” (lymphomatous goiter). All patients were females with goiter and the characteristic histological changes of lymphocytic thyroiditis which soon became known as Hashimoto’s thyroiditis (HT). The autoimmune nature of this disease was established in 1956, when Roitt et al detected autoantibodies against thyroglobulin in patients with HT. One year later, Trotter et al in 1957 identified a second antigen in the microsomal fraction of thyroid homogenates, which proved to be thyroid peroxidase (TPO).

**Clinical Features**

Hashimoto’s thyroiditis most frequently affects middle-aged women, though it is also the most common cause of sporadic goiter in children. It is the most common cause of hypothyroidism in areas of the world, where iodine levels are sufficient. Patients may present with hypothyroidism, goiter or both. Widespread use of thyroid function tests have also identified many cases of Hashimoto’s thyroiditis with subclinical hypothyroidism characterized with positive anti-TPO and antithyroglobulin antibodies in the serum associated with high TSH and normal T4. The goiter, when present, is firm

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**Fig. 5.2.** Graves’ disease; (a) hyperplastic thyroid follicles with irregular outlines and lymphocytic infiltrate (40×), (b) follicles lined by tall columnar thyrocytes (200×), (c) thyroid follicles with depleted colloid and peripheral scalloping of the colloid (100×), (d) thyroid follicles with multiple pseudo-papillary epithelial hyperplasia.
and often lobulated, which may be mistaken for a multinodular goiter or carcinoma. Hashimoto’s thyroiditis presenting as goitrous hypothyroidism has been found to be associated with HLA-D3 and HLA-D5. It may also coexist with other autoimmune diseases including pernicous anemia, diabetes mellitus, Sjögren’s syndrome, chronic hepatitis, adrenal insufficiency, and Graves’ disease. There appears to be a familial predisposition for the development of Hashimoto’s thyroiditis; up to 5% first-degree relatives of patients with Hashimoto’s thyroiditis may have positive antithyroid antibodies in their serum. There is also a high prevalence of autoimmune thyroid disease in patients with Down’s syndrome, familial Alzheimer’s disease, and Turner’s syndrome.

**Pathogenesis of Hashimoto’s Thyroiditis**

Hashimoto’s thyroiditis is a thyroid-specific autoimmune disorder, in which both humoral and cellular immune mechanisms play a role. The inflammatory process is initiated by the activation of thyroid-specific CD4 positive T helper cells. The cause of the activation is not entirely clear; both viral and bacterial infections have been implicated, but there are no conclusive supporting data. Another hypothesis implies that thyrocytes express HLA-DR antigen and become antigen-presenting cells, thereby exposing the thyroid cellular proteins to the CD4 T helper cells, which initiates the formation of autoantibodies against the thyroid autoantigens. Thyroglobulin autoantibodies are prevalent in HT; high titers of anti-Tg IgG can be found in >80% of HT patients. Furthermore, 94% of the Tg positive patients also have antibodies to TPO. Anti-TPO antibodies are complement fixing and thus directly cytotoxic for thyrocytes.

Blocking anti-TSHR antibodies were detected in 10% of HT patients. There is limited evidence that these effects play a major role in the destructive mechanisms of HT.

In addition to the pathway of thyroid cell injury, an alternative pathway involving cytotoxic T (CD8) cells and apoptosis has been more recently proposed. It has been shown that a significant population of intrathyroidal lymphocytes in Hashimoto’s thyroiditis are of CD8 phenotype having a cytotoxic/suppressor activity (reviewed in refs. 56–67). Some studies have shown that follicular cells from tissue samples of Hashimoto’s thyroiditis exhibit strong staining for the death receptor Fas and Fas ligand (FasL), together with a high apoptotic rate as compared normal controls. Bcl-2 inhibits apoptosis; immunohistochemical studies have shown a decreased expression of Bcl-2 in thyroid follicles from Hashimoto’s thyroiditis patients compared to normal controls and also in patients with Graves’ disease. Furthermore, interfollicular lymphocytes exhibit weak staining for FasL and strong expression of bcl-2. These data suggest that in patients with Hashimoto’s thyroiditis, the thyrocytes undergo apoptosis by upregulation of Fas and FasL and downregulation of bcl-2, which is independent of the antibody-mediated thyroid cell injury.

**Pathology of Hashimoto’s Thyroiditis**

The thyroid gland in Hashimoto’s thyroiditis is symmetrically enlarged and has a pale pink to yellow, lobulated cut surface. The accentuation of the lobulations may make the gland appear nodular on gross examination. Characteristic histologic features of Hashimoto’s thyroiditis include atrophy of the thyroid follicles with oncocytic (Hürthle cell) metaplasia of the follicular epithelium and abundant lymphoplasmacytic infiltrate with lymphoid follicles including germinal centers (Figure 5.3a–d). In addition, there may be varying degrees of fibrosis and foci of squamous metaplasia associated within the atrophic follicles. The peri-thyroidal lymph nodes are generally enlarged and show evidence of reactive lymphoid hyperplasia. The milieus of lymphocytic inflammation and regenerative hyperplasia results in increased risk of thyroid carcinoma and lymphoma. In some cases, there may be aggregates of oncocytic cells forming partially encapsulated hyperplastic nodules. The oncocytic cells may show nuclear enlargement and cytologic atypia. The follicular cells may show clearing of the nuclear chromatin and grooves, which may be mistaken for papillary carcinoma. Therefore, strict histologic criteria should be followed in making the diagnosis of papillary carcinoma in the background of Hashimoto’s thyroiditis. On immunohistochemistry, the lymphocytic population is composed of a mixture of B and T cells (Figure 5.4a–b). The T cells are predominantly activated CD4+ helper cells with evidence of HLADRII expression. In addition, there are also CD8+ cytotoxic/suppressor T cells, B cells, and plasma cells. As mentioned earlier, the CD8+ cells are thought to play a role in Fas-FasL-mediated thyroid follicular cell apoptosis and cytotoxicity. The plasma cells show reactivity with immunoglobulin γ (IgG), α (IgA) and μ (IgM) heavy chains and both κ and λ light chains. Immunohistochemistry may also be useful in differentiating a dense lymphoid infiltrate of Hashimoto’s thyroiditis from lymphoma, which may arise in the background of Hashimoto’s thyroiditis. The lymphoid infiltrate in Hashimoto’s thyroiditis is polyclonal as described previously and lacks evidence of light chain restriction on κ or λ. Additional molecular studies and flow cytometry may sometimes be useful in difficult cases.

**Histologic Variants of Hashimoto’s Thyroiditis**

In addition to the classic form of Hashimoto’s thyroiditis, many variants have been described, including the following.

**Fibrous Variant**

This comprises approx 10% of the cases and is usually seen in elderly patients who present with markedly enlarged goiter and hypothyroidism. Gross examination, the gland has a firm consistency due to a marked degree of fibrosis. Microscopically, there is extensive atrophy of the
thyroid follicles with diffuse fibrosis (Figure 5.5a–b), which in some areas has a keloid-like appearance. This fibrotic process is confined within the thyroid capsule, which is an important feature to distinguish it from Riedel’s thyroiditis. In Riedel’s thyroiditis, there is involvement of the surrounding structures such as muscles, nerves, and sometimes the parathyroid glands by the fibrotic process. Other features that help to differentiate are the lack of vasculitis or myointimal proliferation, which can be seen in Riedel’s thyroiditis. Squamous metaplasia may be seen within the follicles. Foci of the classic form of Hashimoto’s thyroiditis with lymphoplasmacytic infiltrate and oncocytic metaplasia may also be seen.

Fibrous Atrophy Variant

This is also referred to as idiopathic myxedema and is characterized by a very small (2–5 g in weight) fibrotic thyroid gland, which is often barely identifiable on gross examination.71 There is widespread destruction of the thyroid parenchyma with replacement by fibrous stroma. The histological features are similar to the fibrous variant of
Hashimoto’s thyroiditis, the only difference being a much smaller gland.\(^5\)

**Juvenile Variant**

This is a form chronic lymphocytic thyroiditis seen in younger patients and is often associated with hyperthyroidism, with later progression to hypothyroidism. The follicular atrophy and oncocytic metaplasia is focal and hyperplastic changes may be in the thyroid follicles.\(^7\)

**Other Forms of Thyroiditides**

**Riedel’s Thyroiditis**

Riedel’s thyroiditis is a rare disorder causing hypothyroidism due to the destruction of the thyroid gland and its replacement by fibrous tissue. This extensive fibrotic process extends into the extrathyroidal tissues of the neck and therefore this entity has sometimes been referred to as *invasive fibrous thyroiditis*.\(^7\) The clinical presenting features include a rapidly enlarging hard neck mass causing compression of the trachea and esophagus mimicking carcinoma.\(^7\) Hypocalcemia due to hypoparathyroidism may sometimes be present because of the fibrous destruction of parathyroid.\(^7\)

The etiology of Riedel’s thyroiditis is not known; autoimmune inflammation has been suggested because of the lymphoplasmacytic infiltrate in the thyroid and the presence of thyroid autoantibodies reported in patients with Riedel’s thyroiditis.\(^7\) In view of the extensive fibrosis, Riedel’s thyroiditis is also considered to be part of disseminated idiopathic fibrosing disorders, which include retroperitoneal and mediastinal fibrosis. These latter disorders have been found to be associated with or may develop after the diagnosis of Riedel’s thyroiditis.\(^7\)

The thyroid gland in Riedel’s thyroiditis is enlarged with a hard consistency because of extensive asymmetrical fibrosis. This has been often referred to as “woody thyroid.” The fibrosis often extends into the extrathyroidal soft tissues.

On the cut surface, the gland is tan gray in color with loss of normal lobulations.\(^7\) Microscopy reveals replacement of the normal thyroid parenchyma by dense sclerotic and acellular fibrous tissue. In addition, a mixed inflammatory infiltrate comprising mainly lymphocytes and plasma cells with few neutrophils and eosinophils may be seen. Vascular changes in the form of intimal proliferation and thrombosis may also be seen. In longstanding cases, the specimen may contain only dense sclerotic fibrous tissue with no residual thyroid follicles, making a diagnosis of Riedel’s thyroiditis in the absence of thyroid tissue difficult. In these cases, a clinicopathological correlation is essential to make the diagnosis of Riedel’s thyroiditis.

The differential diagnosis of Riedel’s thyroiditis includes a fibrosing variant of Hashimoto’s thyroiditis (see discussed above) and anaplastic carcinoma. Although this distinction can be more easily made on thyroidectomy specimens, small biopsies may sometimes pose a problem. Riedel’s thyroiditis is distinguished from a paucicellular variant of anaplastic carcinoma by the absence of nuclear pleomorphism, atypia, and mitoses which are usually seen in the latter.\(^5\)

**Postpartum Thyroiditis**

Postpartum thyroiditis is a rare autoimmune disorder characterized by transient hyperthyroidism followed by persistent hypothyroidism associated with lymphocytic infiltrate in the thyroid occurring within the first year after delivery.\(^7,8\) It occurs up to 10% of the women in the US. It has been regarded as a variant of Hashimoto’s thyroiditis.\(^8\) The autoimmune etiology of postpartum thyroiditis has been supported by the finding of a more common prevalence of the disease among women with HLA-DR3, DR4, or DR5 phenotypes, which is similar to that seen in Hashimoto’s thyroiditis. In addition, postpartum thyroiditis is seen associated with thyroid autoimmune disorders such as type I diabetes mellitus, Graves’ disease, and primary autoimmune hypothyroidism.\(^8\) Postpartum thyroiditis occurs most commonly in women with positive antithyroid peroxidase antibody in early pregnancy; the titers decline during pregnancy and then
rapidly rise again after delivery. The thyroid gland may be slightly diffusely enlarged and histology shows lymphocytic infiltrate associated with some follicular disruption and focal hyperplasia.5

Silent Thyroiditis

This form of thyroiditis has also been referred to as atypical subacute thyroiditis, painless thyroiditis, chronic lymphocytic thyroiditis, and transient hyperthyroidism with lymphocytic thyroiditis.5 It shares abnormal thyroid function with subacute (de Quervain’s) thyroiditis including transiently elevated blood levels of T4 and T3 associated with low radioactive iodine uptake. However, in contrast to the pain and tenderness seen in de Quervain’s thyroiditis, patients present with a painless thyroid enlargement.6 The etiology of silent thyroiditis is uncertain, both autoimmune and viral etiology has been proposed.8 On gross examination, there is slight diffuse enlargement of the thyroid gland. Microscopy shows preservation of the lobular architecture with a varying degree of lymphocytic infiltrate and associated follicular destruction. Oncocytic change may be seen but is uncommon.

Focal Lymphocytic Thyroiditis

This is mostly an incidental finding discovered in either surgically removed thyroid or at autopsy and is characterized by a focal lymphocytic infiltrate with preservation of normal lobular architecture and no significant alteration of thyroid functions. It is seen in 5–20% of adult autopsies, mostly in elderly women.9 On gross examination, no specific changes can be seen. Microscopy reveals preservation of the follicular architecture with foci of lymphocytic infiltrate, which may be associated with germinal center formation in the interfollicular region. Focal lymphocytic thyroiditis may be seen associated with multinodular goiter and thyroid tumors.9

References

destruction associated with Hashimoto’s autoimmune thyroiditis. 


Introduction

Benign nodular disease of the thyroid gland is exceedingly common in most of the world. In fact, some estimates have suggested that up to 70% of the population in the United States will have thyroid nodules detectable by ultrasound. The diagnostic categories for benign nodular thyroids include both multinodular disease and dominant single nodules, which can be of neoplastic or nonneoplastic origin. The benign nodular thyroid diseases are discussed in this chapter, along with the relevant molecular pathogenesis.

Sporadic Multinodular Goiter

Goiter is defined as diffuse or nodular enlargement of the thyroid gland. Goiter can have a variety of different etiologies, but importantly it should not be related to inflammatory processes. Multinodular goiter remains most common in endemic areas, where the disease is strongly associated with iodine deficiency in these selected geographic areas.4 Endemic goiter that develops secondary to iodine insufficiency should affect both genders equally, but in most studies women are more susceptible than men, and there are still familial tendencies for the disease to occur.4 This suggests that there are multiple factors, both environmental and genetic that contribute to the development of goiter. In nonendemic regions, goiter is much more common in women and the prevalence can still be relatively high, ranging between 0.4 and 5%.4 The treatment for symptomatic goiter in particular generally remains surgical, with either a subtotal or total thyroidectomy.31,41,43

Goiter is often accompanied by one or more dominant nodules, which have been traditionally considered to be nonneoplastic nodules. At the histologic level, nodules arising in goiter can have a variety of different appearances. These nodules are usually not fully encapsulated, though they can have fibrosis within the nodule or around the periphery. The growth pattern within the lesion is nearly identical to that in the surrounding thyroid gland. This includes variably sized follicles, some of which are quite large and expanded by colloid. Reactive changes are common, including hemorrhage, cystic degeneration, and hemosiderin laden macrophage deposition. The terminology used for histologically documented nonneoplastic nodules in goiter is “dominant hyperplastic nodule.”

Because of their multiplicity, the absence of a capsule, and the generally macro-follicular growth pattern, nodules in goiter were originally thought to be nonneoplastic and thus nonclonal. However, in several elegant studies which examined the clonality of these lesions using X-inactivation, (HUMARA assay) it has been shown that at least some nodules in multinodular goiter are clonal.1,8,18,28 Follicular adenomas have always been thought to be clonal proliferations, and this has also been shown to be true at the molecular level, through analysis of somatic mutations in oncogenes, loss of heterozygosity analysis of tumors suppressor genes, and through X-inactivation studies.1,11,16,29,39

Hereditary Causes of Goiter

Hereditary, familial, or clustering within families who demonstrate multinodular goiter has been described and been studied for years, but relatively few gene candidates have been proposed. This is likely because goiter, like most other complex diseases, is polygenic. There are both environmental and genetic contributions to goiter as well. The purely genetic contribution is best illustrated through careful twin studies. In epidemiological analysis of monozygotic and dizygotic twin pairs, the genetic contribution to development of goiter has been estimated at 82%.4

Several gene candidates have been proposed to be associated with the development of goiter. The thyroglobulin gene (chromosome 8), multinodular goiter gene (MNG1, chromosome 14), TSH-receptor (chromosome 14q13), the Na’/I’ symporter gene (NIS, chromosome 19p13.2–p12), and Gs alpha subunit gene (chromosome 20q13.2) have all been implicated for potential genetic contributions to goiter.3,7,12,30,32
Congenital Hypothyroidism

Congenital hypothyroidism is a condition that can have clinically devastating consequences if it goes unrecognized. But, newborn screening programs have greatly reduced the ramifications of this condition. Congenital hypothyroidism has both genetic and nongenetic contributions. It can also be a temporary condition caused by maternal factors, such as antibodies or drugs that cross the placenta and affect the newborn.

The presentation of a newborn with congenital hypothyroidism includes post-maturity, large posterior fontanel, and various forms of thyroid dysgenesis.21 The dysgenesis can include agenesis, ectopic glands, or hypoplastic glands, or they can have enlarged thyroid glands.

Congenital hypothyroidism is a heterogeneous disorder with many potential genetic alterations. Defects in the pathways involved in thyroid hormone synthesis, storage, secretion, or utilization are more common and are referred to as primary hypothyroidism.9,14,33 Defects at the pituitary or hypothalamic level that lead to congenital hypothyroidism, termed central hypothyroidism, are extremely rare.9 Most cases are considered to be sporadic, with maximal estimates of 15% of cases being hereditary.21 In the inherited cases, the inheritance patterns vary with the genetic mutations, but include autosomal dominant, recessive, and X-linked possibilities.14

Mutations have been described in many different genes, including the TSH receptor, the thyrotropin receptor, the Na+/I− symporter gene, and many other genes in the thyroid hormone pathways.14,21 Several specific transcription factors have also been implicated in both the sporadic and hereditary forms of thyroid dysgenesis. These include PAX8 (chromosome 2q12–q14), TTF1 (NKX2.1, chromosome 14q13), and TTF2 (FOXE1, chromosome 9q22).17 TTF2 germline mutations are responsible for the thyroid dysgenesis syndrome Bamforth–Lazarus syndrome.33 Mutations in the pendrin gene (SLC26A4, chromosome 7q31) are responsible for the Pendred syndrome, which includes sensorineural hearing defects and goiter and iodine organification defects.24,33

Thyroid goiter is generally seen in patients with dysmorphogenesis. These thyroids have a unique histologic appearance, with bizarre atypical cells and abnormal architecture. Dysmorphic goiter is generally seen in patients with defects in the steps leading to synthesis of thyroid hormone. The genes most commonly implicated are the thyroperoxidase, thyroglobulin, Na+/I− symporter gene, and the NADPH oxidases (such as thyroid oxidase-2, THOX2).9

Follicular Adenoma

Perhaps, the most common neoplastic condition of the thyroid is a benign follicular adenoma.26 The typical follicular adenoma will be an encapsulated lesion with a follicular growth pattern. These tumors can also have oncocytic changes, in which case they may be referred to as “Hürthle cell adenoma.” The lesion should have a reasonably complete capsule, though it may be variably thick and thin. Several other clues to the diagnosis of a neoplasm include the following: the nodule follicles should be different from the thyroid parenchyma outside of the nodule, and the thyroid follicles are often compressed and flattened on the outside of the nodule. Importantly, the cells are bland and remain small and round. They are essentially identical to thyroid follicular cells in the background thyroid gland. The cells in adenomas can also have Hurthle or oncocytic differentiation.

Thyroid follicular adenomas have been extensively studied at the molecular level. One of the first oncogenes discovered in the thyroid gland was the RAS gene, which is part of the RAS-RAF-MEK-MAPK pathway.23 Follicular adenomas have been long known to harbor mutations in all three RAS genes, H-RAS, K-RAS, and N-RAS.11,19,27 The RAS mutations are thought to be early mutations in the follicular neoplasia pathway, and therefore testing for RAS mutations has never had a strong diagnostic role.29

Tumor suppressor gene alterations have been described in both benign and malignant follicular derived tumors. The genes involved are diverse and encompass a spectrum of common tumor suppressor genes. However, it has been shown in several studies that the rate of tumor suppressor gene loss of heterozygosity alterations in follicular adenomas is significantly lower than that seen in follicular carcinoma.16,36,38 This is particularly true when adenomas are compared to follicular carcinomas with poor clinical outcome and higher grade histologic features.17 In several CGH-based studies, follicular adenomas and follicular carcinomas did not have a vastly different profile of losses and gains, and these changes did not appear to be consistent from tumor to tumor.5,37 One study did separate out cases diagnosed as aneuploid “fetal adenoma,” which appeared to have a very consistent molecular profile that included gains of chromosomes 4, 5, 7, 12, 14, 16, and 17 and losses of chromosomes 2, 11, and 15.5 Several studies have examined gene expression profiling to determine whether the expression patterns can differentiate between benign and malignant follicular tumors. Recent evidence does indicate that the profiles are different.4,43 Despite the fact that there is an attempt to narrow the genes to a limited set of the most informative,40 the clinical implications of these findings are still unclear, as the technique remains expensive and difficult to perform for routine tumor classification.

Papillary Hyperplastic Nodules

Some adenomas, or even benign hyperplastic nodules, can have an exuberant papillary growth pattern.25 These lesions are often clinically found to be “hot” or toxic thyroid nodules. They tend to be particularly common in younger women.2,10,20 They have been alternatively termed “papillary hyperplastic nodules” or follicular adenomas with papillary growth.
The striking feature in these lesions is a marked papillary growth pattern. But, in all of these lesions, the most critical diagnostic clue is the absence of nuclear features of papillary carcinoma. The nuclei tend to be small, dark, and basally located in the cells. Other softer signs include edema and follicles within the papillary cores and the fact that the papillae are quite organized and tend to all point toward the center of the lesion. These are very important lesions to recognize, so that a misdiagnosis of carcinoma is not made.

Interesting molecular findings have been described in papillary hyperplastic nodules. More than half of these hyperfunctional nodules, both in a background of goiter and in the setting of solitary nodules, have been shown to harbor clonal mutations in the TSH-receptor. These tumors do not appear to harbor mutations in the RAS genes.

References


Introduction

Thyroid nodules are common and they affect up to 7% of US population; they are often seen in women and the majority are benign. Fine-needle aspiration (FNA) is considered an essential tool in providing a rational approach to the clinical management of thyroid nodules. The results of FNA can determine whether a thyroid nodule should be followed clinically or undergo surgical excision.1,2

FNA Indications, Procedure, Specimen, and Classification

Every patient with a palpable thyroid nodule is a candidate for fine needle aspiration (FNA) and should undergo further evaluation to determine if an FNA is required. Thyroid nodules measuring at least 1.0 cm in dimension may be palpable, and these detectable lesions are considered to be clinically significant. However, some thyroid nodules measuring >1.0 cm may not be readily palpated because of their location in the thyroid gland.2 Non-palpable nodules are generally discovered during radiologic examination of the head and neck for nonthyroidal lesions. Thyroid nodules can be biopsied by palpation and under ultrasound guidance; the latter is becoming the method of choice since it provides precise information regarding the location, size, and structure (solid vs. cystic) of the nodule and is more effective to obtain adequate samples for cytologic interpretation.3

Thyroid FNA specimens are usually prepared as air-dried smears for staining with Romanowsky (Diff-Quik®, Wright-Geimsa, Wright stains) and as alcohol fixed smears for Papanicolaou stains. Additional sample can be prepared as liquid based preparation or as a cellblock.1

Thyroid FNA specimens are usually prepared as air-dried smears for staining with Romanowsky (Diff-Quik®, Wright-Geimsa, Wright stains) and as alcohol fixed smears for Papanicolaou stains. Additional sample can be prepared as liquid based preparation or as a cellblock.1

Cytomorphology of Thyroid Lesions

Nodular Goiter

The term goiter encompasses both nodular and diffuse enlargement of the thyroid, and it clinically can be divided into toxic and nontoxic variants based on the clinical symptoms and thyroid function tests (hypothyroid, euthyroid, or hyperthyroid).5,7

The cytology specimen features from a goiterous nodule depend somewhat upon the preparation method, but generally include copious watery colloid and small, round to oval shaped follicular cells with dark nuclei. The follicular cells are arranged in monolayer sheets, groups with follicle formation or as single cells.8,9 The cytoplasm is usually scant in follicular cells and can show numerous, small blue-black granules (lysosomes containing hemosiderin and lipofuscin pigments).9 Macrophages are usually present and their number depends upon the presence or absence of degenerative changes or a cystic component.9,10 (Figure 7.1) The aspirates from a hyperplastic/adenomatoid nodule tend to be more cellular and contain an admixture of follicular cells and oncotic cells arranged in monolayer sheets in a background of watery colloid and macrophages.9–11

Diffuse Toxic Goiter (Graves’ Disease)

The patients with Graves’ disease (GD) usually do not undergo FNA, except when they have solitary hypofunctioning (cold) nodules on radioiodine scan in the typical background of hyperfunctioning tissue. The specimens from GD are usually cellular, with features similar to hyperplastic goiter, but they also can contain lymphocytes and oncotic cells. Although these FNAs can occasionally display focal nuclear chromatin clearing and rare intranuclear grooves, the other diagnostic nuclear features of papillary carcinoma are not observed.12–15

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American Association of Clinical Endocrinologist and special committees1,2,4,5 (Table 7.1).
**Autoimmune Thyroiditis**

The FNA is usually performed in patients with chronic lymphocytic thyroiditis who present with distinct nodules. The specimens from such cases usually contain Hurthle cells, follicular cells, lymphocytes, and few plasma cells in a background of scant colloid.

The lymphocytes are usually seen in the background, percolating between cell groups and in some cases one may see an intact lymphoid follicle. The Hurthle cells and follicular cells can display nuclear atypia, including chromatin clearing and nuclear grooves. The potentially extensive lymphocytic infiltrate can appear monotonous and can be mistaken for malignant lymphoma arising in lymphocytic thyroiditis.

**Follicular-Patterned Neoplasms**

Thyroid (FNA) cannot distinguish between benign and malignant nonpapillary follicular and Hurthle cell lesions; both benign and malignant lesions demonstrate similar cytomorphology.

**Follicular/Follicular Neoplasm with Oncocytic Features (Hurthle Cell Neoplasm)**

This term is used to describe the FNA features seen in both benign and malignant tumors, including follicular adenoma and carcinoma and oncocytic follicular adenoma and carcinoma. The cytologic diagnosis of “neoplasm” reflects the limitations of thyroid cytology, since the diagnosis of follicular carcinoma can only be made with histologic evidence of capsular and/or vascular invasion. Several authors have shown that, at most, only 20–30% of cases diagnosed as “follicular neoplasm” are diagnosed as malignant on histologic examination, with the remainder representing benign follicular adenomas or cellular adenomatoid nodules.

The FNA of a follicular neoplasm is usually hyper-cellular and show a monotonous population of either follicular or Hurthle cells arranged in loosely cohesive group and hemosiderin laden macrophages. (a) Papanicolaou stained alcohol fixed smear, (b) Papanicolaou stained alcohol fixed smear.
Papillary Thyroid Carcinoma and Its Variants

The cytodiagnosis of papillary thyroid carcinoma (PTC) is based on characteristic nuclear morphology regardless of cytoplasmic features, growth pattern, special stains and immunohistochemical markers. This holds true for a majority of cases of PTC, though some variants of PTC may be difficult to diagnose at the cytologic level.\(^3\text{1,32}\)

The FNA specimen of PTC is usually cellular and shows tumor cells arranged in papillary groups, three-dimensional clusters or as single cells in a background of watery or thick “ropy” colloid. Additional features that can be seen are nuclear or calcific debris, macrophages, and stromal fragments.

The individual tumor cells are enlarged and elongated, or oval in shape, with eosinophilic cytoplasm. The cytoplasmic eosinophilia is more pronounced in Romanowsky stained preparations, but can be indistinct in alcohol fixed Papanicolaou stained preparations and monolayer preparations.\(^8\) The nuclei show nuclear membrane thickening, chromatin clearing, grooves, and inclusions (Figure 7.2). The nucleoli are usually small and eccentric. Some of these features are not specific for papillary carcinoma. Intranuclear grooves and inclusions can be seen in other benign and malignant conditions of thyroid, including Hashimoto’s thyroiditis, nodular goiter, hyalinizing trabecular neoplasm, Hurthle cell tumors, and medullary carcinoma.\(^3\text{3,34}\)

The cytologic interpretation of Follicular variant of papillary carcinoma (FVPTC) can be challenging due to a paucity of diagnostic nuclear features. The cytologic samples from FVPTC usually show enlarged follicular cells arranged in monolayer sheets and follicular groups in a background of thin and thick colloid. The individual tumor cells show nuclear elongation, chromatin clearing, and thick nuclear membranes, but nuclear grooves and inclusions are usually scarce. Thus, a majority of cytologic samples of FVPTC are diagnosed as “suspicious for papillary carcinoma.”\(^3\text{5}\)

The cytologic samples in tall cell variant of papillary carcinoma demonstrate elongated cells with sharp cytoplasmic borders, granular eosinophilic cytoplasm, and variably sized nuclei with the other typical nuclear features of papillary carcinoma.\(^3\text{6}\)

Warthin-Like variant of PTC

Warthin-like variant of PTC can show papillary fragments or cellular groups of oncocytic cells with PTC nuclei infiltrated by a lymphocytes and plasma cells. Aspirates from this form of PTC can be mistaken for chronic lymphocytic thyroiditis. The tumor cells from warthin-like variant, however, have distinct PTC nuclei which do not resemble oncocytic follicular (Hurthle) cells.\(^3\text{7}\)

Medullary Thyroid Carcinoma

Medullary thyroid carcinoma (MTC) arises from the C-cells of the thyroid and constitutes about 10% of all malignant thyroid tumors. This thyroid tumor is unique among thyroid tumors due to its clinical presentation, potential hereditary associations, and variable morphology.\(^6\)

The FNA specimens from MTC can display a spectrum of morphologic pattern similar to surgical pathology specimens. The majority of MTC FNA cases are cellular specimens consisting of round to oval cells arranged loosely cohesive groups or as single cells.

The tumor cells show ample granular cytoplasm with eccentric nuclei imparting a plasmacytoid appearance to tumor cells (Figure 7.3). The nuclear chromatin is similar to that seen in neuroendocrine tumors. Intranuclear inclusions and multinucleated cells can also be seen. The tumor

Fig. 7.2. FNA specimen of papillary thyroid carcinoma showing all major diagnostic features. The cells are enlarged with oval nuclei, intranuclear grooves, eccentric nucleoli, chromatin clearing and intranuclear inclusions (arrowhead) (Papanicolaou stained alcohol fixed smear, 60×).

Fig. 7.3. FNA specimen of medullary thyroid carcinoma showing round to oval tumor cells with eccentric nuclei (plasmacytoid appearance) and eosinophilic cytoplasm (Diff-Quik® stained air dried smear, 40×).
cells can assume a “spindle shape” and appear mesenchymal in origin. Acellular amyloid can be seen, and this substance can be distinguished from the thick colloid by performing a Congo-red stain. The diagnosis of MTC can be confirmed by performing immunostains for calcitonin and thyroglobulin.38

Anaplastic Carcinoma

Anaplastic carcinoma of the thyroid is one of the most aggressive and fatal human tumors. It usually presents in older individuals and is more common in regions of endemic goiter. The aspirates from anaplastic carcinoma usually do not pose any diagnostic difficulties; they can be readily classified as malignant because of extreme cellular pleomorphism and obvious malignant features.7

Role of Special Studies in the Diagnosis of Thyroid Tumors in Cytologic Specimens

Immunohistochemistry

All follicular derived thyroid lesions, whether benign or malignant, commonly express transcription factor (TTF-1) and thyroglobulin. This immunostaining panel is helpful in differentiating primary vs. secondary tumors of the thyroid. The diagnosis of medullary carcinoma can be established in FNA specimens by performing immunostains for calcitonin and calcitonin gene related peptide (CGRP). Medullary carcinoma also stains positive for carcinoembryonic antigen (CEA), chromogranin, and synaptophysin.38

Several reports have been published regarding the use of various immunohistochemical markers that can differentiate papillary carcinoma from other follicular derived lesions of the thyroid. From an extensive list of these markers, the ones that have shown some promise include cytokeratin-19, HBME-1, and Galectin-3. However, none of these have proven to be specific since all can be expressed in some benign lesions of thyroid. In addition, spurious staining of benign thyroid epithelium in chronic lymphocytic thyroiditis can lead to false positive diagnosis of malignancy.39–41 Therefore, it is suggested that if the confirmation of cytologic diagnosis of papillary cancer requires use of immunohistochemistry, then it should be carried out by employing an immunopanel consisting of markers mentioned above in a sample containing enough cells or in cell block preparations.

Molecular Genetics/Diagnosis

In the past decade, the literature on thyroid gland has been focused mainly on the role of various biologic events and genetic determinants in the pathogenesis of various thyroid tumors, and many of these studies have examined the mutations present in FNA specimens.

RET/PTC Oncogenes

Rearrangements of RET gene, known as RET/PTC have been identified in papillary carcinoma of thyroid.42 RET/PTC 1 and 3 are the most common forms that occur in sporadic papillary carcinoma.43 The prevalence of RET/PTC in papillary carcinoma varies significantly among various geographic regions;42,44,45 in US, it ranges from 11 to 43%.42 Several authors have investigated the expression of RET/PTC rearrangements in thyroid aspirates to establish the diagnosis of papillary thyroid carcinoma. Domínguez et al found RET/PTC rearrangements in 25% of FNA specimens of histologically confirmed cases of PTC,46 whereas Sapio et al found 17% and Pizzolanti et al found 6% FNA samples from histologically confirmed PTC showing RET/PTC rearrangements.47,48

It has been reported that RET/PTC expression can also occur in some benign lesions. These include hyalinizing trabecular neoplasm,49 Hashimoto’s thyroiditis50 and hyperplastic nodules and follicular adenoma.51,52 Thus, employing only RET/PTC analysis of FNA specimen to establish the diagnosis of PTC does not appear to be a reliable test.

B-RAF

Recently, an activating mutation in BRAF was described in 29–69% of human papillary thyroid cancers. BRAF mutational analysis of FNA samples has been shown to be of value in the diagnosis of papillary thyroid carcinoma. Pizzolanti et al found BRAF mutations in 11/16 (69%) of the FNA samples of the histologically confirmed PTC. Interestingly, in two cases, the cytologic diagnosis was suspicious but not conclusive for PTC. Similar findings have been reported by other investigators.48 These results appear to justify the role of BRAF mutational analysis to diagnose PTC in inconclusive thyroid FNA specimens. It is well established that BRAF mutations are more common in classic variant of PTC as compared to FVPTC and as mentioned above, the FNA diagnosis of suspicious for papillary carcinoma is most commonly rendered for FVPTC cases because of paucity of diagnostic nuclear features.48,53–57 Thus, the potential utility of BRAF test in suspicious for PTC FNA cases may be of limited value in clinical practice. Some authors have suggested that since BRAF mutations and RET/PTC rearrangements are independent of each other, it may be helpful to analyze both markers in a given thyroid FNA specimen to establish the diagnosis of PTC.48

DNA Microarray Analysis

Recently, DNA microarray analysis of the thyroid FNA samples have been shown to successfully distinguish between majority of the benign and malignant thyroid lesions. Lubitz et al evaluated 22 FNA samples by unsupervised hierarchical cluster analysis using a list of 25 differentially expressed genes. These authors found that their FNA cohort could be separated into three clusters: malignant, benign, and indeterminate.
The benign and malignant groups were in complete concordance with histologic follow-up except one case. Interestingly, in the indeterminate group, two cases were FVPTC on histologic examination. All above-mentioned studies show a great promise in improving the diagnostic efficacy of thyroid FNA, however, in our view, cytomorphology still remains the gold standard in the clinical management of thyroid nodules.

References


Introduction

Papillary thyroid carcinoma (PTC) is a malignant epithelial tumor showing follicular differentiation and distinctive nuclear features.1 Papillary thyroid carcinoma is the most common thyroid carcinoma, accounting for 80% of thyroid carcinomas.2 Numerous variants of PTC have been described, some of which behave more aggressively than conventional PTC. Three distinct molecular alterations are associated with PTC and are mutually exclusive.3 These include somatic BRAF point mutations, RET/PTC rearrangements, and somatic RAS point mutations. These molecular alterations are also associated with particular clinicopathologic features of PTC as well as particular subtypes of PTC.

Clinical Features

PTC can occur at any age, but most tumors develop in patients between 20 and 50 years of age, and women are affected more commonly than men.1 PTC accounts for at least 80% of adult and 90% of pediatric thyroid carcinomas.1 PTC has been identified with Graves, Hashimoto thyroiditis, and hyperplastic nodules and can arise in ectopic thyroid tissue (struma ovarii).

Radiation exposure is a risk factor for the development of PTC.4 PTC in pediatric patients who were involved in the Chernobyl nuclear accident were associated with a short latency, young age, an almost equal sex ratio, aggressive behavior with intraglandular spread, soft tissue invasion, metastases, and a solid growth pattern.5–7 Radiation induced tumors frequently show RET/PTC rearrangements, but show a low prevalence of BRAF mutations.8 Radiographically, PTCs are well demarcated or irregular masses on ultrasound and cold nodules on radioactive iodine scan.

PTC is often treated by near total thyroidectomy with ipsilateral lymph node dissection. Radioactive iodine can also then be administered in attempt to ablate any possible remaining tumor including metastatic sites or suppression of thyroid stimulating hormone secretion without radioactive iodine. The overall prognosis for patients with PTC is excellent, with greater than 90% overall survival.1 PTC has a propensity to metastasize to ipsilateral cervical lymph nodes and may spread to lung.2 Unfavorable prognostic features include older age, male gender, large tumor size, multicentricity, vascular invasion, extrathyroidal extension, distant metastases, aneuploidy, high microscopic grade, and progression to poorly differentiated carcinoma.2 Other poor prognostic features include tumor necrosis, numerous mitoses, and marked nuclear atypia.9 Recognition of the variants of PTC (Table 8.1) is important as the tall cell variant,10–12 columnar variant,13 solid variant, and Hurthle cell variant14 may be more aggressive tumors.

The increasing incidence of thyroid carcinoma in the United States is mainly due to increasing diagnosis of papillary thyroid carcinomas (PTC), particularly small papillary cancers.15 Papillary microcarcinoma is defined as a PTC measuring 1 cm or less. These microcarcinomas are often incidental findings in thyroids removed for benign clinical nodules or thyroiditis,16 and the majority are cured by lobectomy alone. Studies have identified these tumors in 7–35% of autopsies17,18 and in 7% of surgical cases in patients undergoing surgery for presumably benign disease.17 The treatment for papillary microcarcinomas has been controversial. Papillary microcarcinomas presenting clinically as a mass or a metastasis are usually treated as a clinical cancer.16 Up to 5% of these tumors may be associated with capsular invasion or metastases.19 Incidentally, clinically occult papillary microcarcinomas are less aggressive than those presenting clinically.20 Clinically overt papillary microcarcinomas are larger and more frequently show multifocality, extrathyroidal extension, vascular invasion, metastases, and recurrence than those discovered incidentally.20 Familial papillary thyroid microcarcinomas can also behave aggressively.21

Follicular variant of PTC (FVPTC) (Lindsay tumor) is the most common variant of PTC and one of the most challenging to diagnose. The histologic features of these tumors were recognized more than 50 years ago.22,23 These tumors were originally
classified as a type of follicular carcinoma, but were later classified as a type of papillary carcinoma, follicular variant. Recent reports have suggested that this tumor may be over-diagnosed by pathologists. A recent study has shown that even experienced thyroid pathologists have low concordance in diagnoses of FVPTC, although increased concordance was noted in tumors associated with metastases. Follicular variant of PTC behaves and is treated similarly to conventional PTC.

Recognition of the variants of PTC is important as some of them including the tall cell variant, columnar variant, solid variant, and oxyphilic variant may behave more aggressively. The tall cell variant is an uncommon, aggressive variant of PTC, which is more common in elderly males. This aggressive tumor is associated with extrathyroidal disease, recurrence, vascular invasion, metastases, decreased survival, and death in 20–25% of patients. The tall cell variant shows increased expression of c-Met, a factor of invasion which correlates with extracapsular spread, and muscle invasion, than classic PTC and FVPTC. The columnar variant of PTC is a rare, aggressive variant of PTC that occurs over a wide age range, can metastasize widely, and usually does not respond to radioactive iodine or chemotherapy.

The solid variant of PTC is uncommon, comprising only 2.6% (20 of 756) of papillary thyroid carcinomas at the Mayo Clinic between 1962 and 1989. The solid variant of papillary carcinoma was associated with slightly increased distant metastases and less favorable prognosis than classical PTC. An AMES (Age, Metastasis, Extent and Size) risk classification study of 121 PTC found that among variants of PTC, solid cell variant has the highest proportion of high-risk tumor classified by the AMES criteria (75%), followed by tall cell variant with 33.3% of high risk patients, while only 8.3% of classic PTC were classified as high-risk.

The solid cell variant of PTC is more common in children. This variant was the most common subtype of PTC in Belarusian children after the Chernobyl accident, comprising 34% of the PTCs. These tumors were associated with lymph node metastases, extrathyroidal extension, vascular invasion, slightly more distant metastases, and less favorable prognosis than conventional PTC.

The oxyphilic (Hurthle cell) variant of PTC is uncommon. While some have found these tumors to behave similarly to conventional PTC, others have identified more aggressive behavior. A study of 1,552 patients with thyroid carcinoma included 42 cases of Hurthle cell variant of PTC with generally favorable 5- and 10-year survival rates of 94 and 87%. Unfavorable prognostic factors included older age, extrathyroidal extension, tumor stage, and metastases. Tumors with features of both oxyphilic PTC and tall cell variant may behave more aggressively.

The Warthin-like variant of PTC resembles Warthin tumor of the salivary gland with its oxyphilic cells and lymphocytic stroma. Overall, these tumors behave like conventional PTC. Similar to conventional PTC, a Warthin-like variant of PTC has been documented in association with an anaplastic thyroid carcinoma.

The diffuse sclerosing variant of PTC was described in 1985. These tumors are more common in women and in younger individuals. The diffuse sclerosing variant of PTC shows aggressive features with increased cervical lymph node involvement and lung metastases. The overall outcome has been controversial with some studies showing a worse prognosis with decreased disease-free survival. However, others have found that in spite of aggressive histologic features such as large tumor size and extensive lymph node metastases, overall survival is not significantly different from conventional PTC.

### Histologic Features

Grossly, PTCs are often ill-defined, infiltrative, firm, single, or multiple masses with a granular whitish appearance. The tumors are usually 2–3 cm in size but can be extremely small and not identifiable grossly. Classically, PTCs are composed of fibrovascular papillae lined by follicular cells with enlarged, irregular nuclei showing nuclear clearing (“Orphan Annie” nuclei), longitudinal nuclear grooves, and intranuclear holes (cytoplasmic invaginations into the nucleus) (Figure 8.1). The colloid in PTC is often darker than that of the surrounding thyroid, and psammoma bodies are seen in approximately 50% of cases. The stroma is usually abundant, fibrous, and sclerotic, although cystic change is not uncommon. Fine needle aspiration biopsies of PTC are cellular and show papillary structures with branching or sheets of cuboidal or columnar cells with enlarged irregular nuclei, nuclear grooves, and nuclear pseudoinclusions. PTC must be differentiated from Graves disease with papillary hyperplasia as well as thyroid neoplasms. Both Graves and PTC can show a papillary architecture, but the cytologic features of PTC are helpful in separating PTC from Graves. In difficult cases of Graves disease with papillary hyperplasia, p27 protein expression is helpful as Graves disease shows higher levels of p27 expression when compared to PTC.
Histologic variants of PTC are important to recognize as the molecular features and biologic behavior may differ from classic PTC, and they can be mistaken for other tumors (Figure 8.2, Table 8.1). FVPTC can have a microfollicular pattern (Figure 8.2a), a macrofollicular pattern, a diffuse pattern, or mixed patterns. These tumors can be particularly difficult to diagnose on fine needle aspiration biopsy. The diffuse pattern of FVPTC is seen in younger patients, is often multicentric, and shows extrathyroidal extension, nodal metastases, and vascular invasion. Differentiating FVPTC from a follicular neoplasm can be extremely difficult. Important criteria to diagnose FVPTC are nuclear pseudo-inclusions (cytoplasmic invaginations into the nucleus), abundant nuclear grooves, and ground glass nuclei (Figure 8.2b). Immunohistochemical markers, particularly when used in panels, such as HBME-1, galectin-3, and cytokeratin 19 can also be helpful. The tall cell variant of PTC has prominent papillary structures lined by cells in which height of cell at least twice the width, basally located nuclei, abundant eosinophilic, oxyphilic cytoplasm, and a lymphocytic infiltrate. The columnar cell variant shows a prominent papillary architectural pattern with elongated cells with nuclear stratification and scant cytoplasm. Tumors can show both tall cell and columnar features, areas of solid growth, organoid or glandular features, foci of solid spindle-cells, and microfollicular and follicular growth patterns. Encapsulated columnar-cell PTCs have a better prognosis than tumors that are unencapsulated and invasive into adjacent thyroid or extrathyroid tissue. The diffuse sclerosing variant of PTC shows diffuse involvement of one or both lobes of the thyroid, extensive squamous metaplasia, numerous psammoma bodies, interstitial fibrosis, a marked lymphocytic infiltrate, and can show widespread intrathyroid lymphatic invasion (Figure 8.2h). The solid cell variant of PTC has a solid architectural pattern of growth and cytologic features classic of PTC (Figure 8.2e). The oxyphilic (Hurthle cell) variant of PTC has a papillary architectural pattern, oxyphilic cytoplasm, and nuclear features are characteristic of PTC. The papillae are lined by a single layer of oxyphilic cells with nuclear features of PTC (Figure 8.2f). Oxyphilic PTC must be differentiated from Hurthle cell neoplasms showing papillary architecture and oxyphilic papillary hyperplasia in Hashimoto thyroiditis and Graves disease. In 1995, the Warthin-like variant of PTC was described as “a peculiar thyroid tumor of follicular epithelial differentiation with distinctly papillary architecture, oxyphilic cytology, and lymphocytic infiltrates in papillary stalks” was described. The Warthin-like variant of PTC resembles Warthin tumor of the salivary gland with its oxyphilic cells and lymphocytic stroma (Figure 8.2g). Warthin-like PTCs can be mistaken for benign lymphoepithelial lesions of the thyroid, Hurthle cell carcinoma, and tall cell variant of PTC in both cytology and histology specimens. A helpful low-power clue to the diagnosis of Warthin-like PTC is a prominent lymphoplasmacytic infiltrate, but cytologic features classic of PTC are most useful in separating the Warthin-like variant of PTC from benign lymphoepithelial lesions of the thyroid and Hurthle cell carcinoma. The cells in the Warthin-like variant can be elongated, but the height of the cells is not at least twice the width as is seen in the tall cell variant of PTC. The nuclear stratification and scant cytoplasm are helpful in differentiating the columnar variant from the tall cell variant of PTC.

A variety of other variants of PTC have been described. PTC with nodular fasciitis-like stroma can be mistaken for a mesenchymal tumor, a carcinomasarcoma, or an anaplastic thyroid carcinoma. The tumors show a prominent spindle cell stroma, reminiscent of nodular fasciitis in which small tubules and nests of epithelioid cells with cytologic features characteristic of PTC. Foci of squamous differentiation and/or papillae may be seen. PTC can also show a lipomatous stroma. The clear cell variant of PTC is uncommon, but appears to behave like conventional PTC. Metastatic papillary lesions to the thyroid, such as from kidney, nasopharynx, among other sites also need to be differentiated from PTC.
Fig. 8.2. Variants of papillary thyroid carcinoma (PTC). (a) Papillary thyroid microcarcinoma measuring less than 1 cm. (b) Follicular variant of PTC with follicular growth pattern and cytologic features of PTC. (c) The tall cell variant of PTC has prominent papillary structures lined by cells in which height of cell at least twice the width, basally located nuclei, abundant, eosinophilic cytoplasm. (d) Columnar variant of PTC a prominent papillary architectural pattern with elongated cells with nuclear stratification. (e) Solid variant of PTC showing cytologic features of PTC but a solid architectural pattern. (f) Oxyphilic variant of papillary thyroid carcinoma showing cells with nuclear features of PTC, but abundant oxyphilic cytoplasm. (g) Warthin-like variant of PTC with oxyphilic cells showing intranuclear holes and a prominent lymphocytic stroma. (h) Diffuse sclerosing variant of PTC.

**Immunohistochemistry**

PTCs are positive for thyroglobulin, thyroid transcription factor-1 (TTF-1), and cytokeratins and are negative for chromogranin, synaptophysin, and calcitonin. Immunohistochemical and molecular markers have been very useful for diagnosing and predicting the behavior of papillary thyroid carcinomas. Although not specific, a number of immunohistochemical markers (Figure 8.3) have been utilized singly and in panels have shown promise in helping to confirm a diagnosis of
8. Well-Differentiated Papillary Thyroid Carcinoma

PTC including HBME-1, galectin-3, cytokeratin 19, and CITED-1. A study of 67 PTC and a variety of benign thyroid tumors showed expression of galectin-3, fibronectin-1, CITED-1, HBME-1, and cytokeratin 19 were significantly associated with PTC with coexpression of multiple antibodies showing increased specificity for carcinomas, while loss of sets of markers such as galectin-3 or fibronectin-1, and HBME-1 was highly specific for benign lesions. A panel consisting of galectin-3, fibronectin-1, and HBME-1 was most helpful for the diagnosis of PTC. However, galectin-3, cytokeratin 19, and fibronectin as single markers showed focal staining in nonneoplastic thyroid tissue, and the seven neoplasms in the study of undermined malignant potential showed intermediate staining between the benign and malignant tumors. A study from Mayo Clinic also found a panel of markers consisting of HBME-1, galectin-3, and CK19 or a panel consisting of HBME-1, CITED-1, and galectin-3 to be most effective in distinguishing follicular adenoma from FVPTC. Similarly, single markers were useful, but none was completely specific for FVPTC. Nasr et al found HBME1 to be the most sensitive and specific single marker for PTC, but concluded that a combination of HBME1 and CK19 had the greatest diagnostic utility in differentiating PTC from benign mimics. Another study evaluating multiple immunohistochemical markers also found HBME-1 to be the most specific single marker, but again noted increased specificity when panels of markers such as HBME-1, galectin-3, and CK19 or HBME-1, CITED-1, and cytokeratin19 were used. They also noted that while these markers are helpful in separating FVPTC from follicular adenoma, particularly when used in panels, an additional group of diagnostically challenging encapsulated follicular lesions with questionable features of PTC showed intermediate staining patterns indicating that these markers may be less useful in borderline lesions. Thus, HBME-1 appears to be a particularly useful marker in differentiating FVPTC from follicular adenoma, but a panel of immunohistochemical markers including HBME-1, galectin-3, CITED-1, and/or CK19 appears to be most useful.

Genetics of Papillary Thyroid Carcinoma

Three distinct molecular alterations are associated with PTC and are mutually exclusive. These include somatic BRAF point mutations, RET/PTC rearrangements, and somatic RAS point mutations. These molecular alterations are also associated with particular clinicopathologic features of PTC as well as particular subtypes of PTC.

BRAF

In 2002, Davies et al reported somatic BRAF mutations in the majority of melanomas studied and at lower frequency in a wide variety of human cancers, and noted that growth in cancer cell lines with BRAF mutation was independent of RAS function. Numerous studies soon followed regarding somatic BRAF mutation in thyroid tumors. BRAF mutation was found to be the most common genetic abnormality in PTC, occurring in 36–69% of cases. The overwhelming majority of mutations are in exon 15, nucleotide 1799 (V600E mutation), but other mutations have been identified in rare cases.

The BRAF gene codes for a serine/threonine kinase signaling protein, which along with ARAF and RAF1, are involved in the RAS/RAF/MEK/ERK/MAPK signaling pathway that relays signals from the cell membrane to the nucleus regulating expression of genes involved in cell growth, differentiation and cell death. Tumor suppressor genes and thyroid iodide-metabolizing genes are down-regulated, while cancer promoting molecules including VEGF, matrix metalloproteinases, NF-kappaB, and c-Met are up-regulated. The three RAF genes code for cytoplasmic serine/threonine kinases that are regulated by binding RAS. When RAS becomes activated it activates BRAF. Mutation of BRAF is an alternative route for activation of the MAP kinase signaling pathway that is independent of RAS. BRAF mutation V600E mimics the phosphorylation of the activation segment of BRAF, thus BRAF becomes activated independent of RAS. RAS function is not required for the growth of cancer cell lines with BRAF point mutations. Recently therapeutic strategies...
and MEK inhibitors have been proposed to target the MAPK pathway that is aberrantly activated by BRAF mutation.73,74

Clinical and pathologic features are associated with BRAF associated PTC (Figure 8.4). BRAF associated PTC most commonly occur in older patients and are uncommon in children.75-77 Unlike RET/PTC mutation associated PTC, BRAF associated PTC generally are not associated with radiation.75,78 However, the oncogenic AKAP9-BRAF fusion was recently been identified is associated with radiation induced PTC.79 This rearrangement of BRAF via paracentric inversion of chromosome 7q results in an in-frame fusion between the AKAP9 gene and BRAF resulting in a fusion protein that lacks the autoinhibitory N-terminal portion of BRAF and has elevated kinase activity is a novel mechanism of MAPK pathway activation.79

Somatic BRAF mutation has also been associated with more aggressive tumors in some studies and with particular subtypes of PTC.69,70,76,77,80-83 BRAF mutation has been associated with clinicopathological features of PTC that are conventionally known to predict tumor progression and recurrence, including, older patient age, extrathyroidal extension, lymph node metastasis, and advanced tumor stages, treatment failure, and tumor recurrence in patients with conventionally low-risk clinicopathological factors.72 In a study of 96 cases of PTC, BRAF mutations were identified in 42% of cases and associated with older patient age, typical papillary appearance or the tall cell variant, a higher rate of extrathyroidal extension, and more advanced tumor stage at presentation.77 The diagnostic utility of BRAF mutation was evaluated in 71 thyroid fine needle aspiration (FNA) specimens, and BRAF mutation was detected in 31 of 58 PTC and in none of the 13 non-PTC lesions.84 The PTC harboring BRAF mutation had higher extrathyroidal invasion and/or lymph node metastasis than PTC with wild-type BRAF.84 A multicenter study of 219 patients with PTC found a significant association between BRAF mutation and extrathyroidal extension, lymph node metastases, and advanced tumor stage at initial surgery. This association remained significant on multivariate analysis, adjusting for conventional predictors of recurrence, but excluding PTC subtype, but it was lost when tumor subtype was included.80 However, BRAF mutation remained associated with tumor recurrence on multivariate analysis even with PTC subtype, and BRAF mutation was an independent predictor of recurrence in patients with low stage disease and absence of I131 avidity and treatment failure in recurrent disease.80 Another study found that BRAF positive tumors correlated with early recurrences and negative radioiodine scans which are associated with worse outcome as treatment with radioactive iodine is not effective.82 They suggested that this is due to impairment of the sodium iodine symporter targeting the membrane mediated transport of iodine into the thyroid follicular cells.82 Another study found BRAF mutation associated with recurrence in low-risk patients with conventional PTC, but this association was lost in multivariate analyses when factors of age, gender, tumor size, extrathyroidal extension, multifocality, and lymph node metastasis were included.81 A recent study of 500 consecutive cases of PTC found BRAF mutation in 219 cases (43.8%), BRAF mutation was associated with extrathyroidal invasion, multicentricity, nodal metastases, absence of tumor capsule in particular in FVPTC, and micropapillary thyroid carcinomas.85 However not all studies have found BRAF mutation to

**Fig. 8.4.** Direct sequencing for BRAF mutation in papillary thyroid carcinomas. A classic papillary thyroid carcinoma showing wild-type BRAF sequence in forward direction (a) and reverse direction (b). Another classic papillary thyroid carcinoma showing a T to A transversion at nucleotide 1799 in forward direction (c) and reverse direction (d).
be associated with aggressive behavior.\textsuperscript{86,87} In addition to PTC, BRAF mutations are also present in poorly differentiated and anaplastic carcinomas arising from PTC.\textsuperscript{86}

Frequencies of BRAF mutation vary among different variants of PTC. BRAF mutations are frequently identified in the tall cell variant of PTC\textsuperscript{77} and PTC with a papillary or mixed follicular/papillary architecture, including conventional PTC, papillary microcarcinoma, Warthin-like PTC, oncocyctic/oxyphilic/Hurthle cell variant of PTC,\textsuperscript{69,76,89} but are less common in FVPTC.\textsuperscript{60,76} BRAF mutation is common in the tall cell variant of PTC.\textsuperscript{64,77,88,90} BRAF mutations have been identified in up to 67% of cases of tall cell variant of PTC.\textsuperscript{64} BRAF mutations are identified in 75% of Warthin-like variant of PTC.\textsuperscript{76} BRAF mutations are commonly seen in the oxyphilic variant of PTC, occurring in 55% of cases.\textsuperscript{76} BRAF mutations are also identified in the diffuse sclerosing variant of PTC.\textsuperscript{93} A novel BRAF triplet deletion has recently been reported in a PTC with a predominantly solid growth pattern.\textsuperscript{70} The deletion leads to the replacement of a valine and a lysine by a glutamate in the BRAF activation segment (BRAF(VK600-1E)).\textsuperscript{70} The columnar variant of PTC does not appear to show increased frequency of BRAF mutation (unpublished observations).

BRAF somatic point mutations are identified in 28–42% of papillary thyroid microcarcinomas.\textsuperscript{76,91,92} A recent study of tumors from patients with diverse genetic backgrounds showed no differences in BRAF mutation rates in tumors from Russian patients when compared to Japanese patients.\textsuperscript{92} BRAF mutation did not significantly correlate with the gender, age at presentation, metastatic indices or with papillary, mixed papillary and follicular, and solid/trabecular features among the papillary microcarcinomas, however papillary microcarcinomas of follicular morphology had fewer BRAF mutations, while those with co-occurrence with less differentiated components showed more frequent BRAF mutation.\textsuperscript{82}

BRAF mutations are less frequently associated with FVPTC than with other types.\textsuperscript{69,76} Interestingly, a study including 54 cases of FVPTC showed the less common BRAF(K601E) mutation in 7%.\textsuperscript{76} Recent studies have compared genetic alterations of FVPTC with those of classic PTC.\textsuperscript{64,83} A study from Mayo Clinic found BRAF mutation in 38% of PTC, including 20% of FVPTC.\textsuperscript{64} Another study comparing genetic alterations in FVPTC showed BRAF mutation in 1 of 13 (7.6%) cases, RET rearrangement in 5 of 12 cases (41.7%), RAS mutation in 6 of 24 cases (25%), as compared to classic PTC which showed BRAF mutation in 3 of 10 cases (30%), no RAS mutations, and RET rearrangement in 5 of 11 cases (45%). One FVPTC exhibited simultaneous RAS mutation and RET/PTC1 rearrangement.\textsuperscript{83} Another study compared the genetic alterations of 30 cases of FVPTC with 46 non-FVPTC.\textsuperscript{93} The cases of FVPTC showed one RET/PTC rearrangement (3%) and 13 RAS mutations (43%), while the non-FVPTC cases showed 13 RET/PTC rearrangements (28%) and no RAS mutations confirming the high frequency of RAS point mutations in FVPTC.\textsuperscript{93} A recent study microdissecting different components from 44 PTC found that different structural components within the same tumor had identical BRAF mutation status in 93% (41 of 44) of tumors, while only 7% (3 of 44) of tumors showed BRAF mutation only in the papillary component and not in the follicular component. Similarly, 92% (11 of 12) of lymph node metastases showed BRAF mutation patterns identical to the respective primary tumor.\textsuperscript{89} Thus, the cells in PTC appear to be homogeneous in regard to the mutational status, and the results support the reliability of molecular diagnostic testing of PTC in preoperative biopsies as the BRAF status would not depend on which tumor component was sampled.\textsuperscript{93} A study evaluating the diagnostic utility of BRAF mutation in thyroid FNA specimens found a high degree of specificity for BRAF mutation by analysis of FNA specimens.\textsuperscript{84} BRAF mutation analysis can be useful for the diagnosis of PTC in cases of cytoplogically indeterminate fine needle aspiration specimens.\textsuperscript{84}

**RET/PTC**

The RET gene is located on 10q11.2.\textsuperscript{94} There are many types of RET rearrangements, all of which result in the fusion of the tyrosine kinase domain of RET to the 5' portion of different genes.\textsuperscript{95} There are at least ten different RET/PTC rearrangements. The most common rearrangements are RET/PTC1 and RET/PTC3.\textsuperscript{95} RET/PTC1 is the most common overall accounting for two-thirds of RET rearrangements (Figure 8.5). RET/PTC1 is more common in classic PTC with papillary growth, microcarcinomas, and benign behavior.\textsuperscript{98} RET/PTC3 correlates with the solid variant of PTC and more aggressive behavior.\textsuperscript{95,96} A study from an iodine-rich, nonirradiated area of Japan found the overall frequency of RET/PTC rearrangements in PTC to be 28%, with increased frequency in younger patients (41% in patients less than 20 years of age).\textsuperscript{97} The prevalence rate of RET/PTC1 was similar regardless of age, but RET/PTC3 rearrangements were more common among patients less than 20 years-old and in tumors with a solid growth pattern.\textsuperscript{97} A study including 17 patients with multifocal PTC found only 2 (12%) patients had identical RET/PTC rearrangements in multiple tumors.\textsuperscript{98} Fifteen of the 17 (88%) cases showed diverse rearrangements among the tumors in each patient, indicating that individual tumors arise independently in a background of genetic or environmental susceptibility.\textsuperscript{98} An RET/PTC model of thyroid tumor has been reported in transgenic mice.\textsuperscript{99} However, not all transgenic mice with overexpression of RET/PTC in their thyroids develop thyroid tumors, other genetic lesions are likely needed for PTC to develop.\textsuperscript{99}

RET/PTC is associated with radiation-related PTC, both in patients who have received external radiation for disease\textsuperscript{100} and after the Chernobyl disaster.\textsuperscript{101,102} Chernobyl-associated PTC with short latency periods (within 1 year of exposure) are most commonly associated with RET/PTC3, regardless of age.\textsuperscript{96,103–105} Late onset PTC (greater than 1 year after exposure) more often show RET/PTC1.\textsuperscript{96,103–105}
RET/PTC rearrangement is a common genetic alteration in PTC with a prevalence that varies among geographical sites and tumor subtypes. Overall, approximately 20–30% of sporadic adult PTC have RET/PTC rearrangement. RET/PTC rearrangements are associated with younger age, papillary histology, psammoma bodies, and lymph node metastases. RET/PTC rearrangements are the most common genetic alteration in PTC in children. RET rearrangements are more commonly detected in incidentally discovered microcarcinomas than in clinically evident PTC, suggesting that RET plays a role in the initiation of thyroid tumorigenesis but is not required for further progression of the tumor. RET rearrangements are not associated with aggressive features such as large tumor size, extrathyroidal extension, metastases, or with poorly differentiated or anaplastic thyroid carcinomas. Generally, RET/PTC rearrangements are associated with well-differentiated PTC, and the subset of RET/PTC positive PTC do not progress to more aggressive, less differentiated tumor phenotypes.

RET/PTC1 tends to be more common in papillary microcarcinomas, tumors with typical papillary growth, and a more benign clinical course, while RET/PTC3 is more common in the solid variant of PTC and more aggressive tumor behavior. In a study of 21 papillary thyroid microcarcinomas by interphase fluorescence in situ hybridization, RET rearrangements were detected in 52% of microcarcinomas, a significantly higher frequency than that found in clinically evident PTC. The authors concluded that the high frequency of RET rearrangements in microcarcinomas suggests that RET plays a role in the initiation of thyroid tumorigenesis but does not seem to be necessary for the further progression of the tumor. In a series of 201 PTC, RET rearrangements were detected in 81 (40.3%) of PTC and correlated with “classic” morphological features of papillary cancer or with the microcarcinoma subtype. A study evaluated the RET/PTC-1, -2, and -3 by PCR analysis in thyroid microcarcinomas and clinically evident PTC to determine its role in early stage versus developed PTC and to examine the diversity of RET/PTC in multifocal disease. Thirty-nine occult papillary thyroid microcarcinomas were analyzed from 21 patients, and 30 microcarcinomas (77%) had RET/PTC rearrangements, 12 of which were RET/PTC1, three RET/PTC2, six RET/PTC3, and nine had multiple RET/PTC rearrangements. RET/PTC rearrangements were identified in 47% of clinically evident tumors. Of the 17 patients with multifocal disease, identical RET/PTC rearrangements were identified in multiple tumors in two of the patients, while the other 15 patients had diverse rearrangements among their tumors. The authors concluded that RET/PTC rearrangements may have a role in early-stage papillary thyroid carcinogenesis, but they are less important in determining progression to clinically-evident disease, and the diversity of RET/PTC rearrangements among multifocal tumors suggests that individual tumors arise independently in a background of genetic or environmental susceptibility.

Fig. 8.5. Direct sequencing showed RET/PTC1 rearrangement (H4 and RET fusion) in a papillary thyroid carcinoma (a) forward direction; (b) reverse direction). Another papillary thyroid carcinoma showing RET/PTC3 rearrangement (ELE1 and RET fusion). (c) forward direction; (d) reverse direction).
A recent study found RET rearrangements in 35.8% (14 of 39 cases) of cases of FVPTC. The prevalence of RET/PTC1 and RET/PTC3 were almost equal in classic PTC and FVPTC, but the tall cell cases showed only RET/PTC3 rearrangements.\textsuperscript{109} p53 mutations are usually associated with poorly differentiated and anaplastic thyroid carcinoma, but p53 positivity was found in 61% of tall cell PTC when compared with 11% of common PTC.\textsuperscript{110} The diffuse sclerosing variant is seen both in the setting of radiation-induced and in sporadic PTC, with RET/PTC1 being more common than RET/PTC3.\textsuperscript{111} RET/PTC3 is more common in the solid/follicular variant, while RET/PTC1 associated with papillary carcinomas of the classic and diffuse sclerosing variants.\textsuperscript{111} Another study of PTC in children exposed to radiation identified RET/PTC3 in 79% of solid variant of PTC, whereas only 7% had RET/PTC1 rearrangement.\textsuperscript{96}

There are reports of RET/PTC in Hashimoto thyroiditis,\textsuperscript{112,113} but others have found no RET/PTC rearrangements in Hashimoto thyroiditis or PTC arising in the background of Hashimoto thyroiditis in contrast to 33% prevalence in PTC not associated with Hashimoto thyroiditis.\textsuperscript{114} Expression of wild-type RET was found in more than half of the PTC.\textsuperscript{114} If there is an association between Hashimoto thyroiditis and PTC, the molecular basis is thought to be different from the RET/PTC rearrangement.\textsuperscript{114} Also, variability in RET/PTC mRNA levels may contribute to inconsistencies in the reported detection rates.\textsuperscript{115}

RAS

There are three RAS genes, H-RAS, K-RAS, and N-RAS. Mutational activation of RAS proto-oncogenes results in activation of the MAP kinase pathway. Although RAS point mutations occur relatively frequently in follicular thyroid carcinoma, poorly differentiated thyroid carcinoma, and anaplastic thyroid carcinoma, RAS point mutations occur only in a minority of PTC.\textsuperscript{77,93} However, the prevalence of H-RAS, K-RAS, and N-RAS gene mutations in thyroid tumors according to malignancy and histology is controversial.\textsuperscript{116} Different studies have identified different frequencies of mutations. A recent study of 96 unselected PTC found 15% had RAS mutations.\textsuperscript{77} RAS mutations are more common in the FVPTC,\textsuperscript{77,93} occurring in up to 43% of cases.\textsuperscript{93} RAS mutations have correlated with less prominent nuclear features of PTC.\textsuperscript{77} The prognostic significance of RAS mutations in PTC is controversial. A low rate of lymph node metastases has been reported in patients with PTC with RAS mutation.\textsuperscript{77} Others have found RAS mutations to be more common in PTC associated with recurrence or death from disease,\textsuperscript{117} while others have found RAS mutations not related to prognosis.\textsuperscript{118} RAS mutations have correlated with the histologic differentiation of thyroid carcinomas overall, with higher rates of RAS mutation seen in poorly differentiated carcinomas than in well-differentiated carcinomas.\textsuperscript{119} Thus, RAS mutations may be important in the progression from well-differentiated carcinoma to poorly differentiated thyroid carcinoma.\textsuperscript{119} However, others have found RAS mutations to be equally prevalent in benign and malignant thyroid neoplasms and have suggested that it may be an early event in thyroid tumorigenesis.\textsuperscript{120} Hence, the role of RAS mutations in thyroid carcinoma remains unclear.

Epigenetic Studies

Gene methylation is an important mechanism in regulating gene expression, while aberrant gene methylation can result in the inappropriate silencing of genes particularly tumor suppressor genes. PTEN, RASSF1A,\textsuperscript{64} TIMP3 (tissue inhibitor of metalloproteinase-3), SLC5A8, DAPK (death-association protein kinase), and retinoic acid receptor B2 (RAR\textsubscript{B2}) are tumor suppressor genes inappropriately silenced in thyroid carcinomas.,\textsuperscript{121} with TIMP3, SCL5A8, and DAPK particularly important in PTC.\textsuperscript{122} TIMP3 inhibits metalloproteinase-3 hereby inhibiting the degradation of interstitial matrix.\textsuperscript{123} TIMP3 also inhibits angiogenesis by blocking vascular endothelial growth factor (VEGF) binding to VEGF receptor-2.\textsuperscript{123} SLC5A8 is a proposed thyroid apical iodide transporter with proapoptotic functions which is down-regulated in classic PTC and methylated in 90% of classic PTC and 20% of other PTCs.\textsuperscript{125} Low SLC5A8 expression was also associated with BRAF mutation.\textsuperscript{124} DAPK also has proapoptotic functions and is methylated in a variety of cancers including PTC.\textsuperscript{122,125} Aberrant methylation of tumor suppressor genes TIMP3, SCL5A8, and DAPK has been associated with features of aggressiveness in PTC, including extrathyroidal extension, lymph node metastases, multifocality, and advanced tumor stages.\textsuperscript{122,125} Interestingly, methylation of these genes was also associated with BRAF mutation and classic and tall cell subtypes of PTC.\textsuperscript{122} These genes, and others may be epigenetically modified in concert, and silencing of multiple genes increases with tumor progression.\textsuperscript{125} These results suggest that aberrant methylation and hence silencing of TIMP3, SLC5A8, DAPK, and RAR\textsubscript{beta}2, in association with BRAF mutation, may be an important step in PTC tumorigenesis and progression.\textsuperscript{122} Another study found a trend toward hypermethylation of multiple markers with hypermethylation of multiple markers detected in 25% of hyperplasias, 38% of adenomas, 48% of thyroid carcinomas, and 100% of thyroid cancer cell lines with a subset of markers appearing to be modified in concert and increasing methylation with cancer progression.\textsuperscript{125}

Aberrant methylation of thyroid stimulating hormone receptor (TSHR) expression can silence this gene in PTC.\textsuperscript{126} A recent study showed methylation of TSHR gene in 59% of PTC, 47% of follicular thyroid carcinomas, and no methylation in the follicular adenomas and normal thyroid tissues studied.\textsuperscript{126} TSHR was normally expressed at the protein and mRNA level in thyroid tumor cell lines, where the TSHR gene was unmethylated, but it was silenced when the TSHR promoter was hypermethylated. They proposed a potential use of demethylating agents in conjunction with TSH-promoted radioiodine therapy for epithelial thyroid cancers.\textsuperscript{126}
Gene Expression Profiling

Gene expression profiling has identified over and underexpression of a variety of genes in PTC. In an early study of gene expression array profiling of PTC, Huang et al.62 found that PTC showed concordant expression of many genes and distinct clustered profiles. Genes encoding adhesion and extracellular matrix proteins showed increased expression in PTC.62 Genes overexpressed in PTC included MET, LGALS3, KRT19, DPP4, MDK, TIMP1, and FN1, as well as genes not previously identified in PTC such as CITED1, LGALS3, KRT19, DPP4, MDK, TIMP1, and FN1, as well as genes not previously identified in PTC such as CITED1, CHI3L1, ODZ1, N33, SFTPB, and SCEL.62 Tumor suppressor genes, thyroid function proteins, and fatty acid binding proteins were underexpressed.62 The microarrays are useful in identifying markers for further evaluation by other means. The authors demonstrated by immunohistochemical studies that 49 of 52 PTC were positive for CITED1 and 39 of 52 for SFTPBP, while follicular thyroid carcinoma and normal thyroid tissues were negative.62 Genes underexpressed in PTC included tumor suppressor genes, thyroid function-related proteins, and fatty acid binding proteins.62 Another microarray expression analyses of six PTC showed expression changes in four genes not previously implicated in thyroid carcinogenesis, identified two distinct groups of genes that were either over- or underexpressed when compared with normal thyroid, and identified five genes that could collectively distinguish the PTC from follicular thyroid carcinoma.127 PTC showed overexpression of CITED1, claudin-10, IGFBP6 but showed no change in the expression of caveolin-1 or -2.127 However, PTC did not express CLDN10 and had decreased expression of IGFBP6 and/or CAV1 and CAV2.127 Thus, the distinctive expression profiles indicate PTC and follicular carcinomas are different tumors and suggest markers that may be diagnostically useful.127 Although DNA array analyses in papillary thyroid carcinoma are helpful in enabling the analysis of gene expression of thousands of genes simultaneously, limitations of DNA array analyses include the use of different platforms, intra-individual versus inter-individual comparisons, different reference tissues, and differences in data analysis methods complicates comparisons between different studies.128 To date, only a few new molecular markers have been adapted for clinical use from gene expression profiling.

Other Genetic Studies

Other modalities have been utilized to study PTC including microRNA profiling, loss of heterozygosity and cytogenetic analyses. microRNAs are single-stranded, noncoding RNAs, 21–23 nucleotides in length, that negatively regulate gene expression.129 microRNA expression profiles have been studied in PTC.130–132 Different microRNA expression profiles have differentiated PTC cell lines with BRAF mutations as well as those with RET/PTC1 from normal thyroid cell lines.130,132 Recently, a study characterized microRNAs signatures on microRNA obtained formalin-fixed paraffin-embedded PTC and identified 13 upregulated and 26 downregulated microRNAs in PTC compared to multinodular goiter.132

Loss of heterozygosity of tumor suppressor genes demonstrate that classic PTC have the lowest frequency of allelic loss (7%), followed by FVPTC (19%), tall cell (20%), and oncocytic (34%).133 Allelic loss associated with increasing tumor size, but not with age, gender, extrathyroidal extension, or lymph node metastases.133 Cytogenetic analysis of 94 PTC showed clonal chromosomal changes in 37 cases (40%) with structural cytogenetic abnormalities in 18 cases, including rearrangements of chromosomes 1, 3, 7, and 10 as well as novel breakpoint cluster regions.134 The most common breakpoint loci 1p32–36, 1p11–13, 1q, 3p25–26, 7q34–36, and 10q11.2.134 Karyotypic features correlated with some variants of PTC. FVPTC showed some chromosomal aberrations found in thyroid follicular adenomas: a del(11)(q13q13), a t(2;3)(q13;p35), and gains of chromosomes 3, 5, 7, 9, 12, 14, 17, and 20.134 Of the tall cell PTC, four of the seven tumors had clonal cytogenetic changes, 3 (75%) of which were anomalies of chromosome 2 that lead to overrepresentation of the long arm of this chromosome. Noted also in these series was an association between complex karyotypes and tumors with poorly differentiated histotypes.

Hereditary Papillary Thyroid Carcinoma

Introduction

The overwhelming majority of PTC occurs sporadically, but familial cases have been identified.21,135–142 PTC has also been reported with ataxia telangiectasia,143 Gardner syndrome and Cowden syndrome.135 The cribriform-morular variant of PTC is characteristically occurring in the familial setting.

Clinical

The cribriform-morular variant of PTC can occur as a sporadic tumor,144 but characteristically occurs in the setting of familial adenomatous polyposis (FAP) and germ line mutations in the APC gene on chromosome 5q21–22.144–148 The cribriform-morular variant of PTC may be solitary or multiple and occurs predominantly in young women. Although the tumors are often well demarcated, total thyroidectomy has been advocated by some because of the frequent multicentricity of the tumor.146 These tumors behave similarly to conventional PTC.

Histologic Features

The tumor cells have cytologic features of PTC, but are hyperchromatic. The architecture is characterized by cribriform areas with anastomosing bars and arches without intervening
8. Well-Differentiated Papillary Thyroid Carcinoma

stroma and areas solid/trabecular and morular growth (Figure 8.6).147

Immunohistochemistry

The cribriform-morular variant of PTC shows focal positivity for thyroglobulin.1 Beta-catenin is also positive in these tumors.

Genetic Findings

Patients with FAP may present with this thyroid tumor before their colon findings are known.146 Hence, patients diagnosed with the cribriform-morular variant of PTC should be evaluated for the possibility of FAP. A recent study of five young women with the cribriform-morular variant of PTC found two patients with attenuated FAP.148 The CTNNB1 gene is located on chromosome 3p21 which encodes beta-catenin. Germline APC mutation was identified in only one FAP patient, but somatic mutation analysis of exon 3 of the beta-catenin gene (CTNNB1) revealed alterations in seven tumors from all five individuals.148 Two different tumors from two patients with the multicentric PTC had different somatic mutations of the CTNNB1 gene suggesting that genetic alterations in FAP-associated thyroid cancer involve loss of function of APC along with the gain of function of RET/PTC.147

Conclusion

As PTC is the most common endocrine malignancy and is increasing in incidence, molecular genetic discoveries will play an increasingly important role in elucidating the pathogenesis and providing diagnostic and prognostic markers for these neoplasms.

References

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Fig. 8.6. Cribriform-morular variant of papillary thyroid carcinoma (PTC). (a) Cribriform-morular variant of PTC with cribriform structures. (b) Areas of morular growth in a cribriform-morular variant of PTC.


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Introduction

Tumors that arise from follicular epithelial cells of the human thyroid gland are common in clinical practice and can be detected in up to 50% of adults by ultrasonography.1–3 Fortunately, less than 20% of palpable thyroid tumors are carcinomas, and these make up only about 1% of all cancers.4 Approximately 80% of thyroid cancers are papillary carcinomas that have an excellent prognosis with an overall patient survival of 90–95%.5 Another 15% of thyroid carcinomas are follicular and Hürthle cell carcinomas that have a good prognosis with an overall patient survival of 75–80%.4,6–12 Papillary, follicular, and Hürthle cell carcinomas are thought to arise from the same type of precursor thyroid follicular epithelial cell, although each has unique morphologic and clinical features. Well-differentiated thyroid carcinomas have the potential to progress to aggressive clinical disease that may be rapidly lethal, particularly in older patients.

Two overriding clinical problems must be addressed to improve the care of patients with thyroid carcinoma. First, differentiating benign from malignant thyroid tumors must be improved, so that each patient can be treated more specifically and accurately for their particular thyroid disease. Second, identification and treatment of thyroid carcinomas that are resistant to radio-iodine therapy must be improved because no effective therapy for these advanced thyroid cancers is yet available. Recent insights into the molecular pathogenesis of thyroid carcinomas suggest new approaches to these clinical problems. This is important because thyroid carcinoma is the most common cancer of endocrine organs and has been increasing in incidence.13–15

The superficial location of the thyroid gland in the anterior neck has facilitated identification, pathologic evaluation, and experimental analyses of thyroid tumors. As such, the thyroid presents an excellent model of epithelial cell-derived cancer. This model has evolved into a histologic-molecular paradigm because different histologic subtypes of thyroid carcinoma contain different somatic mutations that determine biologic features of the cancers. This chapter is focused on the molecular pathology, genetic alterations, clinical-pathologic correlates, and molecular mechanisms of well-differentiated follicular and Hürthle cell carcinomas. Epidemiologic studies suggest a strong inherited predisposition for thyroid carcinoma16,17 and preliminary data on inherited follicular and Hürthle cell carcinomas are also briefly considered.

Follicular Thyroid Carcinoma

Well-differentiated follicular thyroid carcinoma is a thyroid cancer that has follicular cell differentiation and invasive growth. Follicular carcinomas are most often unilateral, light tan/beige, firm but not hard and variegated in appearance (Figure 9.1a). Pathologic diagnosis of follicular carcinoma is based mainly on morphologic patterns in thyroid tumor sections that have been stained with hematoxylin and eosin.5 The growth patterns typically seen in follicular carcinomas include microfollicular, trabecular, and/or solid architectures (Figure 9.1b). Identification of invasion into blood vessels, the tumor capsule, and adjacent normal thyroid tissue and/or extra-thyroidal tissues is essential for making the diagnosis of follicular carcinoma (Figure 9.1c). Importantly, the nuclei of follicular carcinomas are small, round, regular, well-dispersed, and lack morphologic features of papillary thyroid carcinoma such as nuclear clearing, elongation, overlap, irregularity, grooves, and pseudo-inclusions.5 Even so, the differential diagnosis of follicular from papillary carcinomas can be difficult even for experienced endocrine pathologists.18–20 Well-differentiated follicular carcinomas are often encapsulated (Figure 9.1a) and express differentiation markers such as thyroglobulin (Figure 9.1d). Follicular carcinomas tend to present at slightly older age and lack regional lymph node spread compared to papillary carcinomas.5,10 Although some geographical variation exists, the incidence of follicular carcinoma has declined over the past 35 years,7,11,21 most likely because of: (a) earlier clinical detection and removal of small thyroid tumors,15 (b) evolution of criteria that more broadly define the pathologic diagnosis of papillary carcinoma13
and (c) a reduction in endemic goiter from iodine deficiency, a risk factor for follicular carcinoma that has decreased after the introduction of dietary iodine.\textsuperscript{22,23}

**Hurthle Cell Thyroid Carcinoma**

Hurthle cell carcinoma is a thyroid cancer that has follicular cell differentiation, invasive growth, and histologic features similar to, but distinct from follicular carcinoma. The most recent classification of thyroid tumors by the World Health Organization categorizes Hurthle cell carcinoma as a variant of follicular carcinoma,\textsuperscript{5} but there is considerable evidence to indicate that Hurthle cell and follicular thyroid carcinomas represent distinct biologic entities. The unique features of Hurthle cell lesions, as compared to follicular lesions, include: (a) distinct morphologic features, (b) more frequent resistance to radio-iodine therapy,\textsuperscript{24-26} (c) increased frequency of local recurrence, lymph node metastases and possibly a worse prognosis,\textsuperscript{9,27-29} (d) lack of *RAS* mutations,\textsuperscript{30} (e) lack of *PPARγ* rearrangements,\textsuperscript{30,31} (f) different patterns and higher levels of loss of hetereozygosity\textsuperscript{32-34} and (g) *RET* rearrangements in up to 50\% of cases,\textsuperscript{35,36} (although the exact nature of these Hurthle cell thyroid tumors with RET rearrangements remains to be clarified).\textsuperscript{37}

Well-differentiated Hurthle cell carcinoma presents most often as a unilateral tumor that is encapsulated and soft with a distinctive mahogany brown appearance (Figure 9.2a). The mahogany color results from excessive biogenesis and accumulation of normal and abnormal mitochondria within the tumor cytoplasm (Figure 9.2b). Over production of mitochondria produce a pink, granular (oncocytic) cytoplasm on hematoxylin and eosin staining (Figure 9.2c). It is said that the pink Hurthle “oncocyes” must make up at least 75\% of tumor cells to diagnose a Hurthle cell tumor,\textsuperscript{7} but this is a somewhat arbitrary designation. Round, well-dispersed nuclei that vary in size and have prominent, central purple-pink (amphophilic) nucleoli are characteristic of Hurthle cell carcinoma (Figure 9.2c). Hurthle cell carcinomas exhibit microfolicular, trabecular, and/or solid architectural patterns.
9. Well-Differentiated Thyroid Follicular Carcinoma

and invade blood vessels (Figure 9.2d), the tumor capsule (Figure 9.2d), normal thyroid tissue and/or extra-thyroidal tissues, much like follicular carcinomas. Hürthle cell carcinomas are a biologically more heterogeneous group of thyroid cancers than follicular carcinomas.

Immunohistochemistry

Immunohistochemistry can assist in the pathologic diagnoses of well-differentiated thyroid carcinomas. Both follicular and Hürthle cell carcinomas immunoreact strongly with antibodies against thyroid follicular cell antigens such as thyroglobulin (Figure 9.1d), the major protein precursor of thyroid hormones, and TTF-1 (Figure 9.3a), a transcription factor that regulates the expression of genes in thyroid and lung epithelial cells. Immunoreactivity against thyroglobulin is a diagnostic adjunct that is used to differentiate follicular and Hürthle cell carcinomas from medullary thyroid carcinomas, which are thyroglobulin negative and exhibit diverse morphologic patterns. Thyroglobulin and TTF-1 also help distinguish thyroid from nontumor carcinomas at metastatic sites. The detection of thyroglobulin in the serum is used routinely to screen for recurrence after the primary treatment of thyroid carcinoma.

Additional immunohistochemical assays are employed by some pathology laboratories to help distinguish follicular carcinomas from benign follicular adenomas and hyperplastic thyroid nodules or from the follicular variant of papillary carcinoma. For example, cytoplasmic staining for galectin 3, a β-galactoside-binding lectin, is observed in 30–80% of follicular carcinomas and higher numbers of papillary carcinomas but is low in normal thyroid tissues and many benign thyroid tumors. Galectin-3 immunoreagents tend to cross-react with lymphocytes and therefore may generate background in thyroiditis cases and in papillary carcinomas with lymphocytic infiltration. 30–80% of follicular carcinomas also immunoreact with the HBME-1 antigen in membranous, apical and/or cytoplasmic patterns, whereas HBME-1 immunoreactivity in benign colloid nodules and follicular adenomas is usually weak and focal. Lack of HBME-1 immunoreactivity has been reported to correlate with the presence of the
PAX8–PPARγ gene fusion in follicular carcinomas.

The extent of galectin-3 and HBME-1 immunoreactivity vary considerably in different reports and depends on the antibodies and tissue processing that are used.

Follicular and Hürthle cell carcinomas immunoreact weakly with immunoreagents against the intermediate filament protein CK-19, and this can assist in differential diagnosis from papillary carcinoma in some situations. CK-19 is expressed strongly and diffusely in the cytoplasm of >85% of papillary carcinomas, whereas lighter and focal staining is observed in <15% of follicular and Hürthle cell carcinomas (Figure 9.3b). Membrane staining for claudin-1, a protein of epithelial cell junctions, also may aid in differentiating papillary from follicular and Hürthle cell carcinomas (unpublished data) (Figure 9.3c). Well-differentiated thyroid carcinomas tend to lose expression of these differentiation markers as they progress to poorly differentiated or anaplastic thyroid carcinoma forms.
Follicular and Hürthle cell carcinomas exhibit increased cell proliferation over normal thyroid tissues and benign thyroid tumors, and this can be measured by the nuclear expression of Ki-67, an in situ proliferation marker (Figure 9.3d). Hürthle cell carcinomas tend to be more proliferative than follicular carcinomas. However, Ki-67 staining varies widely in thyroid tumors of the same class, including follicular adenomas and Hürthle cell adenomas, and this has limited the diagnostic utility of Ki-67.

The extent and pattern of immunohistochemical staining for the above antigens are important in their interpretation. Thus, each laboratory must validate their immunohistochemical assays with well-defined series of thyroid tumors. In the past, an endogenous biotin-like activity has often generated background in thyroid tissues when performing immunohistochemistry with the classic avidin–biotin complex.50 This complication has been circumvented in recent years by use of nonbiotin, polymer-based detection reagents. As a general rule, Hürthle cell carcinomas stain less uniformly and with more cytoplasmic background than follicular carcinomas.42,57–59,77

Somatic Mutations

Somatic mutations are fundamental drivers in the pathogenesis of thyroid carcinoma and can be divided into two basic classes: (a) mutations that are present in both thyroid and nonthyroid cancers such as point mutations in RAS (G-protein), BRAF (serine/threonine kinase), and PI3KCA (phosphatidylinositol-3-kinase) and (b) mutations that are specific for thyroid tumors such as rearrangements of PPARγ (nuclear receptor), RET (tyrosine kinase), and NTRK1 (tyrosine kinase) genes (Figure 9.4). RAS and PPARγmutations characterize the majority (70–80%) of well-differentiated follicular carcinomas and appear to be mutually exclusive in individual follicular carcinomas. These mutations are rarely observed in Hürthle cell carcinomas and are thought to arise early in tumorigenesis because they are present in low stage follicular carcinomas and in a subset of follicular adenomas that do not have morphologic signs of invasion. Such follicular adenomas with RAS or PPARγmutations are either true benign thyroid tumors that require additional cellular changes before they progress to invasive carcinoma or are early carcinomas in which invasion has not yet developed or has been missed because of practical limitations of tissue sampling. This distinction may be more academic than practical because both scenarios may portend an increased risk of malignancy relative to thyroid follicular adenomas as a group.

RAS Mutations

RAS point mutations were among the first somatic mutations identified in cancer tissues78–80 and were the first recurrent mutations identified in sporadic thyroid follicular carcinomas. RAS mutations are common in follicular carcinomas (approximately 45%) and follicular adenomas (approximately 20%) but rare in Hürthle cell carcinomas and adenomas,30,81–83 demonstrating that most follicular and Hürthle cell carcinomas develop through different molecular pathways. RAS mutations have also been detected in a small subset (approximately 10%) of thyroid papillary carcinomas38 that tend to have a follicular growth pattern (follicular variant), tumor encapsulation, vascular invasion, and infrequent lymph node spread,80 features that are more common in follicular than papillary carcinomas. It will be important to determine whether follicular-patterned papillary carcinomas with RAS mutations are “hybrid” thyroid carcinomas that possess mixed morphologic, genetic and clinical characteristics of follicular and papillary carcinoma or are follicular carcinomas that have focal morphologic nuclear alterations that lead to the diagnosis of papillary carcinoma.

In well-differentiated thyroid follicular carcinoma, N-RAS mutations seem to predominate over K-RAS and H-RAS mutations and codon 61 mutations in N-RAS may be the most prevalent.10,87,91–93 In contrast, mutations in K-RAS may be more frequent in papillary carcinomas,82,83,94 radiation-associated thyroid carcinomas,83 and aggressive thyroid carcinomas.94 Clinical and pathologic features that appear to correlate with RAS mutations in follicular carcinoma patients include older patient age, higher stage at presentation and larger, less differentiated thyroid tumors.10,85,94–96

The oncogenic contribution of RAS mutations to thyroid carcinoma has been demonstrated experimentally in transgenic mouse models.97–99 However, mutant RAS is insufficient to induce rapid development of follicular carcinoma in vivo or to produce a fully transformed phenotype in thyroid cells in vitro.100–104 Thus, additional cellular alterations appear to be necessary to manifest a fully invasive follicular carcinoma phenotype. RAS proteins are coupled to growth factor receptors such as receptor tyrosine kinases, bind GTP and propagate signals from the thyroid cell membrane to downstream molecular effectors such as BRAF, the mitogen-activatedprotein kinases MEK and ERK, Rac1-Rho, Ral-GDS, and PI3K (Figure 9.4). RAS proteins are activated constitutively by mutations in codon 61 that inhibit intrinsic GTPase activity or by mutations in codons 12 or 13 that tend to increase the affinity of RAS for GTP. RAS induces proliferation, survival, de-differentiation, genomic instability, and transformation in thyroid cells. Interestingly, mutations in RAS have been observed most often in follicular carcinomas, whereas mutations in RET, NTRK1, and BRAF, molecules also involved in the RAS signaling pathway (Figure 9.4), have been observed most often in papillary carcinomas. It will be important to determine how follicular and papillary carcinomas that have different morphologic and clinical tendencies appear to arise from the same RAS mutations. Collaborating mutations and/or other cellular alterations may be involved.
Somatic mutations in PPARγ have been identified in 25–55% of follicular thyroid carcinomas in pathologically well-defined series that separate follicular from Hurthle cell carcinomas. PPARγ mutations have also been identified in follicular carcinomas (~30%) and may be linked functionally to RAS/PI3K/ AKT/PDK1 through PDK1 but additional experiments are required to confirm this connection. On the other hand, the RAS/BRAF/MEK/ERK pathway has been implicated in papillary thyroid carcinoma based on mutations in RET (~25%) and NTRK1 (~10%), RAS (~10%) and BRAF (~40%) in papillary carcinoma tissues. Both RAS/PI3K/AKT/PDK1 and RAS/BRAF/MEK/ERK regulate multiple cellular processes related to tumorigenesis. Activation of these pathways by mutations increases cell proliferation, survival, de-differentiation, genomic instability and transformation and contributes to biologic and clinical phenotypes in follicular and papillary thyroid carcinoma. And contributes to biologic and clinical phenotypes in follicular and papillary thyroid carcinoma. GF growth factor; RET RET receptor tyrosine kinase; NTRK1 NTRK1 receptor tyrosine kinase; PTEN phosphatase and tensin homologue deleted from chromosome 10; PI3K phosphatidylinositol 3’-kinase; PIP3 phosphatidylinositol-3-4-5-triphosphate; PDK1 3-phosphoinositide-dependent protein kinase-1; AKT/PKB AKT serine/threonine protein kinase/protein kinase B; RAS GTP-activated RAS G protein; BRAF BRAF serine/threonine kinase; MEK mitogen activated protein kinase ERK kinase; ERK extracellular regulated kinase; RAC1 RHO RAC1 small GTPase, Rho family; RAL1-GDS RAL1 small G protein; RB retinoblastoma protein; CDKs cyclin-dependent kinases; CDKIs cyclin-dependent kinase inhibitors; HAT histone acetyltransferase; HDAC histone deacetylase; PPRE PPARγ response element; TATA TATA box; mRNA pol II mRNA polymerase II.

PPARγ Mutations

Somatic mutations in PPARγ have been identified in 25–55% of follicular thyroid carcinomas in pathologically well-defined series that separate follicular from Hurthle cell carcinomas. PPARγ mutations are gene rearrangements that were discovered by cloning of a chromosomal translocation t(2;3)(q13;p25) in follicular thyroid tumors. t(2;3) juxtaposes the promoter region and 5′ coding sequences of the PAX8 gene on chromosome 2 with coding and 3′ noncoding sequences of the PPARγ gene on chromosome 3 (Figure 9.5a). The PAX8 promoter is active in thyroid epithelial cells and drives expression of chimeric PAX8–PPARγ fusion mRNA and protein (Figure 9.5a). Another PPARγ mutation encoded by t(3;7)(p25;q34) has been identified recently in <3% of thyroid follicular carcinomas. t(3;7) juxtaposes the promoter region and 5′ coding sequences of the CREB3L2 gene on chromosome 7 with coding and noncoding 3′ sequences of the PPARγ gene on chromosome 3 (Figure 9.5b). The CREB3L2 promoter is active in thyroid epithelial cells and drives expression of chimeric CREB3L2–PPARγ fusion mRNA and...
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Protein119 (Figure 9.5b). These discoveries of PAX8–PPARγ and CREB3L2–PPARγ demonstrate that a family of related PPARγ mutations exists in follicular carcinoma.

PPARγ rearrangements are rare in Hürthle cell carcinomas30,31,106,109,111 arguing that follicular and Hürthle cell carcinomas develop through different molecular pathways. Most studies have also shown that PPARγ rearrangements occur at relatively low frequency in follicular adenomas30,31,106–108,110,120 and the follicular variant of papillary carcinoma,31,105–108 with a couple exceptions.111,121 The biological relationships of follicular adenomas and PPARγ rearrangements to follicular carcinomas with PPARγ rearrangements are not yet clear in part because the histologic diagnoses of follicular-patterned tumors is imprecise and approached somewhat differently by different pathologists.18,19 mRNA expression profiles argue that follicular adenomas and follicular carcinomas with PPARγ rearrangements are closely related, if not identical, thyroid tumors,125 although the latter data requires verification. The possibility that PPARγ rearrangements mark a subset of follicular carcinomas, some even before histologic evidence of invasion is present, supports the idea that the molecular diagnosis of PPARγ rearrangements may be useful in fine needle aspiration biopsies to detect a subset of thyroid carcinomas prior to surgery.123

PPARγ rearrangements can be detected in thyroid follicular carcinomas by reverse transcription-polymerase chain reaction (RT-PCR), immunohistochemistry (Figure 9.6a) or fluorescence in situ hybridization (FISH) (Figure 9.6b). Dual color FISH using a probe set that flanks the PPARγ gene is particularly informative because it can identify PPARγ rearrangements, deletions, amplifications, and clonality in a single assay in clinical or research specimens.31,123 For example, FISH on paraffin sections of follicular carcinomas, demonstrates two alleles in each nucleus for a normal diploid
PPARγ locus, one split allele in each nucleus for a balanced PPARγ rearrangement, and one split allele plus a signal loss for concomitant PPARγ rearrangement and 3p25 deletion (Figure 9.6b). In addition, aneusomy at the PPARγ locus can be detected by increased 3p25 copy number (Figure 9.6b).

Clinical-pathologic correlates of PPARγ rearrangements have been investigated in patients with thyroid follicular carcinoma. Follicular carcinomas with PPARγ rearrangements tend to have well-defined vascular invasion, no lymph node metastases, presentation at younger patient age, solid/nested tumor histology and little 3p25 aneusomy when compared to follicular carcinomas without PPARγ rearrangements. Follicular carcinomas with PPARγ rearrangements metastasize in some cases.

Experimental work has shown that fusion mutations underlie the pathogenesis of cancer and PPARγ rearrangements are no exception. PPARγ is a ligand-activated transcription factor that binds in a protein complex to PPARγ response elements and regulates the transcription of target genes (Figure 9.4). All functional domains of wild-type PPARγ1 are present in PAX8–PPARγ and CREB3L2–PPARγ (Figure 9.5), suggesting that PPARγ-related activities are critical in the oncogenic function of PPARγ fusion proteins. The paired domain of PAX8 and the transactivation domain of CREB3L2 are...
retained in PAX8–PPARγ and CREB3L2–PPARγ, respectively, suggesting that PAX8- and CREB3L2-related functions are also important. Consistent with this possibility, wild-type PAX8 is required for the development of thyroid follicular cell lineage and exhibits transforming activity in vitro. In addition, PAX5 is a frequent target of mutations and rearrangements in B cell lymphoblastic leukemia and the paired domains of PAX3 and PAX7 are rearranged as fusion proteins in alveolar rhabdomyosarcoma. Moreover, the BZIP domain of CREB3L2 is rearranged as a fusion protein in fibromyxoid sarcoma.

Potent growth activities for the PAX8–PPARγ and CREB3L2–PPARγ fusion proteins have been demonstrated. Primary human thyroid follicles and follicular epithelial cell monolayers can be isolated from normal thyroid tissues removed by surgery for thyroid tumors (Figure 9.7a). PAX8–PPARγ and CREB3L2–PPARγ have been shown to stimulate proliferation of primary human thyroid cells by 20–30% on day 3 after electroporation (Figure 9.7b). PAX8–PPARγ also induces proliferation, anchorage-independent growth, and transformation and inhibits apoptosis in immortalized human thyroid cell lines. Initial functional studies indicated that PAX8–PPARγ has little transcriptional activity at PPARγ response elements and inhibits transcription by wild-type PPARγ in a dominant negative manner, suggesting a model in which PAX8–PPARγ acts in part by blocking transcriptional activities of PPARγ. This model is consistent with: (a) the growth inhibitory effects of PPARγ ligands on thyroid and nonthyroid cancer cell lines, (b) the low levels of wild-type PPARγ in thyroid tumor tissues that lack PPARγ mutations, (c) rare inactivating point mutations in wild-type PPARγ in colon carcinoma tissues, and (d) inhibition of PPARγ expression and transcriptional activity in a mouse model of aggressive thyroid follicular carcinoma that arises after overexpression of a mutant thyroid hormone receptor β. Even so, dominant negative activity is probably not the most important functional aspect of PPARγ fusion proteins because: (a) the CREB3L2–PPARγ fusion protein, which contains the same PPARγ domains as PAX8–PPARγ (Figure 9.5), exhibits robust transcriptional activity at PPARγ response elements (unpublished data), (b) transcriptional activity of PPARγ fusion proteins may depend on cell context and type of PPARγ response element examined, (c) expression profiling indicates that some PPARγ-responsive genes are up regulated in follicular carcinomas with PAX8–PPARγ and (d) some inhibitory effects of PPARγ ligands on cell growth result from PPRE-independent effects. Furthermore, PPARδ not PPARγ is the predominant PPAR that is expressed in thyroid cells and tissues. PPARδ regulates thyroid cell proliferation by a cyclin E1-dependent mechanism and controls PPARγ transcriptional responses. Thus, fundamental mechanisms of PPARγ mutations in follicular carcinoma remain to be elucidated. Expression profiling suggests that NORE1A, FGDL1, ANGPTL4, CCND1, CTBP2, EGFR, or other molecular pathways may be involved.

An important aspect of fusion mutations in cancer is that they often identify novel growth pathways and a new thyroid signaling system has been detected by investigation of CREB3L2–PPARγ. Wild-type CREB3L2 is a BZIP transcription factor with a transmembrane domain that is cleaved by intra-membrane proteolysis, thereby releasing CREB3L2 to the nucleus to regulate gene expression. Wild-type CREB3L2 is localized in the cell membrane, cytoplasm, and nucleus of normal thyroid cells based on antisera that are specific to CREB3L2 domains (unpublished data). The CREB3L2–PPARγ fusion protein lacks the DNA binding domain of CREB3L2 and has little ability to stimulate...
transcription from cAMP response elements, in marked contrast to wild-type CREB3L2. CREB3L2–PPARγ also inhibits the native expression of thyroglobulin in primary thyroid cells that have been treated with thyroid stimulating hormone (Figure 9.7c). Thus, CREB3L2–PPARγ likely acts in follicular carcinoma in part by inhibiting cAMP-dependent thyroid genes and disrupts the CREB3L2 pathway. In contrast, the PAX8–PPARγ fusion protein may have less effects on thyroid cell differentiation.

**P13KCA and PTEN Mutations**

Somatic mutations in the P13K/PIP3/AKT/PDK1 pathway have been implicated in well-differentiated follicular thyroid carcinoma. The P13K/PIP3/AKT/PDK1 pathway is activated by growth factors and receptor tyrosine kinases and regulates multiple cell processes related to tumorigenesis. P13K (phosphatidylinositol 3'-kinase) generates PIP3 (phosphatidylinositol-3,4,5-triphosphate) at thyroid cell membranes and PIP3 activates AKT, PDK1, and other downstream signaling molecules (Figure 9.4). Somatic mutations in P13KCA, which encodes the p110α catalytic subunit of P13K, have been identified in 7–13% of thyroid follicular carcinomas and P13KCA copy number changes have been detected in 24–29% of follicular carcinomas. P13KCA mutations in follicular carcinomas may correlate with metastatic disease, but additional patients are needed to verify this association. P13KCA mutations are more frequent in anaplastic thyroid carcinoma than in follicular carcinoma, consistent with an association with clinical aggressiveness. Mutations in exons 9 (helical region) and 20 (kinase region) of P13KCA are common in breast, ovarian, colorectal, and brain cancers and contribute functionally to these malignancies.

PTEN (phosphate and tensin homologue deleted from chromosome 10) encodes a dual-specificity phosphatase that inhibits the P13K/PIP3/AKT/PDK1 pathway. Germ-line mutations or deletions in PTEN cause Cowden Syndrome, which is associated with the development of benign and malignant tumors including breast and thyroid follicular carcinomas. Mice engineered to be heterozygous for PTEN (+/−) develop thyroid and other tumors. Deletions of PTEN have been noted in up to 27% of sporadic follicular thyroid carcinomas and PTEN mutations have been identified in sporadic follicular carcinomas as well. PTEN protein is reduced in a significant fraction of thyroid follicular tumors.

**Other Chromosom al Rearrangements and Deletions**

Moderate numbers of chromosomal alterations have been described in well-differentiated follicular and Hürthle cell carcinomas. Balanced and unbalanced t(7;8)(p15;q24) has been noted in some follicular carcinomas, but the rearrangement breakpoints have not yet been cloned. Loss of heterozygosity and comparative genomic hybridization studies indicate that genetic losses predominate over genetic gains in follicular and Hürthle cell carcinomas. Consistent chromosome losses in follicular carcinomas occur at the chromosomes 2p, 3p, 7q, 8q, 9q, 14q, and 17q. DNA losses are thought to encode tumor suppressor genes and Hunt et al have undertaken a genotyping analyses of polymorphic short tandem repeats in follicular carcinomas to determine whether loss of heterozygosity correlates with useful clinical parameters. The biologic rationale for this approach is supported by high numbers of mutations and deletions identified recently in carcinoma tissues by direct sequencing of long regions of DNA. DNA losses do not correlate consistently with specific thyroid tumor types, suggesting that this approach provides a general measure of genetic instability that may be useful for prognostication of thyroid carcinoma in patients.

**RET Mutations**

RET rearrangements have been identified in approximately 50% of Hürthle cell carcinomas and adenomas, a somewhat surprising finding because RET rearrangements were long considered to be specific for papillary thyroid carcinoma. Although the exact nature of Hürthle cell tumors with RET rearrangements is not yet certain, they may be variants of papillary carcinoma. Papillary carcinomas with oncocytic cytoplasm are recognizable, and Hürthle cell tumors can have nuclear hyperchromasia that may obscure nuclear alterations used for pathologic diagnosis of papillary carcinoma. Interestingly, Hürthle cell tumors with RET rearrangements exhibit frequent lymph node metastases, a feature common to papillary carcinomas.
cancer pathogenesis, provide molecular-based classification of thyroid cancer subtypes and correlate gene expression patterns with important clinical variables. A major finding from mRNA expression profiling studies (Table 9.1) is that follicular carcinomas cluster separately from follicular adenomas and papillary carcinomas based on gene expression signatures. This observation is supported by other techniques such as serial analysis of gene expression and mass spectroscopy of proteins that are expressed in thyroid tumors. mRNA Expression profiles also distinguish Hürthle cell carcinomas from follicular carcinomas with and without PAX8–PPARγ, indicating that mutation status is a primary determinant of gene expression and may correlate more strongly with gene expression than with classic tumor morphology.

### MicroRNAs

Investigations of microRNAs in thyroid carcinoma tissues suggest both a functional role and diagnostic potential. MicroRNAs are small noncoding oligonucleotides that are believed to regulate the expression of multiple target genes. Alterations in the expression of microRNAs in cancer have created great interest because many microRNAs function by binding the 3’ untranslated region of mRNA to control its degradation and translation. Several studies have focused on microRNAs in thyroid papillary carcinoma and shown that engineered overexpression of selected microRNAs can stimulate the growth of follicular or papillary thyroid carcinoma cell lines. Fewer studies have investigated microRNAs in well-differentiated follicular and Hürthle cell carcinomas, but

### Table 9.1. Selected expression profiling studies on well-differentiated follicular and Hurthle cell thyroid carcinoma.

<table>
<thead>
<tr>
<th>Study</th>
<th>Separation</th>
<th>Classifier set</th>
<th>Validation</th>
<th>Selected genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huang et al.</td>
<td>Oligonucleotide array</td>
<td>FC vs. PC</td>
<td>83–143 genes</td>
<td>RTPCR, IHC</td>
</tr>
<tr>
<td>Barden et al.</td>
<td>Oligonucleotide array</td>
<td>FC vs. FA</td>
<td>105 genes</td>
<td>RTPCR, IB</td>
</tr>
<tr>
<td>Aldred et al.</td>
<td>Oligonucleotide array</td>
<td>FC vs. PC</td>
<td>5 genes</td>
<td>qRTPCR</td>
</tr>
<tr>
<td>Chevillard et al.</td>
<td>cDNA array</td>
<td>FC vs. FA vs. PC</td>
<td>23–80 genes</td>
<td>qRTPCR</td>
</tr>
<tr>
<td>Finley et al.</td>
<td>Oligonucleotide array</td>
<td>FC vs. FA vs. PC</td>
<td>627 genes</td>
<td>NA</td>
</tr>
<tr>
<td>Takano et al.</td>
<td>SAGE</td>
<td>FC and HC vs. FA</td>
<td>1 gene</td>
<td>qRTPCR</td>
</tr>
<tr>
<td>Cerutti et al.</td>
<td>SAGE</td>
<td>FC vs. FA</td>
<td>17–73 genes</td>
<td>qRTPCR, IHC</td>
</tr>
<tr>
<td>Weber et al.</td>
<td>Oligonucleotide array</td>
<td>FC vs. FA</td>
<td>3 genes</td>
<td>qRTPCR, IHC</td>
</tr>
<tr>
<td>Fryknas et al.</td>
<td>cDNA array</td>
<td>FC vs. FA</td>
<td>10 gene</td>
<td>qRTPCR</td>
</tr>
<tr>
<td>Baris et al.</td>
<td>cDNA array</td>
<td>HC vs. PC, oncocytic</td>
<td>83 genes</td>
<td>qRTPCR, IHC</td>
</tr>
<tr>
<td>Netea-Maier et al.</td>
<td>Mass spectroscopy</td>
<td>FC vs. FA</td>
<td>37 proteins</td>
<td>IHC</td>
</tr>
</tbody>
</table>

SAGE: serial analyses of gene expression; FC: follicular carcinoma; PC: papillary carcinoma; FA: follicular adenoma; HC: Hürthle cell carcinoma; RTPCR: reverse transcription polymerase chain reaction; qRTPCR: quantitative reverse transcription polymerase chain reaction; IHC: immunohistochemistry; IB: immunoblot.
both upregulation (Figure 9.8) and downregulation of microRNA expression have been observed. In these studies, the expression microRNAs in follicular tumors, Hürthle cell tumors, and papillary carcinomas appears to be more similar to each other than in medullary thyroid carcinomas, as would be expected from their common origin from thyroid follicular cells. Unsupervised hierarchical clustering based on microRNA expression also segregated thyroid tumors into the classic histologic categories of follicular tumor, Hürthle cell tumor, papillary carcinoma, and medullary carcinoma, supporting the idea that microRNAs contribute to thyroid cancer phenotype. Separation of follicular from Hürthle cell carcinomas by microRNA expression provides additional evidence that these are distinct thyroid cancer groups. Clustering of follicular adenomas with follicular carcinomas and Hürthle cell adenomas with Hürthle cell carcinomas is consistent with the progression of some adenomas to carcinoma, a possibility that requires further investigation. Interestingly, deregulation of miR-200A may be particularly important in follicular carcinomas with the PAX8–PPARγ fusion and distinct microRNA expression patterns correlate with specific BRAF, RET, or PAX8–PPARγ mutations, although there is considerable variability in the expression of microRNAs in thyroid tumors of the same histologic type.

Inherited Well-Differentiated Thyroid Carcinoma

Epidemiologic studies indicate that thyroid carcinoma arising from follicular epithelial cells occurs in families more often than expected by chance. Most studies have suggested a predisposition to familial papillary carcinoma, but follicular carcinoma is an inherited component of Cowden Disease that results from germ-line mutations in the PTEN tumor suppressor gene. In addition, families with Hürthle cell carcinomas and other oncocytic tumors have been linked to the 19p13.2 chromosomal locus, although the mutation responsible for this linkage is not yet identified.
Summary

Recent advances in the molecular pathology of thyroid tumors, including well-differentiated follicular and Hürthle cell carcinomas, have identified mechanisms of thyroid carcinogenesis and suggested new approaches to help improve the diagnoses, prognostication, and treatment of thyroid cancer in patients. Somatic mutations are driving molecular events in well-differentiated thyroid carcinoma, and similarities in the mutations in thyroid carcinoma and myeloid leukemia are remarkable—common N-RAS mutations,241–244 PPARγ compared to RARα rearrangements245,246 in the same nuclear receptor family and RET compared to FTL3247,248 receptor tyrosine kinases. Mutations in both myeloid leukemia and thyroid carcinoma are primary determinants of gene expression, morphology, and clinical phenotype and speak to a shared molecular pathogenesis of cancers arising in very different tissues. Approaches that have been successful in diagnosis and treatment of leukemia may be worthy of consideration in thyroid carcinoma. For example, molecular diagnosis and disease monitoring of mutations are now standard-of-care in many leukemia patients.249–251 In thyroid carcinoma, molecular diagnostic assays that reflect mutation status, whether based on RTPCR, FISH, DNA sequencing, expression signatures, or other techniques, will be implemented and validated to diagnose thyroid carcinomas more precisely and accurately or other techniques, will be implemented and validated to diagnose thyroid carcinomas more precisely and accurately.216–220

References


193. Frisk T, Kytola S, Wallin G, Zedenius J, Larsson C. Low frequency of numerical chromosomal aberrations in follicular


10
Poorly Differentiated and Undifferentiated Thyroid Carcinomas

Jennifer L. Hunt and Virginia A. LiVolsi

Introduction

The definition of poorly differentiated thyroid carcinoma has been the source of great controversy in the pathology literature. Many definitions and schemes have been proposed, but little consensus exists about what defines a poorly differentiated thyroid carcinoma (PDTCA). Because of this, the clinical behavior is not well defined and the clinicopathologic features affecting prognosis remain largely unknown.

In contrast, anaplastic thyroid carcinoma (ATC, also called “undifferentiated thyroid carcinoma”) is pathologically fairly well characterized; clinically it affects a unique group of patients, which further enables a straightforward diagnosis.

This chapter will discuss the clinical and histologic features, immunohistochemical staining patterns, and the molecular genetics of each of these two thyroid tumor categories.

Poorly Differentiated Thyroid Carcinoma

No standardized definitions exist for the grading of the differentiation of thyroid carcinoma, other than the dogma that follicular carcinoma and papillary carcinomas are considered to be well-differentiated tumors and anaplastic carcinoma is considered to be undifferentiated. Grading of tumors that exist in the spectrum between these two extremes is surrounded by some degree of controversy both in the literature and in practice. Grading is further complicated by the fact that PDTCAs may be derived from well-differentiated tumors and varying amounts of these well-differentiated elements can be represented in the histologic sections of any particular tumor.

As with other organ systems, tumor differentiation does play a key role in prognostication for thyroid tumors. But, in thyroid carcinoma, the independent prognostic value or independent impact of differentiation on prognosis is less clear. In fact, the influence of standard variables, such as age, gender, tumor size, extrathyroidal extension of tumor, and metastasis is so strong that prognosis is heavily influenced by these factors. The strongest argument for assessing differentiation can be found in the many papers that have demonstrated survival disadvantage when higher grade histologic features are identified. Even when poorly differentiated areas are entirely well contained within otherwise well-differentiated tumors, the presence of this more aggressive morphology likely impacts survival. Unfortunately, in treatment protocols for thyroid carcinoma, there are no additional therapies that have proven to be of great value when poorly differentiated components are identified. Although chemotherapy and external beam radiation therapy have been used in some series, dramatic improvement in survival has not been shown and these treatments are usually reserved for the palliative setting.

When tumors are well-differentiated, tumor histologic grading is probably not essential. Patients with these well-differentiated tumors tend to have an excellent survival and differences based on traditional nuclear and histologic grading will not impact greatly on prognosis. When tumors transition into less well-differentiated lesions, however, it is more important to note the presence of histologically aggressive features. The pathologist should therefore be familiar with the features of poorly differentiated tumors.

Histology

The growth pattern of a thyroid tumor provides one of the most important diagnostic features for differentiation in thyroid carcinomas. However, some classification schemes include specific subtypes of thyroid cancer as PDTCAs, i.e., tall cell papillary carcinoma or Hurthle cell carcinoma. These latter entities have been fairly well described both morphologically and be clinicopathologic features and hence should not be added to the mix of PDTCAs. Growth patterns that are considered to be less well-differentiated include insular, trabecular, and solid growth. Various studies have demonstrated that these growth patterns are associated with a worse prognosis. Unfortunately, the studies are...
highly variable about what is considered the minimal amount needed to classify a tumor as “poorly differentiated”.

Various proposed cut-offs have included >10%, >40%, and >70%. Because of these inconsistencies, it is difficult to compare the different studies and to arrive at a conclusion about the true impact of these growth patterns on prognosis.

In 1984, Carcangiu and Rosai described 25 cases of a new form of PDTCA that had a poor prognosis and a unique histologic appearance; they designated this tumor as “insular carcinoma”. The insular growth pattern shows large nests of cells with small round nuclei. The nests are surrounded by a rich vascular network. This tumor designation has now been used for several decades, but it is now controversial whether this is a distinct tumor type or simply a pattern of poorly differentiated tumors, or to the methods used for detection, or they may simply reflect the fact that PDTCA remains a very heterogeneous tumor. The results for expression of p53 in insular carcinoma in PDTCA, and low p27KIP1 expression and high proliferative activity, at least focally.

In terms of tumor markers, the results for PDTCAs are sparse. The best studied genetic alteration is in the p53 gene. The results for expression of p53 in insular carcinoma in particular have been mixed, with some studies showing significant expression and others showing no expression. These discrepancies may be related to the original classification of the tumors, or to the methods used for detection, or they may just reflect the fact that PDTCA remains a very heterogeneous group of tumors. Another tumor marker that has been studied is the KIT gene, which is a receptor tyrosine kinase. The KIT gene has been best studied at the molecular level in gastrointestinal stromal tumors, which harbor both over-expression and somatic mutations. In these tumors, remarkable results have been achieved when patients are treated with targeted therapies against receptor tyrosine kinase. Expression of KIT is seen in some PDTCAs, but is highly variable. Importantly, the KIT expression is not associated with mutations in the commonly mutated exons for the KIT gene. Therefore, it is unlikely that agents targeting receptor tyrosine kinases will be of value in treating PDTCA. Very few other immunohistochemical markers have shown any prognostic significance in PDTCA.

Another immunomarker that may be helpful is Ki67 since PDTCAs often have significant mitotic activity and this stain may allow numerical assessment of this activity.

Very little work has been done with PDTCAs and the other oncogene mutations that are associated with thyroid carcinoma, such as BRAF, RAS, and the tumor translocations. There do appear to be different expression profiles between well-differentiated tumors and PDTCAs. Specifically, PDTCAs appear to have alterations of key activators and negative regulators in the MAP-kinase pathways. There is also evidence that cyclin D1 and e-cadherin are over-expressed in PDTCAs, and low p27KIP1 expression and high proliferative rates have been associated with a poorer prognosis. Finally, CTNNB1, which is the gene that encodes beta-catenin, has been shown to harbor mutations and altered expression by immunohistochemistry in PDTCAs.

Anaplastic Thyroid Carcinoma

The terminology for ATC has changed over the years. In the 1930s, ATCs were designated as sarcomas, but in the 1950s to 1960s, it was recognized that these tumors were actually carcinomas with spindle and giant cells (sarcomatoid carcinoma). Some ATCs have an epithelioid pattern (at least in part) and usually these resemble large cell lung carcinoma or even frank squamous differentiation may be noted. The association with a well-differentiated carcinoma in many cases also lends support to the notion that these are carcinomas. A particular example is the entity of spindle cell squamous cell carcinoma arising in association with tall cell variant papillary carcinoma.

Many tumors that had been designated as “small cell anaplastic thyroid carcinoma” before the use of immunohistochemistry were reclassified as medullary carcinomas and lymphomas when markers for these tumors came into use. What was uniformly recognized throughout the decades was the aggressive behavior of ATC.

ATCs represent an extremely aggressive tumor that is at the extreme end of the differentiation spectrum; they have also been designated as “undifferentiated thyroid carcinoma”. J.L. Hunt and V.A. LiVolsi
These tumors usually present in patients older than 60 years of age, often with a rapidly enlarging neck mass. Patients may come to medical attention because of respiratory distress, which may even necessitate an emergent tracheostomy. The major differential from the clinical perspective is lymphoma (markedly different therapy and prognosis).

Undifferentiated carcinomas frequently develop in patients who have a history of thyroid disease. This can include multinodular goiter or possibly even a history of a well-differentiated carcinoma. Some patients will have a component of a well-differentiated thyroid carcinoma in their tumor specimens, when there is adequate material for representative sampling. The well-differentiated tumors most commonly reported in combination with ATC are papillary carcinoma, Hurthle cell carcinoma or follicular carcinoma. Concordant well-differentiated components are present in up to 50% of well sampled ATCs.

The prognosis for ATC is dismal. Survival for ATC is usually measured in months, with mean survival in larger series ranging from 3 to 6 months. However, there are rare cases that have a better prognosis. These are usually restricted to otherwise well-differentiated tumors with early incidental anaplastic transformation or rare cases that can be completely removed with negative margins.

### Histology

ATCs can exhibit various morphologies from very bland to the highly pleomorphic tumors. They can be composed of a wide variety of cell types, as well, including spindle cells, giant cells, squamoid cells or even more rare variant morphologies like the rhabdoid subtype. These variant morphologies do not have significant differences in prognosis. An unusual morphologic feature of ATC is its propensity to invade into vessels. The invasion can have a unique appearance that resembles colonization or replacement, but not destruction of the vessel wall by anaplastic tumor cells.

The major differential diagnoses for ATC will include both benign and malignant tumors. The benign lesions that are in the differential diagnosis are spindle cell metaplasia, post-FNA spindle cell nodules, nodular fasciitis like areas, and Riedel’s thyroiditis. All of these entities can have spindle cell areas of varying cellularity. However, they should not harbor the pleomorphic cells and mitotic activity of ATC. The malignant tumors in the differential diagnosis for ATC include sarcomas, carcinoma with thymus like differentiation (CASTLE), spindle epithelial tumor of thymus origin (SETTLE), which are both discussed in chapter 12 and squamous cell carcinoma of the head and neck secondarily involving the thyroid. Other entities in the differential diagnosis include solitary fibrous tumor, neural and smooth muscle tumors, and metastases from sarcomas. Some of these may be resolved with an immunohistochemical staining panel (Table 10.1).

### Immunohistochemistry

Immunohistochemical stains are often necessary in the work-up of ATC because this tumor is frequently essentially undifferentiated by light microscopy. But, there are some significant disadvantages of immunohistochemistry, again related to the undifferentiated nature of these tumors. Up to 30%
of ATC will not stain for any epithelial markers, and most will not stain for markers of thyroid origin, such as TTF-1 or thyroglobulin. It may be necessary to utilize a number of anticytokeratin antibodies to demonstrate epithelial differentiation. High molecular weight cytokeratins are somewhat more frequently found in ATCs than the low or intermediate molecular weight moieties (P.J. Zhang MD, personal communication). Recently, it has been shown that Pax8 expression may also be helpful in resolving difficult cases of ATC, since up to 79% of these tumors will be positive for this marker. ATCs, no matter what the morphological appearance, should be considered to be carcinomas, since older studies of ultrastructure of these lesions showed epithelial characteristics. The morphology and the clinical history may be necessary to resolve these difficult differential diagnoses.

Immunohistochemistry targets for potential prognostic and therapeutic value have also been investigated. One study of ATC suggested that these tumors express targets that are associated with potential targeted therapies, including beta-catenin (in 41%), aurora A (in 41%), cyclin E (in 67%), cyclin D1 (in 77%), and EGFR (in 84%). Over-expression of platelet derived growth factor and Her2/neu have also been seen in a subset of ATC.

Molecular Genetics

Much of the early work in the molecular mutational analysis of ATC was done in an attempt to prove its origin from well-differentiated carcinomas. Several different studies have demonstrated that ATC and well-differentiated carcinoma that occur concurrently have similar mutational profiles in terms of both tumor suppressor gene mutational profiles and somatic oncogene mutations.

The presence of specific mutations in ATC may have diagnostic applications. As can be seen from the above discussion of the variable and non-specific immunohistochemical staining pattern of these tumors, some cases remain very difficult to classify. Mutational assessment has also been suggested as potentially promising for the incorporation of targeted therapies into the fairly unsuccessful treatment regimens for this aggressive disease. Early studies in cell lines do suggest that some of the targeted therapies can control cell growth of ATC, but initial human trials have not been promising for showing objective responses.

Oncogenes

The oncogene mutations that are seen in both papillary carcinoma and follicular derived carcinomas are also seen in ATC. This includes the BRAF point mutation, which is found in a highly variable number of ATCs, ranging from 10 to 50%. Up to 30% of ATCs have been found to harbor RAS point mutations. The finding of these mutations in ATCs probably reflects the fact that many of these high-grade tumors derive from well-differentiated precursor lesions, even when the well-differentiated component is no longer histologically apparent.

Other mutations, including both copy number changes and somatic point mutations have also been seen in ATC. For example, CyclinD1 copy number alterations have been noted, as have losses of 7q and 13q, using a comparative genomic hybridization (CGH) approach.

Activation of the PI3K/Akt pathway by various copy number alterations and mutations are seen in up to 81% of ATCs. Another gene that appears to be involved in progression of ATC is the CTNNB1 gene, encoding for beta-catenin. Both altered expression and somatic mutations have been identified in CTNNB1 in ATCs.

Tumor Suppressor Genes

ATC has been show to have a much higher burden of somatic mutations than any of the other thyroid carcinomas, particularly in terms of tumor suppressor genes. These can be evidenced by CGH studies demonstrating consistent loss mutations at specific tumor suppressor gene locations, and by loss of heterozygosity alterations where targeted analysis can be done for specific tumor suppressor genes. The most commonly studied tumor suppressor gene in ATC is the p53 gene. Between 50 and 100% of ATCs will harbor point mutations in the p53 gene, and many will have loss of heterozygosity of the p53 gene as well. These tumors also frequently have over-expression of p53 at the immunohistochemical level, though there is not always correlation between expression and mutation. This contrasts with well-differentiated thyroid carcinomas, which have low rates of p53 alterations.

Epigenetics

ATC has not been extensively studied for changes or alterations in promoter methylation. The one gene that has been identified as having promoter methylation in ATC is the RASSF1A gene, which is tumor suppressor gene located on chromosome 3p21.3. Approximately one-third of ATCs have promoter methylation of the RASSF1A gene, which parallels the rate seen for PTC; follicular carcinoma appears to have a much higher rate of RASSF1A promoter methylation.

References

10. Poorly Differentiated and Undifferentiated Thyroid Carcinomas


11
Medullary Thyroid Carcinoma

Ronald A. DeLellis

Introduction

Medullary thyroid carcinoma (MTC) is currently defined as a malignant thyroid tumor with evidence of C-cell differentiation. While earlier reports had alluded to the existence of this tumor type, Robert Horn in 1951 reported a series of 7 cases of a thyroid cancer characterized by sharply defined rounded or ovoid compact cell groups of moderate size in a background of hyalinized connective tissue. He noted that “while not pursuing the rapid course characteristic of the giant cell, spindle cell and small cell thyroid carcinoma, these tumors have by no means the favorable prognosis of malignant adenoma and papillary tumors”. The major histopathologic features of this tumor, including the presence of stromal amyloid deposits, were defined in 1959 by Hazard and coworkers who suggested the term, medullary thyroid carcinoma (MTC). In 1966, Williams proposed that MTCs were derived from the thyroid parafollicular cells based on comparative studies in dogs and other animals. Bussolati and Pearse in the following year demonstrated the parafollicular cell origin of calcitonin, and subsequent studies confirmed the presence of calcitonin in tumor extracts and in the serum of affected patients.

Medullary carcinomas comprise up to 10% of all thyroid malignancies in most large clinical series. In patients with nodular thyroid disease subjected to serum basal and pentagastrin stimulated calcitonin studies, the prevalence of MTC ranges from 0.24 to 2.85% (mean 0.61%) based on nine reported series, primarily from Europe. The studies of Pacini et al have further underscored the high prevalence of MTC in more than 1,300 patients with nodular thyroid disease. In their study, MTC represented 0.57% all thyroid nodules and 15.7% of all incidentally discovered thyroid carcinomas. Cheung et al have shown that the cost effectiveness of routine calcitonin screening in patients with nodular thyroid disease is comparable to measurement of thyroid stimulating hormone, colonoscopy, and mammographic screening.

Clinical Features

Medullary thyroid carcinomas may occur sporadically or in association with the multiple endocrine neoplasia (MEN)2 syndromes which are inherited as autosomal dominant traits (Table 11.1). Sporadic tumors account for 75% of cases, while MEN2 associated cases account for the remainder. Sporadic MTC is primarily a tumor of middle aged adults with a female to male ratio of 1.3:1. Generally, most patients present with a painless tumor nodule that is typically cold on scintigraphic scan. In the series reported by Kebebew et al, nearly 75% of patients presented with a thyroid mass, while 15% had symptoms of dysphagia, dyspnea, or hoarseness. Cervical lymph node metastases may be present, and in some cases, distant metastases may also be evident. Affected patients may present with a variety of signs and symptoms in addition to the thyroid mass. Patients with metastatic disease, for example, may have severe diarrhea or flushing, and these symptoms may be related to the high circulating levels of calcitonin. In Kebebew’s series, approximately 10% of patients presented with systemic symptoms. The tumors may produce a wide array of peptides, amines, and prostaglandins that have been implicated in the development of diarrhea, flushing, and other systemic symptoms. They may also produce ACTH and proopiomelanocortins, which have been implicated in the development of Cushing’s syndrome. This syndrome occurs in approximately 0.6% of patients with medullary thyroid carcinoma and survival following the recognition of the syndrome is poor. Although very high levels of calcitonin may be present in patients with these tumors, hypocalcemia is virtually nonexistent.
Medullary carcinomas are highly penetrant in the MEN2 syndromes, and it has been estimated that more than 90% of carriers will develop thyroid tumors.\textsuperscript{16} MEN2A accounts for more than 75% of all heritable MTC cases. Affected patients also have pheochromocytomas in approximately 40–60% of cases and parathyroid abnormalities, (predominantly chief cell hyperplasia) in 10–30%. Some kindreds with MEN2A or familial MTC (FMTC) may also have cutaneous lichen amyloidosis which typically appears as pruritic plaques over the upper back.\textsuperscript{19} The studies of Verga and coworkers have suggested that pruritis has a pivotal role in the development of cutaneous lichen amyloidosis with the amyloidosis developing as a consequence of repeated scratching.\textsuperscript{20} Hirschsprung’s disease has been associated with MEN2A in a few families.\textsuperscript{21}

Patients with MEN2A generally present with thyroid tumors between the ages of 25 and 35. It should be noted, however, that the age of presentation has become progressively younger with the development of biochemical and genetic screening studies.\textsuperscript{16} The mean age at diagnosis has become substantially younger with the development of biochemical and genetic screening studies.\textsuperscript{16}

\textsuperscript{a}Mendelian inheritance in man number.

\textsuperscript{b}The mean age at diagnosis has become substantially younger with the development of biochemical and genetic screening studies.

\textsuperscript{c}MEN2A is also known as Sipple syndrome.

\textsuperscript{d}MEN2B has been referred to as Wagenmann-Froboese syndrome.

Pathological Features

Medullary carcinomas vary in size from those that are barely visible to those that replace the entire lobe of the thyroid.\textsuperscript{1,2} The term “medullary microcarcinoma” has been used to describe those tumors measuring less than 1 cm in diameter (Figure 11.1).\textsuperscript{23} The tumors are generally sharply circumscribed but nonencapsulated. Occasionally, however, they may be surrounded by a fibrous capsule. On cross-section, the resected parathyroid glands typically show evidence of chief cell hyperplasia, which is indistinguishable from that observed in patients with other forms of familial or sporadic hyperplasia.

MEN2B is the most phenotypically distinctive type of the MEN2 syndromes\textsuperscript{16,24} and accounts for approximately 5% of the familial forms of MTC. A significant proportion of these cases represent de novo RET mutations. Affected patients have pheochromocytomas in 40–60% of cases, but parathyroid abnormalities, in contrast to MEN2A, are absent. Additionally, patients with MEN2B have neuromas of the tongue and/or ganglioneuromatosis of the gastrointestinal tract with or without a marfanoid habitus. The MTCs in this syndrome typically have an early onset and an aggressive clinical course. The studies of Leboulleux and coworkers indicate that the stage at diagnosis of MTC is the major prognostic factor in these patients.\textsuperscript{25}

Occasional kindreds with familial MTC syndromes may manifest thyroid tumors exclusively, and these patients are classified as familial MTC (FMTC).\textsuperscript{26} These patients typically present at the same age as those with nonfamilial MTCs. In fact, a significant proportion of patients presenting with apparent sporadic MTCs will prove to have FMTC on the basis of molecular analysis.\textsuperscript{27}
many of the tumors are tan to pink with a generally soft consistency. Others are quite firm and fibrotic with areas of granular yellow discoloration that represent focal calcifications. The smaller tumors generally occur at the junction of the upper and middle thirds of the lobes in which C-cells normally predominate. When the tumors become very large, they may replace the entire lobe and extend into the perithyroidal soft tissues and trachea. While sporadic tumors most commonly present as unilateral lesions, the heritable tumors characteristically involve both lobes of the gland and are typically multicentric.

Sporadic and familial medullary carcinomas typically exhibit a wide spectrum of histological patterns that may mimic other primary thyroid tumors, including follicular, papillary, and undifferentiated carcinomas. The prototypic medullary carcinoma has a lobular, trabecular, insular, or sheet-like growth pattern with evidence of focal extension of tumor into the adjacent normal thyroid (Figures 11.2 and 11.3). Individual tumor cells may be round, polygonal, or spindle shaped with frequent admixtures of these cell types (Figure 11.4). In the round and polygonal cells, the nuclei have coarsely clumped or speckled (salt and pepper) chromatin and generally inconspicuous nucleoli. Occasional nuclear pseudoinclusions similar to those seen in papillary carcinomas may be present. Binucleate cells are common and multinucleate giant cells may be evident. Spindle cells have elongated nuclei with chromatin distribution similar to that seen in the polygonal and round cells. Most MTCs exhibit moderate degrees of pleomorphism but mitotic activity is generally low.

The cytoplasm varies from eosinophilic to basophilic and appears finely granular. In some cases, however, the cytoplasm is clear. Occasional mucin positive cytoplasmic vacuoles may be present in some cases. Zaatari et al have found mucicarmine positive deposits in up to 40% of cases. In 17% of cases, mucicarmine positivity was present extracellularly, while in 8% the deposits were intracellular. Both intra- and extra-cellular mucin deposits were present in 17% of cases. Ultrastructurally, the tumors contain dense core secretory granules, in which calcitonin and other peptides are stored.9

Foci of necrosis and hemorrhage are uncommon in small tumors, while larger tumors exhibit these features more commonly. Lymphatic and vascular invasion may be seen at the advancing front of the tumor. In advanced cases, foci of lymphatic invasion may be present in the contralateral lobe. Occasionally, nodal and distant metastases may be present in association with tumors measuring less than 1 cm (Figures 11.5 and 11.6).

Some medullary thyroid carcinomas contain prominent areas of fibrosis (Figure 11.7). Stromal amyloid deposits are present in up to 80% of cases. The amyloid deposits are Congo red positive and show typical green birefringence in
polarized light, while crystal violet stained sections demonstrate metachromasia in the amyloid deposits. Ultrastructurally, the amyloid deposits have a fibrillar ultrastructure that is identical to that seen in other forms of amyloidosis. Antibodies to calcitonin demonstrate positive staining within the amyloid. Although earlier studies had suggested that the amyloid in these tumors was derived from the calcitonin precursor, more recent studies utilizing mass spectrometric analysis indicate that full length calcitonin is the sole constituent of amyloid in medullary carcinoma. In some instances, the amyloid may elicit a foreign body giant cell reaction. Calcification of the amyloid may occur and occasional tumors may contain psammoma bodies. A few medullary carcinomas may consist almost exclusively of amyloid and such cases may be difficult to distinguish from amyloid goiters (Figure 11.8).

Medullary carcinomas are typically argyrophilic with the Grimelius method with the most intensely positive cells having a dendritic morphology. The Masson Fontana stain is usually negative although occasional cases may contain scattered argentaffin positive cells.

In fine needle aspirates, medullary carcinomas are variably cellular depending on the amount of stromal fibrosis or amyloid deposition. Cells are usually present singly or in loosely cohesive groups with poorly defined cell margins (Figure 11.9). Aspirates typically appear pleomorphic and while some cells are small and round, others may be cuboidal, polyhedral, or spindle shaped. Occasional tumors may be dominated by one cell type. The nuclei tend to be located eccentrically within the cytoplasm, and this feature imparts a plasmacytoid appearance to the tumor cells. The chromatin is coarsely granular and nucleoli are usually small and inconspicuous. Occasional nuclear pseudoinclusions may be present.

The cytoplasm is generally pale and fibrillary in Papanicolaou stained preparations with occasional areas of process formation. In Wright–Giemsa stained slides, cytoplasmic granularity may be evident, and in some instances, the granules...
Medullary Thyroid Carcinoma appear metachromatic. Amyloid may be indistinguishable from colloid in Papanicolaou stains, but a Congo red stain can be performed to confirm the presence of amyloid. The diagnosis of medullary carcinoma should be confirmed with immunostains for calcitonin or chromogranin.

Medullary thyroid carcinomas demonstrate positivity for thyroid transcription factor-1 (TTF-1), a property that they share with tumors of follicular cell origin. Stains for thyroglobulin are typically negative, although entrapped normal thyroid follicles may stain positively. Medullary carcinomas are typically positive for low molecular weight cytokeratins and typically exhibit a cytokeratin 7 positive/cytokeratin 20 negative phenotype. Vimentin is variably present within the tumor cells, and some tumors contain subpopulations of neurofilament positive cells.

The tumors are typically positive for calcitonin (Figure 11.10) and a wide spectrum of generic neuroendocrine markers including chromogranin and synaptophysin. Chromogranin is a sensitive marker for medullary carcinoma and, in fact, may be more sensitive than calcitonin for the identification of this tumor type. Other products found in these tumors include calbindin-D28K and polysialic acid of NCAM.

Calcitonin is present in 80–90% of medullary carcinomas (Figure 11.10). Although many cases show extensive calcitonin staining throughout the tumor, others may show only focal and weak reactivity. The calcitonin gene related peptide is also commonly present. Some tumors which are negative for calcitonin peptide and the calcitonin gene related peptide usually give positive signals for calcitonin messenger RNA using in situ hybridization.

A variety of other peptides have been localized within these tumors, and their presence has been confirmed by radioimmunoassays of tumor extracts. Both somatostatin and gastrin releasing peptide are present in subpopulations of normal C-cells, and both of these peptides are commonly expressed in medullary carcinomas. Generally, somatostatin immunoreactivity is present in single cells or in small cell groups, which most often comprise less than 5% of the tumor cell population. The somatostatin positive cells often have a dendritic morphology with branching cell processes extending between the adjacent tumor cells. Somatostatin receptors are also commonly present within these tumors.

Other peptides that are commonly present include ACTH, leuenkephalin, neurotensin, substance P, vasoactive intestinal peptide, and chorionic gonadotropin. In rare instances, the tumors may contain subpopulations of cells immunoreactive for glucagon, gastrin, and insulin. Both catecholamines and serotonin may also be present.

Galectin-3 has been reported in 92% of heritable medullary thyroid carcinomas. The staining is focal in 26% of the carcinomas and diffuse in the remainder. Interestingly, galectin-3 is reduced or absent in nodal metastases, while foci of C-cell hyperplasia are negative for galectin-3.

CEA levels are typically increased in the plasma of patients with MTC and correlative immunohistochemical studies have demonstrated that nearly 100% of the tumors exhibit CEA positivity. Several groups have demonstrated that a subset of MTCs may lose the ability to synthesize and secrete calcitonin while maintaining their capacity for CEA synthesis. In fact, calcitonin negative areas in medullary carcinoma are frequently positive for CEA. The finding of decreasing levels of calcitonin in the face of increasing levels of CEA generally predicts an aggressive clinical course.

Numerous variants of medullary carcinoma have been described. Rarely, medullary carcinomas may exhibit a true papillary pattern in which the component tumor cells are aligned along fibrovascular stalks. In the cases described by Kakudo et al., the tumors also exhibited solid foci characteristic of typical medullary carcinoma. The presence of thyroglobulin serves to distinguish these tumors from papillary carcinomas of follicular origin. A pseudopapillary pattern is considerably more common and results from artifactual separation of groups of tumor cells with groups of tumor cells...
appearing to be attached to stromal elements of the tumor with intervening “empty” spaces (Figure 11.11).

The follicular variant is composed wholly or in part of follicular structures and may resemble follicular cell neoplasms. The tumor cells form follicles lined by cells that resemble those seen in the more solid areas of the tumor (Figure 11.12). The lumina of the follicles may appear empty or may contain an eosinophilic material resembling colloid. Although the origin of the colloid-like material is unknown, it most likely represents calcitonin and other secreted proteins. Immunostains for calcitonin have revealed variable degrees of reactivity within the colloid areas while stains for thyroglobulin are negative.

The small cell variant is characterized by the presence of cells with round to ovoid hyperchromatic nuclei and scant cytoplasm (Figure 11.13). Some of the tumors may resemble neuroblastoma. The presence of calcitonin, calcitonin gene related peptide, or other peptides (somatostatin, gastrin releasing peptide) is characteristic of C-cell differentiation in these cases. The small cell variant may exhibit a compact, trabecular or diffuse growth pattern. Mitotic activity may be high and foci of necrosis may be present. This variant may occur in a pure form or may be admixed with more typical foci of tumor. Of note, amyloid may be absent from the small cell variant. The tumor cells may be negative for calcitonin but stains for CEA are usually strongly positive.

Eusebi and coworkers have reported two cases of small cell thyroid carcinoma that were classified as apparent primary oat cell carcinomas. Both tumors were positive for chromogranin and synaptophysin but were negative for thyroglobulin and calcitonin by immunohistochemistry and for calcitonin messenger RNA by in situ hybridization. On the basis of these studies, Eusebi and coworkers concluded that such cases should be separated from small cell medullary carcinomas and should be classified as primary small (oat) cell carcinomas of the thyroid.

The giant cell variant is characterized by a predominance of multinucleate giant cells. Typically, the giant cell areas are admixed with foci of more typical medullary carcinoma. The tumor giant cells are typically positive for calcitonin.

The clear cell variant is characterized by cells with optically clear cytoplasm. Landon and Ordonez described a medullary carcinoma composed predominantly of large polygonal cells with clear cytoplasm (Figure 11.14). In other areas, the tumor was composed of spindle shaped cells with eosinophilic cytoplasm. The clear cells were negative for mucins and did not contain appreciable amounts of glycogen. The tumor stained positively for calcitonin and contained dense core secretory granules ultrastructurally.

The melanotic variant is composed of cells with varying amounts of melanin pigment (Figure 11.15). Marcus and coworkers described a case of nonfamilial medullary thyroid
Medullary Thyroid Carcinoma

A medullary thyroid carcinoma that contained collections of dendritic argentaffin positive cells. The dendritic cells were negative for calcitonin by immunohistochemistry while the remaining cells within the tumor were positive. Ultrastructurally, the argentaffin positive cells contained typical melanosomes. Beerman and coworkers demonstrated both melanosomes and calcitonin containing secretory granules within the same tumor cells.

The oncocytic variant is composed of cells with mitochondria rich cytoplasm. In the case reported by Dominguez-Malagon, 60–70% of the tumor cells were of the oncocytic type, while the remainder of the tumor had features of conventional medullary carcinoma. The tumor cells had a trabecular arrangement and were separated by an amyloid free fibrous stroma. At the ultrastructural level, the tumor cells contained numerous mitochondria and few membrane bound secretory granules. Tumor cells showed strong positivity for calcitonin, chromogranin, and CEA but were negative for thyroglobulin.

The squamous variant is characterized by the presence of cells with varying degrees of squamous metaplasia. The case reported by Dominguez-Malagon was calcitonin negative but showed diffuse positivity for neuron specific enolase and chromogranin and focal positivity for CEA. Focal deposits of stromal amyloid were present in this case. Schröder and coworkers noted foci of squamous metaplasia in metastatic medullary carcinoma.

The amphicrine variant is characterized by the presence of cells with varying degrees of mucinous change. Combined alcian blue and Grimelius stains revealed that approximately 5% of the tumor cells were both alcianophilic and argyrophilic. Ultrastructurally, the tumor cells contained both mucin deposits and membrane bound secretory granules.

Occasional medullary carcinomas may be encapsulated and may be arranged in a broad trabecular pattern with an amyloid negative hyalinized stroma (paraganglioma-like variant). Huss and Mendelsohn reported two such cases that presented as encapsulated neoplasms resembling hyalinizing trabecular adenoma. In contrast to the latter tumors, however, both cases were calcitonin positive. Rare examples of true paragangliomas have been reported within the thyroid.

Occasional medullary carcinomas may have pseudosarcomatous features and may be reminiscent of angiosarcomas. This variant has been described as the angiosarcoma-like or pseudoangiomatous variant.

Virtually all neoplasms of C-cells have been considered to represent carcinomas although occasional examples of C-cell adenomas have been reported. In 1988, Kodama and coworkers reported 2 cases of C-cell adenoma, each of which measured 4 cm in diameter. Both tumors were composed of cells that were fusiform to cuboidal with small elliptical nuclei. Neither tumor contained amyloid or foci of calcification. In contrast to most medullary carcinomas, stains for CEA were negative, and there was no evidence of increased CEA levels in the serum. Calcitonin levels were markedly elevated, and the tumor cells stained strongly for this peptide. The term “C-cell adenoma” has been suggested for completely encapsulated C-cell tumors; however, until more is known about the biology of these tumors, it is best to regard them as encapsulated medullary carcinomas.

The term medullary microcarcinoma has been used to describe those tumors measuring less than 1 cm in diameter (Figure 11.1). These tumors may exhibit nesting, trabecular, or diffuse growth patterns. Occasional microcarcinomas may have a microfollicular growth pattern resembling small follicular adenomas or adenomatous foci. Most of the reported cases have been incidental findings in thyroids removed for other reasons or in patients with nodular thyroid disease who have been screened for calcitonin abnormalities. However, occasional microcarcinomas may give rise to nodal and distant metastases. Microcarcinomas may occur sporadically or in association with the MEN2 syndromes.

Fig. 11.14. Medullary thyroid carcinoma. This tumor is composed of clear cells (Hematoxylin and eosin).

Fig. 11.15. Medullary thyroid carcinoma. This tumor is pigmented and contains melanin deposits in many of the cells (Hematoxylin and eosin).
Differential Diagnosis

Medullary carcinomas may mimic the entire spectrum of benign and malignant thyroid tumors. Papillary/pseudopapillary and other variants may have nuclear pseudoinclusions and psammoma bodies; however, they lack the nuclear clearing that is typically seen in papillary carcinomas. Additionally, medullary carcinomas are positive for chromogranin, calcitonin, and synaptophysin, while papillary carcinomas are negative for these markers. Since medullary carcinomas may contain follicles, they must be distinguished from follicular neoplasms, which are typically positive for thyroglobulin and negative for calcitonin, chromogranin, and synaptophysin. Poorly differentiated carcinomas of insular type are composed of nests and insulae of follicular cells and may contain microfollicles. These tumors are also positive for thyroglobulin and negative for calcitonin, chromogranin, and synaptophysin. Medullary carcinomas of spindle and giant cell types must be distinguished from undifferentiated carcinomas of follicular origin. The latter tumors are negative for neuroendocrine markers, calcitonin, thyroglobulin, and TTF-1.

The distinction of small cell medullary carcinoma from malignant lymphomas may be difficult, particularly in small biopsy or cytopathological samples. The demonstration of CD45 and other markers of T- and B-cells establishes the diagnosis of lymphoma. Small cell carcinoma of the lungs and other sites may metastasize to the thyroid and may mimic medullary carcinoma of small cell type. Since TTF-1 is expressed both in lung and thyroid tumors, the presence of this marker is not a useful discriminant. Moreover, calcitonin and generic neuroendocrine markers may be present in both tumor types. In these instances, careful clinical and radiological examination may be helpful in identifying a pulmonary primary. Since mucin may be present in medullary carcinomas, these tumors must also be distinguished from mucinous carcinomas that have metastasized to the thyroid. The demonstration of calcitonin in a mucinous tumor within the thyroid establishes its C-cell origin.

Oncocytic medullary carcinomas must be distinguished from oncocyctic tumors of follicular cell origin and oncocytic parathyroid neoplasms. The former will be positive for thyroglobulin, while the latter will be positive for parathyroid hormone. The hyalinizing trabecular tumor is typically encapsulated, as are some variants of medullary carcinoma. A trabecular pattern is present in both tumor types and both may exhibit areas of hyalinization resembling amyloid. However, the stroma of medullary carcinoma is positive for amyloid, whereas the stroma of hyalinizing trabecular tumors stains only for collagen. While medullary carcinomas stain positively for calcitonin and CEA, these markers are negative in hyalinizing trabecular tumors. It should be noted that occasional hyalinizing trabecular tumors may be positive for generic neuroendocrine markers.

Medullary carcinomas may be difficult to distinguish from paragangliomas. The latter tumors are positive for chromogranin and synaptophysin but are negative for calcitonin. A population of S-100 positive sustentacular cells is typically present in paragangliomas. Occasional intrathyroidal parathyroid adenomas also may be difficult to distinguish from medullary carcinomas. Parathyroid tumors are also positive for parathyroid hormone and chromogranin, but stains for calcitonin are negative and should serve to distinguish these tumor types.

Heritable Forms of Medullary Thyroid Carcinoma and C-cell Hyperplasia

The histopathological features of the tumors in patients with heritable medullary carcinoma are virtually indistinguishable from those occurring sporadically, except for their bilaterality and multifocality. Studies based on enhanced calcitonin secretory responses to calcium and pentagastrin in patients at risk for the development of heritable forms of these tumors established that the tumors were preceded in their development by C-cell hyperplasia (Figures 11.16 and 11.17). Detailed clinical and pathological studies have shown that C-cell hyperplasia is characterized by increased numbers of C-cells within follicular spaces. With further progression, C-cells fill and expand the follicles to produce foci of nodular hyperplasia. Transition of this phase of C-cell growth to medullary carcinoma is heralded at the light microscopic level by fine areas of fibrosis between the proliferating C-cells (Figures 11.16b and 11.17c). With further progression, there are increasing degrees of fibrosis between the groups of neoplastic C-cells (Figures 11.16c and 11.17d). At the ultrastructural level, the earliest feature of malignancy is characterized by the extension of intrafollicular C-cells through the follicular basement membranes into the interstitium of the gland. McDermott and coworkers confirmed these observations using an immunoperoxidase technique for the demonstration of type IV collagen in the follicular basement membranes.

The distinction of normal C-cell distribution from the earliest phases of C-cell hyperplasia is both difficult and controversial. Although initial studies suggested that the presence of 10 C-cells per low power field constituted sufficient evidence for C-cell hyperplasia, more recent studies indicate that this diagnosis should be made when there are at least 50 C-cells per low power field. In some regions of the lobes, particularly in the vicinity of solid cell nests, the numbers of C-cells may exceed 50 per low power field in occasional normal individuals. In a quantitative image analysis study, Guyetant and colleagues demonstrated that C-cells were more numerous in male patients than in females, correlating with the known higher plasma calcitonin levels in men than in women. Moreover, this study demonstrated the 33% of the adult population, including 15% of women and 41% of men, had evidence of CCH as defined by having at least three microscopic fields (100×) with greater than 50 C-cells.
Fig. 11.16. C-cell hyperplasia and medullary thyroid carcinoma in a patient with MEN2A. (a) C-cell hyperplasia. The central follicle is surrounded by a collar of C-cells. (b) Early medullary thyroid carcinoma arising in association with C-cell hyperplasia. The C-cells in the left portion of the field are surrounded by collagen which is indicative of early stromal invasion. (c) Microscopic focus of medullary thyroid carcinoma. The tumor cell nests are surrounded by a dense fibrous stroma (Hematoxylin and eosin).

Fig. 11.17. C-cell hyperplasia and medullary thyroid carcinoma in a patient with MEN2A. (a) Diffuse C-cell hyperplasia is characterized by an increased number of C-cells. (b) C-cell hyperplasia. The proliferation of C-cells around this follicle is eccentric. (c) Microscopic medullary thyroid carcinoma. The proliferating C-cells have elicited a mild stromal reaction. (d) Small medullary thyroid carcinoma. The tumor cell groups are separated by a prominent fibrous stroma (Immunoperoxidase stain for calcitonin).
These findings suggest that either a substantial portion of the population has CCH or that the criteria for the definition of this entity are inaccurate. Interestingly, abnormal pentagastrin tests are found in only approximately 5% of the normal population as compared to the 30% frequency of CCH, as defined histologically. These observations suggest that there may be considerable variation in C-cell distribution among normal individuals and that these variations may not be accompanied by hypercalcitoninemia.

In addition to its presence in MEN2 syndromes, CCH has been reported in a number of other conditions including hypercalcemia, hypergastrinemia, Hashimoto’s thyroiditis, and adjacent to a variety of follicular cell tumors, including follicular adenomas and carcinomas and papillary carcinomas (peritumoral C-cell hyperplasia) (Figure 11.19). This type of hyperplasia has been referred to as physiologic or secondary C-cell hyperplasia, in contrast to MEN2-associated CCH, which has been referred to as primary or “neoplastic” hyperplasia. Perry and coworkers have reported that physiologic CCH is primarily a diffuse process characterized by increased numbers of normal C-cells which can be diagnosed with confidence only on the basis of calcitonin immunostains.98 “Neoplastic” CCH, on the other hand, involves the proliferation of dysplastic C-cells and can be diagnosed frequently on the basis of H&E stains alone. While “neoplastic”/MEN2-associated CCH is generally regarded as a precursor of MTC, the neoplastic potential of physiologic CCH remains unknown, notwithstanding the rare cases of MTC reported in patients with chronic hypercalcemia.

Carney and coworkers in 1978 proposed that CCH associated with MEN2 syndromes represents a preinvasive carcinoma or carcinoma in situ.80 Further evidence for the neoplastic nature of CCH has come from Diaz-Cano’s molecular studies of microdissected foci of thyroid glands from patients with MEN2A.81 These studies have shown that foci of CCH are monoclonal with inactivation of the same allele in both thyroid lobes. Moreover, the foci have different secondary alterations involving the tumor suppressor genes p53, RB1, WT1, and NF1. These findings, together with the downregulation of apoptosis, are consistent with an intraepithelial neoplastic process and suggest that early clonal expansions precede migration of C-cell precursors into each thyroid lobe.

Although CCH has been regarded as a precursor and marker of MEN2 associated medullary carcinoma, this view has been challenged by several recent reports. In a series of 30 patients with nodular thyroid disease and abnormal pentagastrin stimulated calcitonin levels, Kaserer et al reported 19 patients (F:M;14:5) with MTC and 11 males but no females with CCH only, as defined by greater than 50 C-cells per low power field.82 Six of 16 patients with sporadic MTC had concomitant CCH, and three of these patients proved to be new MEN2 index cases, as proven by genetic studies. On the basis of these studies, Kaserer et al concluded that CCH was an unreliable marker for heritable MTC and that CCH had a preneoplastic potential in the absence of RET germline mutations.

In a second study of 16 familial and 34 sporadic MTCs, Kaserer et al noted CCH in 16/16 (100%) of familial cases and in 24/34 (71%) of sporadic cases.83 Among familial cases, CCH was of neoplastic type in 85% (14/16), nodular type in 6% (1/14), and focal type in 6% (1/14). In sporadic cases, on the other hand, neoplastic CCH was present in 18% (6/34), nodular hyperplasia in 21% (7/34), diffuse hyperplasia in 18% (6/34), and focal hyperplasia in 14% (5/34). There was no evidence of CCH in 29% (10/34) of the sporadic cases. On the basis of this study, Kaserer et al concluded that many sporadic MTCs develop on a background of CCH.83 Since there is such a wide variation in C-cell counts in normal thyroid glands, two possible interpretations of these
studies are that the observed “CCH” in sporadic tumor cases represents the upper limit of normal C-cell distribution or that it is analogous to the secondary CCH found adjacent to follicular and papillary tumors. Indeed, Mears and Díaz-Cano have reanalyzed the data in the Kaserer study.84 They concluded that multifocal and bilateral invasive MTCs associated with expansile intraepithelial neoplasia (nodular and neoplastic hyperplasia) represent familial disease in 98% of cases. The probability of sporadic disease associated with nodular and neoplastic C-cell hyperplasia was calculated as 1.87%.84

In summary, C-cell distribution has a remarkably wide variation in normal individuals. The current criterion of 50 C-cells per 100x microscopic field is, in all likelihood, an underestimate since up to 40% of normal males would have CCH according to this definition. MEN2-associated CCH has characteristic topographic, cytological, and molecular features, which permit its distinction from secondary C-cell hyperplasia in most cases, and this can be confirmed by the presence of germline RET mutations. Whether physiologic/secondary CCH has a significant preneoplastic potential remains an unanswered question.

### Molecular Pathology

A major breakthrough in the study of the molecular origins of familial MTC syndromes was the observation that the putative MEN2A gene mapped to the pericentromeric region of chromosome 10.85,86 The availability of a number of DNA markers to this region permitted the use of restriction length polymorphisms to identify carriers of this disorder.87,88 Subsequent studies were successful in mapping the MEN2A and 2B gene to the specific region of chromosome 10 (10q11.2) that contained the RET proto-oncogene and in demonstrating germline missense RET mutations in affected individuals.89–100

The RET (rearranged during transfection) proto-oncogene was discovered in 1985 by its ability to transform NIH 3T3 cells.101,102 The PTC oncogene, a naturally occurring RET rearrangement was subsequently discovered in 1990 in a papillary thyroid carcinoma (PTC).103 The PTC oncogene develops as a result of translocation involving the 31 area of RET, which contains the tyrosine kinase domain and the 51 area of several genes that are normally expressed in thyroid follicular cells. More than 10 different RET/PTC rearrangements have been identified to date.104–106 These rearrangements induce transformation in vitro and result in constitutive tyrosine kinase activation. There is a considerable variation in the frequency of RET rearrangements in papillary thyroid carcinomas in different geographic areas and in different age groups, ranging from 3 to 67%.106,107 At least, some of this variability may be related to different pathogenetic mechanisms for the development of these tumors. Studies of transgenic mice have demonstrated that thyroid targeted expression of the RET/PTC oncogene leads to the development of thyroid carcinoma with features of papillary carcinoma, while tissue culture studies have shown that RET/PTC has a direct role in the development of the nuclear features of these tumors.108,109

In addition to its role in the development of papillary thyroid carcinoma, RET is overexpressed in neuroblastomas, MTC, and pheochromocytomas.110,111

The RET proto-oncogene contains 21 exons and encodes a cell surface tyrosine kinase receptor with a cadherin ligand binding site and a cysteine-rich extracellular domain, a transmembrane region and two cytoplasmic tyrosine kinase domains.102,112 The cadherin binding site plays an important role in cell signaling, while the cysteine rich extracellular domain is important for receptor dimerization. RET is expressed in a variety of neural crest derivatives (C-cells, adrenal medulla, extra-adrenal paraganglia, sympathetic and enteric ganglia), parathyroid gland and urogenital tract.100,113 Mouse homozygous for a targeted RET mutation develop to term but demonstrate both renal agenesis or dysgenesis and absence of enteric neurons, but do not have evidence of neoplastic disorders.114

The ligands for RET are members of the glial cell line derived neurotrophic factor family and include glial cell line derived neurotrophic factor (GDNF) and neurturin (NTN).115–121 GDNF is a crucial survival factor for central and peripheral neurons and is required for the development of the kidney and enteric neurons.100 The receptors for GDNF family are membrane bound adaptor molecules and RET which form a trimeric receptor system. Glial derived neurotrophic factor binds GDNF Family Receptor alpha-1 (GDNFR-alpha), a glycosyl-phosphatidylinositol-linked protein, that is now known as GFR alpha-1, with high affinity while NTN binds GFR alpha-2. Additional members of the GFR alpha family include GFR-alpha 3 and -alpha 4.120 Persephin and artemin are additional GDNF-like neurotrophic factors. Upon activation of RET receptors, phosphorylation of intracellular tyrosines leads to the stimulation of downstream signaling pathways including the RAS/ERK, ERK5, p38MAPK, PLCγ, PI3-kinase, JNK, STAT, and SRC cascades.122

Activating germline mutations in the RET proto-oncogene resulting in aberrant activation of RET receptors have been characterized in MEN2A, MEN2B, and FMTC (Table 11.2).16,92–100 The mutations are of the missense type and affect primarily exons 10 and 11 with less frequent mutations affecting exons 13, 14, 15, and 16. Most of the mutations affect the cysteine rich extracellular domain of RET (MEN2A, FMTC). Codon 634 mutations account for approximately 80% of MEN2A patients while codon 609, 618, and 620 mutations account for more than 60% of FMTC cases and those patients with the Hirschsprung’s phenotype. In patients with MEN2A, the codon 634 mutations show a strong genotype–phenotype correlation with the development of pheochromocytoma and hyperparathyroidism.98 Substitution of cysteine with several other amino acids increases the formation of active RET dimers resulting
in their transforming potential. Mutations affecting codons 768 or 804 (tyrosine kinase domain) may also occur (Table 11.2).

Mutations affecting the tyrosine kinase domain (codon 918) are found in patients with MEN2B and in a subset of sporadic medullary carcinomas. This mutation results in the replacement of methionine with threonine. Codon 883 mutations resulting in the substitution of phenylalanine for alanine have also been demonstrated in a small subset of patients with MEN2B. The MEN2B mutations result in RET activation in its monomeric form. These mutations are present in the catalytic core region of the tyrosine kinase domain, and it has been suggested that they induce a conformational change in this region leading to an increased tyrosine kinase activity or alteration in its substrate specificity. Mutations commonly detected in MEN2A and 2B have high transforming activities for NIH 3T3 cells, suggesting a metastatic clone. Somatic mutations in codons 630, 634, 676, 766, 768, 804, and 833 have been observed considerably less commonly than codon 918 mutations in sporadic medullary carcinomas.

In a survey of prophylactic thyroidectomies in 75 children and adolescents with proven RET mutations, MTC was found in 61% and C-cell hyperplasia alone was found in 39%. Three patients had nodal metastases. Testing for RET mutations is now the standard of care for patients with MEN2. For patients with MEN2A, a prophylactic thyroidectomy is recommended between the ages of 5 and 10 years. For patients with MEN2B, thyroidectomy before the age of 3 or earlier is recommended. The therapeutic strategy for those patients with mutations associated with FMTC remains to be determined.

The frequency of RET germline mutations in cases of apparent sporadic MTC has been assessed in several studies. In one study, 6 of 101 individuals harbored mutations involving codons 609, 611, 618, or 634. Four of the affected patients were the probands of previously unrecognized kindreds, while the remaining two patients were examples of de novo mutations. Combining the results of several studies, it has been estimated that approximately 7% of patients with apparent sporadic tumors have germline mutations. These findings indicate that analysis of RET should be performed in all patients presenting with apparent sporadic medullary carcinoma.

Somatic mutations in codon 918 have been observed in up to 70% of sporadic MTCs. This mutation, which is identical to the 918 germ line mutation in MEN2B, results in the substitution of threonine for methionine. Although Zedenius et al. and Eng et al. have suggested that sporadic tumors with this mutation pursue a more aggressive course, this point has been controversial. The studies of Eng et al. have further suggested that the codon 918 mutation can arise as an event in tumor progression within a single tumor or in a metastatic clone. Somatic mutations in codons 630, 634, 766, 768, 804, and 833 have been observed considerably less commonly than codon 918 mutations in sporadic medullary carcinomas.

The relationship of physiological/secondary CCH to the development of nonfamilial MTC is controversial. Recent studies based on laser capture microdissection studies of 24 cases of CCH either isolated or associated with nonfamilial MTC failed to show RET mutations in foci of CCH, despite the presence of codon 918 mutations in 3 concomitant MTCs.

RET mutations involving codons 620, 630, 634, and 918 have been found in 10–20% of sporadic pheochromocytomas. In contrast, RET mutations have not been detected in parathyroid hyperplasias or adenomas, pituitary adenomas, pancreatic endocrine tumors, carcinoids, or neuroblastomas.

RET mutations are also responsible for up to 40% of the autosomal dominant forms of Hirschsprung’s disease and a subset of patients with sporadic disease. Mutations are scattered throughout the gene and result in the loss of function of the RET protein. Rarely, FMTC or MEN2A may be present in association with Hirschsprung’s disease, and

### Table 11.2. RET mutations in MEN2 syndromes.

<table>
<thead>
<tr>
<th>Site</th>
<th>Codon</th>
<th>Syndrome</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysteine-rich domain</td>
<td>609</td>
<td>MEN2A, FMTC</td>
<td>0–1</td>
</tr>
<tr>
<td></td>
<td>611</td>
<td>MEN2A, FMTC</td>
<td>2–3</td>
</tr>
<tr>
<td></td>
<td>618</td>
<td>MEN2A, FMTC</td>
<td>3–5</td>
</tr>
<tr>
<td></td>
<td>620</td>
<td>MEN2A, FMTC</td>
<td>6–8</td>
</tr>
<tr>
<td></td>
<td>630</td>
<td>MEN2A, FMTC</td>
<td>0–1</td>
</tr>
<tr>
<td></td>
<td>634</td>
<td>MEN2A, FMTC</td>
<td>80–90</td>
</tr>
<tr>
<td>Transmembrane domain</td>
<td>768</td>
<td>FTMC</td>
<td>Rare</td>
</tr>
<tr>
<td></td>
<td>790</td>
<td>MEN2A</td>
<td>Rare</td>
</tr>
<tr>
<td></td>
<td>791</td>
<td>FTMC</td>
<td>Rare</td>
</tr>
<tr>
<td></td>
<td>804</td>
<td>MEN2A/FMTC</td>
<td>0–1</td>
</tr>
<tr>
<td></td>
<td>844</td>
<td>FMTC</td>
<td>Rare</td>
</tr>
<tr>
<td>Tyrosine kinase domain</td>
<td>883</td>
<td>MEN2B</td>
<td>Rare</td>
</tr>
<tr>
<td></td>
<td>891</td>
<td>FMTC</td>
<td>Rare</td>
</tr>
<tr>
<td></td>
<td>918</td>
<td>MEN2B</td>
<td>3–5</td>
</tr>
<tr>
<td></td>
<td>922</td>
<td>MEN2B</td>
<td>Rare</td>
</tr>
</tbody>
</table>

R.A. DeLellis
in 5 kindreds with this association, mutations in codons 618 and 620 were found.\textsuperscript{146,147} In addition to RET, a variety of other genes including GDNF, NTN, endothelin-B and endothelin-B receptor have been implicated in the pathogenesis of Hirschsprung’s disease.

Treatment and Prognosis

Surgical treatment of patients with medullary thyroid carcinoma is guided by the pattern of spread of these tumors, which includes central compartment lymph nodes followed by ipsilateral and then contralateral cervical lymph nodes.\textsuperscript{146,147} Approximately 50% of patients with palpable primary tumors, in fact, will have evidence of cervical nodal metastases. Both sporadic and heritable MTCs, therefore, are generally treated by total thyroidectomy with central lymph node dissection. In those patients with palpable primary tumors, some surgeons advocate the use of ipsilateral and even contralateral cervical lymph node dissections to optimize the chances for complete local control. Postoperative reduction of basal and stimulated calcitonin levels as well as other biomarkers is highly effective in providing information on biochemical care.

Radioactive iodine, external beam radiation therapy, and conventional chemotherapy generally have not been effective in the treatment of patients with recurrent or metastatic disease. Newer approaches include the use of tyrosine kinase inhibitors that target RET in addition to vascular endothelial growth factor receptors and additional kinase inhibitors in patients with advanced tumors.\textsuperscript{148} The challenge for investigators is in analyzing the effect to which RET is being inhibited and correlating these findings with a variety of biomarkers indicative of disease status and outcome data.\textsuperscript{148} Approaches for RET negative tumors include the use of angiogenesis inhibitors, proteasome inhibitors, and cytotoxic chemotherapy in combination with tyrosine kinase or angiogenesis inhibitors.

The survival of patients with these tumors is strongly correlated with stage.\textsuperscript{149} In general, surgery provides cure in nearly all patients whose tumors measure less than a few millimeters in diameter, in 90% when tumors are less than 1 cm and in 50% with tumors measuring more than 1 cm.\textsuperscript{150} Overall, 10-year survival rates range from 75 to 85%. In Kebebew’s series of 104 patients with sporadic and heritable MTC, 49.4% of the patients were cured, 12.3% had recurrent tumor, and 38.3% had persistent tumor.\textsuperscript{17} The mean follow-up time was 8.6 years with 10.7% and 13.5% cause specific mortalities at 5 and 10 years, respectively. In this series, 32% of the patients with heritable tumors were diagnosed by genetic or biochemical screening studies. These patients had a lower incidence of cervical node metastases than patients with sporadic tumors, and 94.7% were cured at last follow-up. Patients presenting with systemic symptoms of diarrhea, bone pain, and flushing had widespread metastatic disease, and one third of these patients died within 5 years.

As with other thyroid tumors, patient age (greater than 50 years) and stage are the most important prognostic factors. In the study reported by Kebebew et al, age and stage were the only two independent prognostic factors.\textsuperscript{17} Male sex approached statistical significance in univariate analyses and most likely represents a minor risk factor. While patients with cervical lymph node metastases were more likely to have recurrent or persistent disease, this parameter was not associated with a significantly higher mortality.

The type of MTC (sporadic MTC, FMTC, MEN2A, MEN2B) has been reported to be an important prognostic factor in some studies. However, when controlling for stage or in multivariate analyses, the subtype of MTC does not appear to be an important prognostic factor.\textsuperscript{25} Numerous histological and immunohistochemical parameters, including cellular composition (spindle cell vs. round cell), pleomorphism, extent of amyloid deposition, immunohistochemical features, and extent of calcitonin staining, have been examined as potential prognostic parameters, and none has proven to be significant in multivariate analyses.\textsuperscript{151} Koperek et al have demonstrated that desmoplasia is a reliable and reproducible parameter to predict nodal metastatic potential.\textsuperscript{152} However, additional studies will be required to confirm this hypothesis.

Both calcitonin and CEA serum doubling times have been used as prognostic indices in patients with MTC.\textsuperscript{153} When calcitonin doubling time was less than 6 months, 5 and 10-year survivals were 25% and 8%, respectively, while doubling times between 6 months and 2 years were associated with 5 and 10-year survivals of 92% and 37%, respectively. In contrast, patients with calcitonin doubling times of greater than 2 years were all alive at the end of the study.

The presence of somatic RET mutation, particularly involving codon 918, has been linked to poor prognosis.\textsuperscript{136,137} Elisei and coworkers have shown that somatic RET mutations involving codon 918 were more frequent in large tumors and in those tumors with nodal and distant metastases.\textsuperscript{154} Among all prognostic factors found to be correlated with a worse outcome, only advanced stage at diagnosis and the presence of RET mutations showed an independent correlation ($p<0.0001$ and $p=0.01$, respectively). Survival curves of MTC patients showed a significantly lower percentage of surviving patients in the group with RET mutations ($p=0.006$).

Mixed Medullary and Follicular Carcinomas

Tumors with evidence of follicular cell and neuroendocrine differentiation span a spectrum that includes mixed medullary and follicular/papillary carcinomas, medullary carcinoma with thyroglobulin immunoreactivity, and thyroglobulin positive tumors that coexpress neuroendocrine markers.\textsuperscript{155-170} Among the latter group are occasional poorly differentiated (insular) carcinomas, mucopidermoid tumors, and hyalinizing trabecular tumors.\textsuperscript{171-173} For example, poorly differentiated
carcinomas of the insular type may coexpress thyroglobulin and somatostatin while hyalineizing trabecular tumors may stain positively for neuron specific enolase, chromogranin A, neurotensin, or somatostatin and may contain electron dense neuroendocrine type granules.\textsuperscript{171,172}

Mixed medullary and follicular (or papillary) carcinomas represent a rare and controversial entity. They account for 0.13–0.15% of all thyroid tumors and most cases have been sporadic; however, rare examples of familial mixed tumors have also been reported.\textsuperscript{170} The median age is 48 years and the mean size of reported tumors is 3.7 cm. Nodal metastases have been reported in nearly 75% of cases. According to the of WHO Classification of Endocrine Tumours, mixed tumors are defined as “showing the morphological features of both medullary carcinoma together with immunoreactive calcitonin and the morphological features of follicular (or papillary) carcinomas with immunoreactive thyroglobulin”.\textsuperscript{174}

Although this definition may seem relatively straightforward, the distinction of true thyroglobulin immunoreactivity from passively absorbed or phagocyctosed thyroglobulin by tumor cells is often difficult. For example, positive staining for thyroglobulin was observed in 65% primary MTCs, particularly at the junctions of tumor with normal thyroid, but in none of 8 lymph node metastases in one case series.\textsuperscript{53}

The first documented case of a true mixed medullary and follicular carcinoma was reported in 1982.\textsuperscript{159} Both thyroglobulin and calcitonin were present both in the primary tumor and in its nodal metastases. Ljungberg and coworkers described a group of differentiated thyroid carcinomas with morphological and immunohistochemical evidence of follicular cell (positivity for thyroglobulin) and C-cell differentiation (positivity for calcitonin, neurotensin or somatostatin).\textsuperscript{157,158} These workers concluded that differentiated thyroid carcinomas could be regarded as a spectrum of tumor types with pure follicular carcinomas and pure medullary carcinomas representing the two ends of the spectrum, while tumors with biphasic features represented a broad intermediate group.

In a series of 14 cases of thyroglobulin and calcitonin positive MTCs, thyroglobulin and calcitonin were colocalized in the same neoplastic cells in 8 cases using a double immunolabeling procedure.\textsuperscript{159} Three of the 8 cases also contained the calcitonin gene related peptide. On the basis of these observations, Holm and coworkers concluded that a common stem cell was the most likely origin of these tumors.\textsuperscript{159} Alternative explanations have suggested that mixed follicular and medullary carcinomas represent collision tumors.\textsuperscript{160} Using in situ hybridization and immunohistochemistry, Papotti et al demonstrated that most mixed tumors had separate patterns of calcitonin and thyroglobulin gene expression although rare cells with concurrent calcitonin and thyroglobulin expression were found in 2 of 11 cases of their series.\textsuperscript{170}

Tumors with mixed papillary and medullary components have also been described. The phenotypic features of the medullary and papillary component are distinct.\textsuperscript{161} The papillary components are thyroglobulin positive but are negative for calcitonin and CEA. The medullary components, on the other hand, are calcitonin and CEA positive but negative for thyroglobulin. Both components have been recognized in the primary tumors and in metastatic sites. The existence of such tumors has raised the same histogenetic issues as those of the mixed follicular and medullary carcinomas.

Volante and coworkers have utilized molecular approaches to study a series of 12 mixed medullary and follicular/papillary carcinomas. Following laser capture microdissection, cases were analyzed for mutations in the RET proto-oncogene and allelic losses of nine loci of six chromosomes together with studies of clonality based on the analysis of the androgen receptor gene. These studies supported the view that the two components were derived from different cells since the components of 7 cases consistently showed differences in patterns of RET mutations, allelic losses, and clonal composition. According to their “hostage” hypothesis, Volante et al postulated that entrapped nonneoplastic follicles were stimulated by medullary carcinoma derived trophic factors leading to hyperplastic follicular foci. The nature of the trophic factors, however, remains to be determined. Subsequently, acquired genetic defects in the follicular cells lead to their neoplastic transformation and the development of papillary or follicular carcinoma components that have the capacity to metastasize. These studies have also demonstrated that a subset of “mixed tumors” is composed of a medullary carcinoma containing hyperplastic follicles, based on the fact that such follicles are often polyclonal or oligoclonal.

Rarely, families with RET point mutations develop both MTC and papillary thyroid carcinoma (PTC). These observations suggest that under certain circumstances RET point mutations rather than rearrangements can drive the development of papillary thyroid carcinoma. The mutant RET found in such families scored constitutive kinase activity and mitogenic effects for cultured thyroid follicular cells (PCC13) although at a significantly lower level than RET/PTC1.\textsuperscript{173} These findings indicate that RET point mutants can behave as dominant oncogenes for thyroid follicular cells under some circumstances.

It is likely that mixed medullary and follicular/papillary carcinomas represent a heterogeneous group.\textsuperscript{168} In addition to those tumors explained by the “hostage” hypothesis, some mixed tumors may represent collision tumors, while a subset may represent medullary carcinomas with thyroglobulin-expressing cells that might be derived from a common stem cell.\textsuperscript{168,170}

References


11. Medullary Thyroid Carcinoma


Introduction

There are a number of tumors of the thyroid gland that do not fit into the conventional categories of follicular and C-cell derived lesions. These tumors tend to be very rare, and the literature regarding molecular alterations in these lesions is sparse. However, as the pathologist may encounter these tumors rarely in practice, they will be reviewed in this chapter.

Mucoepidermoid Carcinoma

Mucoepidermoid carcinoma (MEC) of the thyroid gland is a rare tumor that represents less than 0.5% of all thyroid gland malignancies. The patients affected by this tumor have a broad age range, but they are more common in women than in men. There are no clinical features which will distinguish this tumor from other more typical thyroid gland lesions.

Histologically, MEC has a unique appearance, but with features similar to salivary gland derived MEC. The cellular components include both epidermoid type cells and mucin containing cells. The tumor cells grow in compact nests and clusters that may be surrounded by dense fibrosis. The glandular spaces are lined by mucinous cells that can be either cuboidal or can have goblet cell features. One case has been described that demonstrated ciliated cells as well.

Thyroid MECs usually stain positive for TTF-1 and thyroglobulin, at least focally. They will also often stain positive for CEA, and they are negative for calcitonin.

At the molecular level, thyroid MEC has only been rarely studied for molecular mutations. One study of salivary gland MECs also included three cases of thyroid MEC in analyzing the CRTR1/MAML2 (also known as MECT1/MAML2) translocation. This translocation has been identified in approximately 50–60% of salivary gland derived MECs, with higher rates seen in lower grade lesions. Interestingly, one of the three thyroid MECs was reportedly positive for the translocation.

Sclerosing Mucoepidermoid Carcinoma with Eosinophilia

Sclerosing mucoepidermoid carcinoma with eosinophilia (SMECE) is another very rare tumor of the thyroid gland that remains incompletely understood. Patients with these tumors tend to present with a mass lesion. The average age at presentation is around 52 years of age, and there is a strong female predominance. Approximately half of the patients with SMECE will develop recurrent disease or distant metastases.

Most cases of SMECE occur in a background of chronic lymphocytic thyroiditis, particularly those cases with fibrosis (so-called fibrosing Hashimoto’s thyroiditis). At low power, the tumor cells grow in small nests and islands, anastomosing cords, and narrow strands. There are four populations of cells that can be identified. There is a squamoid component that demonstrates typical intracellular bridges and can have squamous pearl formation. The glandular component has mucous cells that occur either individually or as part of mucinous cysts. There can also be a population of clear cells that are usually found to be rich in glycogen. Finally, there are abundant inflammatory cells in the background of these tumors, including eosinophils, lymphocytes, and plasma cells. SMECE can occasionally be associated with a conventional papillary carcinoma.

Immunohistochemical staining for SMECE demonstrates uniform positivity for cytokeratin and variable positivity for TTF-1. p63 has been shown to be positive in these tumors. Thyroglobulin is usually negative and calcitonin is always negative. SMECE has not been studied at the molecular level.

Hyalinizing Trabecular Tumor

Hyalinizing trabecular tumor (HTT) is an unusual lesion in the thyroid that has been the subject of some controversy over the past decade and has had several different names.
The tumor was initially described as a paraganglioma-like adenoma of the thyroid because of the unique histologic appearance, but then assumed the more commonly used name of hyalinizing trabecular adenoma. Because of controversy regarding whether these lesions are variants of papillary carcinoma, many pathologists have adopted the terminology of “hyalinizing trabecular tumor” or “hyalinizing trabecular neoplasm”. Despite this cautiousness, a recent study of 119 lesions demonstrated that 118 were typical hyalinizing trabecular lesions and no cases had recurrence, metastasis or poor outcome with good long-term follow-up.

HTTs have a characteristic morphology. The growth pattern is strictly trabecular and should not include any follicular growth. There is usually deposition of an eosinophilic extracellular material that stains with collagen type IV and laminin; this can be located central to radiating trabeculae or can be deposited in the stromal compartment of the tumors. Because of the growth pattern and the eosinophilic hyaline material, medullary carcinoma should be ruled out with negative calcitonin and positive thyroglobulin stains before making the diagnosis of HTT. They nuclei can have a resemblance to those seen in papillary carcinoma, with intranuclear inclusions, enlargement, clearing, and grooves. In fact, this lesion can represent a major pitfall in cytology. Inclusions in the cytoplasm have been described (“yellow bodies”) that have a refractile nature and represent giant lysosomes on ultrastructural studies; these have also been seen in papillary carcinomas. HTT has the immunohistochemical profile of a follicular derived process, with positive staining with TTF-1 and thyroglobulin stains and negative staining with calcitonin; chromogranin, and NSE can also be positive. One study demonstrated negative cytokeratin-19 staining in HTT and while all papillary carcinoma were positive, though another study found the lesions to be positive for CK19. Another study demonstrated galectin-3 staining in 40% of HTTs, while 83% of papillary carcinomas were positive. True paragangliomas of the thyroid gland are quite rare; they have a refractile nature and represent giant lysosomes on electron microscopy.

Carcinoma with Thymus-Like Elements

This very rare tumor has only been reported in about 30 cases in the literature. CASTLE tends to occur in adults, particularly in the fifth decade. Many of these tumors are slow growing and indolent, but occasional aggressive tumors have also been reported. Grossly, these tumors have a lobular appearance, with multiple lobules of tumor surrounded by fibrous bands. Histologically, the tumor has a unique appearance that can include squamous-like differentiation or features that resemble lymphoepithelial carcinoma or basaloid squamous cell carcinoma. In the lymphoepithelial type of pattern, there are vesicular nuclei growing in a syncytial pattern with variable lymphoplasmacytic infiltrates. The tumor cells are negative for thyroglobulin and calcitonin, and may be positive with markers for thymic differentiation, such as CD5.

Spindle Epithelial Tumor with Thymus-Like Differentiation

SETTLE tumors usually arise in young patients, often in children, with the average age of cases reported in the literature is 18 years. Most of these tumors are reported to have a relatively benign clinical course, but up to 17% of cases are reported with metastases, and 13% of patients are reported to die from disease. The tumors are usually biphasic in nature, with the two components including a cellular spindle cell component that is composed of elongated cells with some deposition of fibrous tissue. The second component, which can merge indistinguishably with the spindle cell component, has a characteristic appearance that includes large nuclei, vesicular nuclei, and mitotic activity.

Tumors with Thymus-Like Differentiation

Two types of tumors of the thyroid gland have been reported to have thymus-like differentiation: carcinoma with thymus-like elements (CASTLE) and spindle epithelial tumor with thymus-like differentiation (SETTLE). Both of these tumors are extremely rare, with very few cases having been reported in the literature. Therefore, experience with these lesions is quite limited and represents the result of examination of only case reports. SETTLE usually is reported to have a reasonably good prognosis, though there are several case reports of patients who have metastases. It has been postulated that these tumors are derived from remnants of thymus within the thyroid gland, or possibly from remnants of the ultimobranchial body.

Because of histologic overlap with and coexistence of HTT and papillary carcinoma, some investigators have studied the mutational profiles of HTT to investigate genetic similarities with papillary carcinoma. In several series, between 30% and 66% of HTTs have proven to have the RET-PTC translocation that is characteristic of papillary carcinoma. The presence of this mutation in HTTs has been used as evidence that HTT is directly related to papillary carcinoma. Interestingly, recent studies of BRAF mutations in HTT has shown that these tumors do not harbor the characteristic point mutations that is seen in papillary carcinomas. Additionally, RAS mutations have not been identified in HTTs.
component, is a glandular or epithelial component. These glandular cells can even be mucinous in nature, though more commonly they consist of non-descript tubules and cystic spaces. Sometimes respiratory type epithelium can be seen. In other cases, well developed epithelial cells are not seen and are termed “monophasic”, but collections of intracellular fluid and cystic mucinous areas may be seen even in these predominantly spindle cell tumors. There is usually low mitotic activity.

At the IHC level, SETTLE is usually negative for thyroglobulin and TTF-1, and should always be negative for calcitonin and CEA. Similar to thymomas, these tumors are positive for cytokeratins and bcl-2. SMA and vimentin have also been reported to be positive. Electron microscopy demonstrates that the tumor has prominent desmosomes and tonofilament bundles, which are seen in thymomas as well. Although this tumor has been postulated to also arise from thymus inclusions or other thymic derivatives, this origin has been disputed since CD5 and CD20, both of which can be expressed in some thymic lesions, are negative in SETTLE.

The molecular mutational profile of these tumors has been largely unexplored. One case has been investigated at the molecular level and was positive for a KRAS mutation and negative for p53 mutations. The differential diagnosis for SETTLE includes other spindle cell neoplasms, including most importantly medullary carcinoma, synovial sarcoma, and anaplastic carcinoma. One recent study used fluorescent in situ hybridization to study a series of 11 cases of SETTLE and 2 unresolved cases for the SS18/SSX1 and SS18/SSX2 translocations that is seen in synovial sarcoma. All 11 of the SETTLE cases were negative for the translocation and the 2 other cases were positive, suggesting that they were true synovial sarcomas of thyroid origin.

References


Introduction

Primary lymphoma of the thyroid gland is a relatively uncommon disease. The term “primary thyroid lymphoma” (PTL) is a nonspecific, broad term referring to any lymphoma restricted to the thyroid gland. Further classification of PTL is clinically necessary and should be based on histologic, phenotypic, and molecular features using the current World Health Organization (WHO) classification system. Both Hodgkin and many types of non-Hodgkin lymphomas can occur as a PTL. However, the most common types of PTL are extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphomas) and diffuse large B-cell lymphomas, NOS (DLBCL).

The molecular genetic changes of PTL vary with the type of lymphoma. In many cases, it is unclear whether PTL are truly distinct entities from their systemic counterparts. For example, follicular lymphomas of the thyroid are identical to systemic follicular lymphomas histologically and phenotypically, and even show identical molecular abnormalities (i.e., IGH/BCL2 translocations). Therefore, in many instances, the molecular genetics of the PTL are simply that of the systemic counterpart of the particular subtype.

MALT lymphomas and DLBCL of the thyroid, although histologically and phenotypically indistinguishable from their counterparts, at other sites show distinct molecular pathogenesis from MALT lymphomas and DLBCL of other sites. The association with chronic lymphocytic thyroiditis has long been established by epidemiologic studies. Recent studies have implicated numerous molecular lesions and pathways, including proliferation and antiapoptotic targets, in the pathogenesis and progression of chronic lymphocytic thyroiditis to MALT lymphoma in the thyroid. However, the data is currently still limited. In this chapter, we review, specifically, MALT lymphomas and DLBCL of the thyroid.

Background

PTL is an uncommon disease accounting for approximately 1–5% of all primary thyroid neoplasms\textsuperscript{1,2} and 2–7% of extranodal lymphomas.\textsuperscript{1,3} Characteristically, PTL presents in older females (M:F 2.5–30, median age 59.5–66.3 years) most commonly as a thyroid mass.\textsuperscript{4,6} Thyroid dysfunction can be present especially with concurrent chronic lymphocytic thyroiditis, which has been observed in a third to >90% of the cases.\textsuperscript{4,5} At presentation, PTL are most commonly low stage (IE or IIE) and demonstrate a relatively good response to combined modality therapy (CMT) including surgical, radiation, and/or chemotherapy.\textsuperscript{7–9} Localized PTL treated with CMT has overall good 5-year survival ranging from 70 to 90%.\textsuperscript{7–9} DLBCL and high grade histologic subtypes show a relatively worse prognosis when compared to MALT lymphomas and low grade histologic subtypes.\textsuperscript{4–6,10} In PTL with mixed histologic types, especially DLBCL and MALT lymphomas, the prognosis is dictated by high grade histology.\textsuperscript{4,6} Of the prognostic factors, the histologic type, stage, and patient performance status are consistently significant.

Although almost all histologic types of lymphomas, including Burkitt lymphoma,\textsuperscript{5} follicular lymphoma,\textsuperscript{4,5} Hodgkin lymphomas,\textsuperscript{5,11} and rarely T-cell lymphomas have been reported in the literature as a PTL, the vast majority (70–90%) of PTL are of the DLBCL, MALT lymphoma, or combined features of DLBCL and MALT lymphoma histology.\textsuperscript{4,5}

Histologically, DLBCL and MALT lymphomas of the thyroid are indistinguishable from lymphomas of the same histologic type involving other sites. Although by no means specific, morphologic features including prominent, destructive lymphoid lesions, prominent plasmacytic differentiation, and lymphocyte “stuffing” of the glandular lumen can be seen in MALT lymphomas involving the thyroid.\textsuperscript{12} Generally, MALT lymphomas of the thyroid show a nodular lymphoid infiltrate of predominantly small lymphocytes.
with slightly irregular nuclei, mature chromatin, and moderate clear cytoplasm, so-called “monocytoid” morphology (Figure 13.1). Although it is not unusual for plasma cell differentiation or plasmacytoid morphology to be present, MALT lymphomas can rarely show extreme plasma cell differentiation, mimicking an extramedullary plasmacytoma. In cases with prominent plasmacytic differentiation, Dutcher bodies and cytoplasmic immunoglobulin accumulations can be seen (Figure 13.2). Thus, diagnosis of plasmacytoma at this site should only be made after exclusion of even a minor component of MALT lymphoma and should be reserved for resection specimens.

DLBCL of the thyroid can be seen concurrently with MALT lymphomas (Figure 13.3) as single or multiple foci, or

Fig. 13.1. Low grade MALT lymphoma of the thyroid. (a) Lymphoid proliferation showing destructive infiltration of thyroid follicular architecture (H&E, ×100). (b) The lymphoid proliferation consists of small lymphocytes with irregular nuclei, mature chromatin, and scant to moderate clear cytoplasm, consistent with monocytoid morphology. Occasional scattered larger lymphocytes can be present (H&E, ×200).

Fig. 13.2. Low grade MALT lymphoma of the thyroid with prominent plasma cell differentiation. (a) Lymphoid aggregates of small lymphocytes with prominent plasma cell proliferation. No identifiable residual thyroid architecture is present (H&E, ×100). (b) There is a distinct interface between the lymphocytes and plasma cells, but gradual interfaces with plasmacytoid lymphocytes can also be seen (H&E, ×400). (c) Prominent plasma cell differentiation can mimic plasma cell neoplasms. Dutcher bodies are present (H&E, ×400). (d, e) Plasma cell differentiation can be highlighted with immunohistochemistry for kappa and lambda light chains. The plasma cells in this case are kappa light chain restricted (d, kappa IHC; e, lambda IHC).
as the predominant histologic type. Cytologically, DLBCL of the thyroid are large transformed cells with irregular nuclei, vesicular chromatin, prominent nucleoli, and scant cytoplasm (Figure 13.3). Morphologic variants including centroblastic, immunoblastic, and anaplastic can be seen either focally or as the predominant histology. Focally, Burkitt morphology with intermediate-sized cells and regular to slightly irregular nuclei, fine chromatin, and scant basophilic cytoplasm can be present. When large areas are present, DLBCL need to be differentiated from Burkitt lymphoma, which can rarely present involving thyroid (Figure 13.4). Burkitt lymphomas should show the characteristic phenotype (CD20+, CD10+, BCL-2−, and Ki-67 proliferation index >95%) with translocation of the MYC gene and the absence of other translocations (i.e., BCL2 or BCL6). Lymphoepithelial lesions are frequently present especially in cases with concurrent areas of MALT lymphoma. In some cases, large transformed cells are seen infiltrating epithelium and glands (Figure 13.5).

DLBCL, MALT lymphoma, and combined DLBCL and MALT lymphomas of the thyroid will all frequently show concurrent chronic lymphocytic thyroiditis. In these cases, follicular hyperplasia with or without colonization by neoplastic cells can be present. In some cases, the follicular hyperplasia can be so extensive as to mimic follicular lymphoma.

Morphology and immunophenotyping are the diagnostic mainstays for PTL. Flow cytometric analysis is the preferred method for phenotyping, but is limited by availability and the requirement for fresh tissue. In the absence of fresh samples, immunohistochemical staining using formalin fixed, paraffin embedded tissue can be used as an alternative. Primary thyroid DLBCL and MALT lymphomas are phenotypically similar to DLBCL and extranodal MALT lymphomas of other sites. Table 13.1 shows the characteristic phenotype of the various common histologic types of PTL.

Genetics

The molecular pathogenesis of PTL, as previously mentioned, is dependent on the specific histologic and phenotypic subtype. For many subtypes of PTL (i.e., Burkitt lymphoma, follicular lymphoma, etc.), the molecular changes, although not well-characterized in thyroid, are identical to their systemic counterparts. However, the two most common PTL, primary MALT lymphoma, and DLBCL of the thyroid have different clinical and molecular findings. Most importantly, unlike MALT lymphomas of other sites including gastric, pulmonary, and orbital, primary MALT lymphomas of the thyroid gland rarely show abnormalities involving the MALT1 or BCL10 gene. Primary thyroid MALT lymphomas, however, show a close association to chronic lymphocytic thyroiditis including Hashimoto thyroiditis and Sjogren syndrome. Similar to the association of gastric MALT lymphomas to Helicobacter infection, chronic antigen stimulation appears...
Fig. 13.4. Burkitt lymphoma of thyroid. (a) There is a monomorphic proliferation of intermediate lymphoid cells with round nuclei, homogenous chromatin, and scant cytoplasm. No identifiable underlying thyroid architecture is present. Numerous mitotic figures and apoptotic bodies are present. Macrophages impart a “starry sky” appearance (H&E, ×200). (b) The lymphocytes are B-cells expressing CD20, CD10, and BCL-6 (CD20 IHC, ×100). (c) There is no coexpression of BCL-2 (BCL-2 IHC, ×100). (d) The Ki-67 proliferation index approaches 100% (Ki-67 IHC, ×100).

Fig. 13.5. Lymphoepithelial lesions. (a) Low power view of lymphoepithelial lesions with predominantly small lymphocytes infiltrating and disrupting thyroid follicles (H&E, ×100). (b) Higher power view of a thyroid follicle “stuffed” with larger transformed lymphocytes (H&E, ×200). (c) Cytokeratin stain highlights residual thyroid follicular epithelium disrupted by the lymphoma cells (CK IHC, ×200). (d) CD20 stain shows the large lymphocytes infiltrating and filling the lumen of a thyroid follicle (CD20 IHC, ×200).
to play a role in the lymphomagenesis of MALT lymphomas of the thyroid. The molecular changes of DLBCL in thyroid are not well understood, but an association with underlying MALT lymphomas of the thyroid and chronic lymphocytic thyroiditis has been noted.4–6 Studies have suggested numerous molecular pathways, including cytokine induced proliferation,18,19 inhibition of apoptosis,20–23 and loss of cell cycle regulation,23–27 might be involved in the pathogenesis of these lymphomas.

### MALT Lymphomas

The association between MALT lymphoma and chronic antigenic stimulation is seen in the prototypic gastric MALT lymphoma and *Helicobacter* infection.28–31 Infections by other organisms have been suggested in MALT lymphomas arising at other sites (Table 13.2). In recent studies, the molecular changes of MALT lymphomas have been further elucidated. These studies have implicated the activation of the NF-kB pathway as the common pathway in the pathogenesis of MALT lymphomas.32–35 Translocations of the *MALT1* and *BCL10* genes have been characterized in a subset of MALT lymphomas (Table 13.3).13,14 The most common *API2–MALT1* translocation results in upregulation of MALT1 and has been shown to participate in the activation of NF-kB pathway upon antigen stimulation.36 However, MALT lymphomas of the thyroid rarely show translocation of *BCL10* and *MALT1*. The t(3;14)(p13;q21) translocation of *FOXP1* gene results in its deregulation. FOXP1 belongs to the Forkhead box (Fox)
family of transcription factors and is involved in the regulation of B-cell development. This translocation was initially found in a significant proportion of MALT lymphomas of the thyroid. However, subsequent studies have not confirmed this to be a significant molecular change in MALT lymphomas of the thyroid.

The close association between chronic lymphocytic thyroiditis and MALT lymphomas of the thyroid has implicated chronic antigen stimulation as a factor in the lymphomagenesis of MALT lymphomas in the thyroid. Patients with PTL will frequently have serum antibodies directed toward the thyroid. The relative risk of MALT lymphoma in patients with Hashimoto thyroiditis is estimated to be as high as 67. Molecular analysis of the immunoglobulin heavy chain (IGH) by PCR have identified clonal B-cell populations in a small subset of Hashimoto thyroiditis. These B-cell clones are frequently seen in a background of polyclonal B-cells or as multiple clones, and are believed to be localized. When cases of Hashimoto thyroiditis are compared with subsequent MALT lymphomas, sequencing of the IGH gene shows homology between the B-cell clones seen in Hashimoto thyroiditis and the lymphoma, suggesting that the lymphoma arises from clones within the Hashimoto thyroiditis. In addition, analysis of VH immunoglobulin gene usage shows a preference for VH3 and VH4 families, and may provide clues to the antigens that may be responsible for the development of MALT lymphoma. In one study, 89% of MALT lymphomas of the thyroid showed usage of VH3 (50%) or VH4 (38%) families. A subset of the VH genes in the MALT lymphomas showed sequence homology to the antibody of thyroglobulin and thyroid peroxidase, suggesting these lymphomas are derived from lymphocytes associated with anti-thyroid antibodies as seen in Hashimoto thyroiditis.

Although chronic antigenic stimulation is likely to be an important initiating factor in the lymphogenesis of MALT lymphomas of the thyroid, secondary molecular changes are believed to be necessary. Studies comparing chronic lymphocytic thyroiditis with MALT lymphomas of the thyroid have suggested secondary molecular changes involving apoptosis signal transduction may be involved. In one study examining the Fas gene, an accumulation of Fas gene mutations was identified in PTL, especially MALT lymphomas. These Fas mutations were most frequently frameshift mutations resulting in truncated forms of Fas protein with reduced response to Fas ligand (FasL) and apoptotic signal transduction. Death-associated protein-kinase (DAP-kinase) gene, a serine/threonine kinase involved in apoptosis induced by interferon-gamma, tumor necrosis factor-gamma (TNF-gamma), and FasL, was shown to have increased methylation in MALT lymphomas versus chronic lymphocytic thyroiditis. Survivin, another protein belonging to a family of antiapoptotic proteins, has been reported to be highly expressed in PTL, including MALT lymphomas. Molecular changes resulting in increased proliferation have also been suggested. One study found increased mRNA transcripts of IL-7, a stimulatory cytokine, in PTL.

One can see that the molecular pathogenesis of primary MALT lymphoma of the thyroid is not well-understood. The close association with chronic lymphocytic thyroiditis suggests that chronic antigenic stimulation probably plays the role of an initiating factor. Although there is data suggesting additional secondary molecular genetic changes, these are currently not well characterized. However, a defect of apoptosis signal transduction pathways appears to be involved.

**Diffuse Large B-Cell Lymphoma**

At least a subset of DLBCL of the thyroid is believed to arise from preexisting MALT lymphomas of the thyroid. In one study of 108 cases of thyroid lymphoma, up to one-third of the cases morphologically showed DLBCL with areas of MALT lymphoma. In some cases, there is not always a distinct separation between the areas of DLBCL and MALT lymphoma. Although morphologically there appears to be a close association between DLBCL and MALT lymphomas of the thyroid, the few molecular studies examining these lymphomas have shown, as expected, molecular heterogeneity between DLBCL and MALT lymphomas. The usage of the VH gene family has been evaluated and showed a preferential usage of VH4 immunoglobulin gene in MALT lymphomas of the thyroid, while the majority (89%) of the DLBCL of the thyroid used the VH3 immunoglobulin gene, suggesting that at least a subset of the DLBCL of the thyroid is de novo and develops in the absence of a preexisting MALT lymphoma.

The molecular progression of MALT lymphomas to DLBCL of the thyroid is an area of interest. However, the current understanding of this progression is limited. Microsatellite instability suggests genetic instability may play a role, but data is limited to small studies. Mutations in oncogenes and tumor suppressor genes including KRAS and p53 have also been implicated. Dysregulation of the cell cycle appears to play some role. Most studies evaluating expression by immunohistochemical techniques suggest dysregulation of cell cycle regulators in PTL correlate with the aggressiveness of the lymphoma. However, most of the data is limited to small studies and have not been adequately reproduced.

**Special Techniques**

Morphology and immunophenotyping by flow cytometry or immunohistochemistry remain standard diagnostic procedures for PTL. However, molecular techniques can be helpful in difficult cases.
Immunoglobulin Heavy Chain

PCR analysis of the \textit{IGH} gene rearrangement is a well-established technique for identifying clonal B-cell populations. \textit{IGH} gene rearrangement analysis can be helpful in differentiating reactive infiltrates of chronic lymphocytic thyroiditis from the low-grade MALT lymphomas. However, \textit{IGH} gene rearrangements can be absent in up to \textit{30\%} of DLBCL and MALT lymphomas of the thyroid.\textsuperscript{39}

In addition, as previously discussed, minor clonal populations can be seen in chronic lymphocytic thyroiditis. These minor clones are infrequent, not reproducible, and are usually minor populations within a polyclonal background.\textsuperscript{15,16} Currently, determining VH gene usage and sequencing are diagnostically unnecessary.

Fluorescent In Situ Hybridization

The utility of fluorescent in situ hybridization (FISH) varies with the type of PTL. In PTL types with characteristic molecular changes (Table 13.1), FISH can be diagnostically useful. For example, FISH for the \textit{BCL2/IGH} translocation can be useful for differentiating follicular lymphoma of the thyroid from a follicular hyperplasia. However, in types of PTL lacking specific molecular genetics detectable by FISH, like MALT lymphomas, the utility of FISH is limited.

Cytogenetics

Cytogenetic karyotyping is helpful, but has limitations as a diagnostic test. Karyotyping can identify clonal population that may show specific or nonspecific genetic changes. Generally, the presence of clonal genetic abnormalities is evidence supportive of a neoplastic process. However, cytogenetic karyotyping requires fresh tissue sampled from involved areas. In addition, karyotyping is a time and labor-intensive procedure with delayed results. Low-grade lymphoproliferative disorders are not always detected by karyotyping and when detected, can show only nonspecific changes not helpful for subclassification. The yield and utility of cytogenetic karyotyping in PTL varies with the availability of FISH and specific histologic type.

Summary

The term “primary thyroid lymphoma” refers to a heterogeneous group of lymphomas of predominantly B-cell lineage, although rare T-cell lymphomas have been reported. PTL are rare entities and current data has been limited to predominantly small studies. The difficulty in differentiating PTL from systemic lymphomas involving thyroid further complicate studies in the area. Although it includes a wide spectrum of entities with widely disparate clinical, morphologic, and phenotypic features, the vast majority fall into DLBCL and MALT lymphoma histological types. The molecular changes of most PTL are unknown, but seem to correspond with their systemic counterparts. However, there is evidence that MALT lymphomas and DLBCL of the thyroid follow alternative pathways of lymphomagenesis. Additional studies are necessary as the molecular pathogenesis of the most common histologic types of PTL, MALT lymphoma and DLBCL, are still largely unknown.

References

39. Sato Y, Nakamura N, Nakamura S, et al. Deviated VH4 immunoglobulin gene usage is found among thyroid mucosa-associated lymphoid tissue lymphomas, similar to the usage at other sites, but is not found in thyroid diffuse large B-cell lymphomas. Mod Pathol. 2006;19:1578–1584.
Section 3
Parathyroid Diseases
Introduction

Parathyroid pathology is usually manifested clinically as hyperparathyroidism (HPT), resulting from parathormone (PTH) excess. PTH is the chief hormone product of the parathyroid glands and can be overproduced in several complexly interrelated settings, termed primary (PHPT), secondary (SHPT), and tertiary hyperparathyroidism (THPT). PHPT can arise sporadically or from inherited syndromes that include multiple endocrine neoplasia type 1 (MEN1), multiple endocrine neoplasia type 2A (MEN2A), isolated familial hyperparathyroidism (FHPT), and familial hyperparathyroidism-jaw tumor syndrome (FHJT). SHPT is secondary to hypocalcemia, renal insufficiency, and/or severe vitamin D deficiency. THPT by definition occurs with a history of renal failure and long-standing SHPT.1

The disease processes governing PTH excess can be the result of a solitary enlarged gland (adenoma), two enlarged glands (double adenomata), diffuse enlargement of all glands (hyperplasia), or malignant growth (carcinoma). The rate of multiglandular disease varies widely by HPT disease type, namely 5–33% for sporadic PHPT,2–7 20–100% for inherited PHPT,8 100% for SHPT, and about 97% for THPT.9 The rate of parathyroid carcinoma is uniformly low (<1%), and multiglandular carcinoma is exceedingly rare.10

The number and the locations of parathyroid glands can also vary widely. Anatomically most people have 4 glands, but supernumerary glands are common, in fact up to 13% of people have 5–9 parathyroid glands.11 Derived embryologically from the third and fourth branchial pouches, enlarged parathyroid glands can reside in many locations in the neck and upper mediastinum. Although surgery is usually the best treatment for PHPT and for selected patients with SHPT and THPT, its success depends upon locating the enlarged, hyperfunctional gland(s). The dual complexities of HPT treatment, therefore, lie in correct identification of the HPT type, which determines the likelihood of gland heterogeneity, and in correct surgical management to achieve biochemical cure of the disease process.

Diagnosis

Inappropriate or unregulated overproduction of PTH leads to abnormal calcium homeostasis. Persistently high levels of PTH in patients with normal renal function cause an increase in renal resorption of calcium, phosphaturia, increased resorption of bone, and increased synthesis of 1, 25-dihydroxyvitamin D$_3$ (1,25 (OH)$_2$D$_3$).12 The elevated 1,25 (OH)$_2$D$_3$ in turn augments intestinal calcium absorption.12 The evident symptoms of longstanding excess PTH are directly related to these physiologic actions and to the effects of consequent hypercalcemia on neuron and muscle function.

Nephrolithiasis still complicates PHPT, but less frequently than in prior decades. Earlier studies reported renal stones in up to 40% of PHPT patients,13 but with the advent of routine screening for hypercalcemia this percentage has declined to 15–20%.14,15 Hypercalciuria results from mismatch of the filtered and reabsorbed calcium and is one of several causative factors for renal stones, which in PHPT are usually composed of calcium oxalate or calcium phosphate.16 Longstanding hypercalcemia and hypercalciuria can lead to calcific disease of the renal parenchyma (nephrocalcinosis), and if left uncorrected ultimately results in a decreased glomerular filtration rate and renal insufficiency.16 The deleterious effect on renal function is not reversed by curative parathyroid resection.16

PTH and calcium independently affect cardiovascular function.17 PTH is vasodilatory, and has chronotropic and inotropic effects on the heart, while hypercalcemia is associated with vascular and valvular calcifications, hypertension and left ventricular hypertrophy.17 In recent European series describing PHPT patients with high mean serum calcium, 82% had left ventricular hypertrophy, 46% had aortic valve calcifications, 39% had mitral calcifications, and with surgical correction of HPT the ventricular hypertrophy improved with concurrent stabilization of valvular sclerosis.18,19 In other European studies, there is a well demonstrated increased risk of cardiovascular related mortality20 and myocardial infarction21 in PHPT which normalizes within 1 year following...
curative resection. However, such findings are not yet replicated in US populations.\textsuperscript{17}

PTH excess causes abnormally high bone turnover. Although the dramatic PHPT sequelae of brown tumors, fractures, and osteitis fibrosa cystica are uncommon now in industrialized countries,\textsuperscript{22} dual-energy X-ray absorptiometry (DEXA) bone scans routinely show reduced cortical bone density in mild PHPT.\textsuperscript{23,24} In PHPT, there is a decrease in bone density at sites rich in cortical bone, such as the distal radius, which is in contrast to the deficiencies of vertebral spine cancellous bone seen in postmenopausal women with osteoporosis. The resultant diminished bone density of PHPT does predispose to fracture of the distal forearm, vertebrae, ribs, and pelvis.\textsuperscript{25} Following curative surgery for PHPT, patients demonstrate improvement in vertebral and hip bone mineral density of 12–20%.\textsuperscript{15,26}

Patients with PHPT may have considerable sensation of early fatigue. Classically, advanced PHPT disease was associated with proximal muscle weakness affecting the lower extremities and characterized by neuropathic atrophy of type-2 muscle fibers.\textsuperscript{27} In the modern era, this degree of neuromuscular dysfunction is rare, yet over half of PHPT patients will report some degree of muscle cramping or paresthesia.\textsuperscript{28}

There is robust evidence not only for an increased prevalence of neuropsychiatric symptoms in PHPT, including depression, anxiety, malaise, and impaired cognition, but also that these symptoms demonstrably improve with normalization of PTH and calcium levels.\textsuperscript{29-33} As yet, there is no universally accepted instrument to evaluate the neuropsychiatric symptoms of PHPT in relation to the degree of biochemical disease or to prove a cause and effect relationship.\textsuperscript{17,34}

Historically, patients with advanced PHPT presented to physicians with nephrolithiasis, osteitis fibrosa cystica, muscle atrophy, hyperreflexia, and other overt symptoms including the extreme of coma.\textsuperscript{35} In the modern era, the most common presentation of PHPT is the incidental finding of hypercalcemia first noted on routine biochemical screening, and usually termed “asymptomatic”.\textsuperscript{36} However, most patients with PHPT are by no means asymptomatic. In order of frequency, the subtler sequelae of HPT include bone mineralization deficits for 44% of patients (osteoporosis or osteopenia), neuromuscular complaints for 33% (fatigue, lethargy, muscle aches and bone aches), neuropsychiatric dysfunction for 15% of patients (insomnia, memory loss, depression, and mood disturbance), hypertension for 6%, gastrointestinal complaints for 4% (gastroesophageal reflux, peptic ulcer disease, dyspepsia and pancreatitis), and polyuria for <1%.\textsuperscript{36} Because such symptoms were not noted to be among the “classic” sequelae described in early HPT literature, they have not always been described systematically and are still underreported in some studies today.\textsuperscript{37}

Estimates of the incidence of PHPT have increased in previous decades, but this trend is certainly related to improvements in disease recognition. The most recent overall incidence rate for PHPT is 21–28 cases per 100,000 population annually in North America and England.\textsuperscript{38-40} Of note, PHPT incidence rises to 190 cases per 100,000 population annually for Caucasian women greater than age 60.\textsuperscript{41} PHPT is two to three times more common in women than men, and the incidence increases for both sexes with age.\textsuperscript{42} Despite the relatively high frequency of PHPT in the general population, mortality is low accounting for an estimated 0.3 deaths per million per year in the United States.\textsuperscript{42} The morbidity of PTHP and its impact on the US health care system remains less well described.

Biochemical Diagnosis of PHPT

Hyperfunctional parathyroid pathology is typically suggested by hypercalcemia, whether found by investigation of identified symptoms or incidentally on routine biochemical screening. Because hypercalcemia can also be caused by thiazide diuretic use, or spuriously by the ischemia of prolonged tourniquet time, PHPT diagnosis rests first on confirmation of hypercalcemia. Total serum calcium should be reevaluated in the fasting state, off calcium supplements and after at least one week of discontinuation of thiazide diuretics.\textsuperscript{33,44} Serum phosphorus is often low to low-normal in PHPT. Most patients have a normal albumin level thus measurement of ionized calcium offers minimal diagnostic advantage.\textsuperscript{35}

After hypercalcemia is confirmed, the second step in PHPT diagnosis is the demonstration of an elevated or inappropriately high-normal serum PTH level.\textsuperscript{33,44} Biologically active PTH circulates mostly as an 84 amino acid peptide but serum carboxyterminal degradation fragments of varying lengths are also present and may additionally be detected depending on the assay type used.\textsuperscript{45} Accurate PTH measurement currently requires use of an immunoradiometric (IRMA) or immunochemiluminescent (ICMA) assay which measures “intact” PTH using antibodies directed simultaneously at both the amino- and carboxy-terminal regions.\textsuperscript{46} The level of serum calcium is under sensitive feedback control via the calcium-sensing receptors in parathyroid glands. Hypercalcemia reduces PTH secretion; therefore, the concurrent elevation of serum calcium and PTH levels strongly suggests the diagnosis of PHPT (Figure 14.1).

Bisphosphonate use and chronic vitamin D deficiency are two common causes of secondary elevation in PTH, which may confound PHPT diagnosis. Low vitamin D levels indicate nutritional deficiency and should prompt medical correction. In this regard, many patients with PHPT have a subclinical initial presentation with normal or high-normal serum calcium level, becoming hypercalcemic only when vitamin D therapy is initiated.\textsuperscript{47} Other conditions causing PHPT diagnostic uncertainty include hypercalcemia of malignancy, vitamin D toxicity, chronic granulomatous disease, and milk-alkali syndrome, and these can usually be identified on history with, if necessary, measurement of vitamin D levels and/or a PTH related-peptide level (PTHrP).\textsuperscript{48}

Diagnostic dilemma may also be due to the rare, autosomal dominant disorder of familial benign hypocalciuric
hypercalcemia (FBHH), which arises from an inactivating mutation in the calcium-sensing receptor of the parathyroid glands and kidneys.\(^49\) This syndrome produces mild hypercalcemia, mild elevation, or lack of suppression in PTH level, low 24-h urine calcium excretion, and a Ca/Cr clearance ratio below 0.01.\(^44\,50\) FBHH has no known adverse sequelae other than unnecessary parathyroid surgery, which illustrates the importance of asking about family history of early onset asymptomatic hypercalcemia and measuring urine calcium excretion in patients with mild biochemical PHPT.

Biochemical Diagnosis of SHPT and THPT

SHPT typically arises in the setting of renal insufficiency but also can be due to any cause of chronic hypocalcemia and/or vitamin D deficiency. Most patients receiving dialysis for end-stage renal disease have some degree of SHPT especially if 1,25 dihydroxy vitamin D\(_3\) is not adequately replaced or hyperphosphatemia is medically uncontrolled. SHPT is a complex, multifactorial process. One proximate cause is the significant loss of functional renal parenchyma in chronic renal disease leading to diminished production of 1,25-dihydroxy vitamin D\(_3\) which results in diminished intestinal absorption of calcium.\(^51\,52\) Another factor is the hyperphosphatemia of renal failure.\(^51\) The cumulative effect of low 1,25-dihydroxy vitamin D\(_3\) and high phosphorus is a persistent hypocalcemic state.\(^51\,52\) These physiologic conditions combine to stimulate PTH production and parathyroid gland hyperplasia. The biochemical diagnosis of SHPT requires demonstration of normal or low-normal serum calcium levels, elevated PTH, and high phosphorus levels (Figure 14.1).\(^52\)

As with PHPT, the spectrum of clinical SHPT has changed over time with more astute detection and with earlier treatment of patients on renal replacement therapy. Historically, symptoms of bone pain, myopathy, tendon rupture, and extraskeletal calcifications were common.\(^52\) Today, most patients with SHPT are relatively asymptomatic, but some complications of SHPT persist and can require close attention including early coronary artery calcification, cardiomypathy, pruritis, uremic calcific arteriolopathy (calciphylaxis), and neuropsychiatric issues.\(^52\)

In the setting of prolonged SHPT, diffuse parathyroid gland hyperplasia can progress to nodular hyperplasia, which can ultimately transform from a polyclonal or oligoclonal process to a monoclonal parathyroid neoplasm with autonomous function.\(^52\) This loss of feedback regulation is termed THPT, and is defined as hypercalcemia in a patient with a history of renal failure.\(^1\) THPT can occur before renal transplantation, persist after transplantation, or can even arise de novo after transplantation.\(^54\,55\) As with primary hyperparathyroidism, THPT is marked biochemically by both elevated serum calcium and PTH levels (Figure 14.1).

### Treatment

**Surgery for PHPT**

Once PHPT has been diagnosed biochemically, the current treatment standard rests on a distinction of whether symptoms are present. Although percutaneous alcohol ablation can temporarily palliate PHPT in selected high-risk patients,\(^56\) surgery provides the only cure of PHPT. Parathyroid exploration is cost effective and is the current standard for symptomatic PHPT patients.\(^37\,43\,44\,57\) As detailed earlier, however, the definition of symptomatic has varied and is still incompletely defined. The goal of surgery for PHPT is to prevent progression to the more serious complications of renal failure, osteoporotic fracture, and cardiac mortality. Nonetheless, after curative surgery, even asymptomatic patients will enjoy a 60% increase in general health at 1 year with objectively measured improvements in fatigue, weakness, bone pain, joint pain, forgetfulness, mood swings, thirst, irritability, and depression among other PHPT disease-specific complaints.\(^30\)

For asymptomatic PHPT patients, certain risk factors predict disease progression. The National Institutes of Health sponsored a consensus conference in 1990 to define guidelines for surgical intervention in asymptomatic PHPT. The panel recommended surgical treatment of PHPT for patients with any of the following: nephrolithiasis, osteitis fibrosis cystica, neuromuscular symptoms (documented proximal weakness, atrophy, hyperreflexia, gait disturbance), a life-threatening
Table 14.1. NIH criteria for parathyroid exploration.

<table>
<thead>
<tr>
<th>Age</th>
<th>&gt;50 years*</th>
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<tbody>
<tr>
<td>Serum level of calcium</td>
<td>&gt;1.0 mg/dL above upper limit of normal</td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td>Reduced by 30% compared to normal age-matched</td>
</tr>
<tr>
<td>24 h urinary calcium excretion</td>
<td>&gt;400 mg</td>
</tr>
<tr>
<td>Bone mineral density</td>
<td>t-score &lt;-2.5 at any site</td>
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*Or patients that are unable to provide consistent follow-up.

An episode of hypercalcemia, age <50, serum calcium level 1–1.6 md/dL above the normal range, 30% reduction in creatinine clearance compared to age-matched controls, 24 h urine calcium excretion >400 mg, or forearm bone density reduced more than 2 standard deviations below the density of age-, gender-, and race-matched controls (z-score). The summary statement of a subsequent 2002 national workshop held to further discuss asymptomatic PHPT advised broader criteria for surgery, namely a calcium level 1.0 mg/dL above the normal range or a bone mineral density at any site reduced by more than 2.5 standard deviations over unmatched controls (t-score) (Table 14.1). Both conferences advised careful long-term follow-up of patients being managed with observation and both cited patient willingness to participate in such follow-up to be a strong prompt for surgery. For asymptomatic PHPT patients being observed, the NIH consensus conference advises twice-yearly physician evaluation and serum calcium level, as well as annual 24-h urine calcium, creatinine clearance, serum creatinine level, and bone density testing.

Testing of the NIH criteria for surgery versus surveillance has not yet been systematically performed. We now know that during 15 years of surveillance, 37% of asymptomatic PHPT patients who do not originally meet NIH criteria will progress to meet criteria for surgery and that progression is more likely for PHPT patients diagnosed at <50 years. In an interesting 2004 report, Eigelberger et al objectively evaluated 178 surgically cured PHPT patients, 103 of whom met at least one of the NIH criteria for surgery, and also compared their outcomes to 63 nonPHPT patients who had thyroid surgery. They observed that nonclassic PHPT symptoms including fatigue, bone pain, nocturia, and depression were indeed much more frequent in PHPT than in thyroid disease. Moreover, such symptoms occurred in PHPT regardless of whether the patient met NIH criteria for surgery and resolved postoperatively in many patients regardless of NIH criteria status. Multivariate analysis of practice patterns among endocrine surgeons show a lack of consensus on the threshold for surgery in asymptomatic patients which is felt to lead to increased cost and burden on the health care system. Cost efficacy studies also favor operative management for asymptomatic PHPT even for patients who do not meet NIH criteria for surgery. In summary, based on multiple objective studies of symptom resolution after curative surgery, we consider the subtler symptoms of PHPT including fatigue, musculoskeletal aches and pains, back pain, weakness, polydipsia, nocturia and memory loss, to be real, valid and potentially reversible. The emerging consensus nationally is that all patients with asymptomatic PHPT should consult with an experienced surgeon before selecting a treatment option.

Nonoperative Management of PHPT

Overall, medical management can achieve only partial, inconsistent reduction in calcium levels, and is not known to prevent the more dire sequelae of PHPT. Calcium restriction currently is not recommended for patients being observed since it can further raise PTH levels; daily replacement doses of 1,000–1,200 mg elemental calcium are suggested. The antiresorptive properties of estrogens can decrease the effects of PTH on bone demineralization, but the doses required are so high that exogenous estrogen treatment is not currently recommended. Selective estrogen receptor modulators such as raloxifene are reported to reduce serum calcium and markers of bone turnover short-term, however the long term effects are not known. Bisphosphonates such as alendronate can be associated with improvement in bone mineral density at the lumbar spine, femoral neck, and hip in patients with PHPT and osteoporosis but also can increase PTH levels, and the use of such agents in the medical treatment of PHPT is still under investigation.

The extracellular calcium-sensing receptor (CaR) was identified and cloned in 1993 by Brown and colleagues and is a G-protein coupled membrane receptor that is activated by high extracellular Ca2+ to control PTH secretion. Molecules that interact with parathyroid CaRs and mimic Ca2+ are termed calcimimetics and function to activate CaRs, suppress PTH secretion and/or block parathyroid gland hyperplasia. The two distinct types of calcimimetics identified to date are termed type I and type II. Type I calcimimetics are full agonists of CaRs and include various inorganic and organic polycations; this class lacks the specificity and safe therapeutic window to be used clinically. Type II calcimimetics are small organic compounds that allosterically modulate the CaR to increase its sensitivity to activation in the presence of extracellular calcium. The compound AMG 073 (cinacalcet HCl) may achieve normocalcemia and modest reduction of PTH levels in some patients with asymptomatic PHPT. However, the longterm effects of cinacalcet in treating PHPT are unknown and cinacalcet HCl is not currently approved for the use in the treatment of PHPT except in an investigational setting.

Preoperative Localization

Parathyroid imaging does not play a role in the diagnosis of PHPT. Once the decision has been made for surgery, the routine use of preoperative imaging has been shown to increase the success rate, decrease the operative time, and decrease the major complications of parathyroid exploration. Because the anatomic location of parathyroid glands can
vary widely, hyperfunctioning glands can be difficult to locate intraoperatively and this is even truer with reoperation in a hostile surgical field. Among the imaging studies available, the most sensitive, specific, and cost-effective are ultrasonography and single photon emission computed tomography (SPECT) $^{99m}$Tc-sestamibi. Use of $^{99m}$Tc-sestamibi nuclear imaging and ultrasound together can synergistically improve correct localization by 10–14% and are useful in operative planning (Figure 14.2). Ultra-sonography is now well-established to provide additional benefit in detecting concurrent thyroid pathology. No current imaging test can reliably exclude multiglandular parathyroid disease, however, and even a single bright focus on sestamibi SPECT imaging does not reliably exclude hyperplasia and is associated with multiglandular disease in 8% of patients undergoing initial exploration.

Operative Strategy

Successful parathyroid surgery is defined as durable normalization of the serum calcium level, ie cure of PHPT. Operative failure is the most frequent risk of parathyroid exploration and carries a high morbidity and cost. Because the therapeutic intent in PHPT is to remove the source(s) of PTH overproduction, the central issues in exploration are identifying the presence or absence of gland heterogeneity (adenoma versus hyperplasia) and locating the abnormal gland(s). Common locations for enlarged superior parathyroid glands include the tracheoesophageal groove, the retropharyngeal space, and the superior middle mediastinum; enlarged inferior glands can be intrathyroidal, undescended at the carotid bulb, within the thymus, or elsewhere in the superior anterior mediastinum.

Unfortunately, histologic criteria are not clinically reliable in distinguishing adenoma from multiglandular disease and in one recent study incorrectly predicted the number of abnormal parathyroid glands 62% of the time. Intraoperative use of a gamma-probe for localization of abnormal parathyroid glands with $^{99m}$Tc-Technetium has been examined but remains controversial. Currently, only two techniques are proven to reliably exclude multiglandular disease in PHPT. The first of these, bilateral 4-gland exploration, is the “gold standard” approach and involves dissection and visualization of all parathyroid glands. Normal parathyroid glands are 30–45 mg in size, while suppressed parathyroid glands in PHPT can be considerably smaller, thus 4-gland exploration requires surgeon expertise in assessment of gland size. Enlarged parathyroid glands are resected and are weighed intraoperatively by the pathologist along with histopathologic confirmation that it is indeed parathyroid tissue (and not brown fat, lymph node, thymic, or thyroid tissue) that has been removed. This information can be used intraoperatively to deduce the presence of missing or supernumery abnormal parathyroid glands. Should the patient have more than 2 enlarged glands (>double adenomata), they are considered to have hyperplasia, and a subtotal parathyroidectomy is performed, leaving a viable 50–100 mg remnant of one enlarged gland in situ and marked with a titanium clip.

The second technique validated to exclude multiglandular disease is intraoperative PTH monitoring, which is now widely used because it facilitates both focused dissection and shorter operative times. In this method, the serum PTH,
which has a short half-life of 3–5 min in most patients, is measured just prior to and just after resection of an enlarged parathyroid gland using a modified rapid assay developed by Dr. Irvin at the University of Miami. If the postresection PTH does not drop adequately, the presence of further hyperfunctional parathyroid glands is deduced, and further exploration is performed. Although nationally the most widely used criterion for an adequate PTH drop is a simple 50% decrease at 10 min postresection, stricter criteria are in use in several centers; we require that PTH also drop into the normal range, to diagnose an appropriate response to parathyroid resection. Intraoperative PTH monitoring facilitates a safe, successful focused dissection, allows optimization of the postoperative calcium supplementation, and is particularly useful in successfully managing reexploration, double adenomata, hyperplasia, intrathyroidal adenoma, or supernumerary adenoma, and in patients with a history of lithium use. To summarize the operative strategy with PTH monitoring, “the localization tells you where to start looking and the PTH drop says you can stop looking.”

Surgical Cure

As stated earlier, cure after parathyroid exploration for PHPT is defined as durable normalization of the serum calcium (>6 months after surgery). Operative failure is usefully categorized as either persistent or recurrent HPT. In persistent HPT, there is an elevated serum calcium and PTH within 6 months postoperatively, and this usually represents a missed adenoma, while in recurrent HPT hypercalcemia arises >6 months after initial exploration, and the situation generally indicates multiglandular disease that was unrecognized, or rarely parathyromatosis and/or parathyroid carcinoma.

Immediately after successful surgery for PHPT, many patients will show elevation of PTH despite correction to normal serum calcium levels. This phenomenon is well described, common (estimated occurrence of about 30% (8–40%) postoperatively), in our recent experience occurs much less frequently when patients routinely take calcium and vitamin D supplements for 6 months postoperatively, and thus is likely to be secondary to chronic dietary deficiency in calcium and vitamin D. All normocalcemic patients are thus recommended to receive maintenance calcium and vitamin D supplementation for 6 months after surgical cure of PHPT. In a small proportion of patients (0.7%), a normocalcemic elevation in PTH at 6 months postoperatively can be the first indicator of recurrent PHPT, and such patients should thus be followed long-term.

Reoperation

The causes of failed initial parathyroid exploration include surgeon inexperience, ectopic or supernumerary parathyroid gland location, multiglandular disease, and parathyroid carcinoma. The indications for initial and reoperative exploration differ. The risks of initial exploration for PHPT are few and are generally limited to operative failure (1–5%), permanent hypoparathyroidism (0.5–2%), permanent recurrent laryngeal nerve injury (1% or less), and cervical hematoma (0.3%) (reviewed in106). Because the risks of reoperation are higher across the board, and include bilateral recurrent nerve paralysis, surgery is usually reserved for clearly symptomatic patients or those with nephrolithiasis, osteoporosis, calcium level >12 mg/dL, or hypercalcemia. After initial failed surgery, the first step is biochemical reconfirmation of the PHPT diagnosis including 24 h urinary calcium excretion. Histologic reexamination of the tissues removed at the initial exploration, review of prior records, precise localization, DEXA bone mineralization testing, laryngoscopy, careful family history, and a frank discussion of the risks and benefits with the patient are also necessary prior to reexplanation.

Parathyroid Surgery in Inherited Syndromes

Hereditary syndromes resulting in HPT require special attention since the setting of the genetic defect involved can have a significant impact on both surgical approach and outcome. Further, hereditary HPT patients require screening for other clinical manifestations of the respective syndrome.

In MEN1, the penetrance of PHPT is close to 100%, and the associated pancreatic islet and pituitary tumors occur much less frequently. The syndrome is secondary to genetic alteration of the MEN1 gene on chromosome 11q13, a tumor suppressor gene that encodes the ubiquitously expressed nuclear protein product menin. Multiglandular parathyroid disease is assumed in all patients. If MEN1 is missed on family history, or in a patient who represents a new mutation, persistent HPT is likely and recurrent HPT is certain. The successful diagnosis of MEN1 in patients presenting with apparent sporadic PHPT is greatly facilitated by use of a simple 6-question panel, which focuses on a personal or family history of neck surgery, nephrolithiasis, brain tumors, peptic ulcer disease, high calcium levels and/or pancreatic tumors. MEN1 accounts for about 26% of patients found to have parathyroid hyperplasia at exploration for presumed sporadic PHPT and is more likely in patients who are young and male. MEN1 PHPT is currently treated either by total parathyroidectomy with nondominant forearm autotransplantation, or by subtotal parathyroidectomy, and patients require counseling preoperatively to assist in decision-making because these operations have differing morbidity rates. Cryopreservation of parathyroid tissue resected during parathyroid surgery should be performed to allow future treatment with preserved grafts in the event of hypoparathyroidism.

MEN2A is a hereditary syndrome expressed as medullary thyroid cancer (MTC), pheochromocytoma and PHPT, and arising from mutations in the tyrosine kinase RET. The penetrance of PHPT in MEN 2A is approximately 30%, and it
usually results in mild HPT presenting in mid adulthood. Because the degree of parathyroid gland enlargement can be asymmetrical in MEN2A, surgical management is yet controversial and not all patients will require subtotal parathyroidectomy. The unique feature of MEN2A is that 90% of patients will develop MTC prior to PHPT; total thyroidectomy for MTC thus can be followed decades later by difficult reoperative parathyroid exploration. If not already performed, total thyroidectomy is mandated at the time of parathyroid exploration.

Familial hyperparathyroidism-jaw tumor syndrome (FHJT or HPT-JT) arises from mutation of the tumor suppressor gene HRPT2 which encodes the parafibromin protein product. The primary clinical features are HPT, fibro-osseous lesions of the mandible and maxilla, and renal lesions including cysts, hamartoma, and Wilms tumor. HPT is expressed in 80% of adults and tends to present at a relatively young age (mean 32 years). Parathyroid gland enlargement is often asymmetric and glands may also be cystic in nature. The surgical approach is the same as that for MEN2A with resection of all enlarged glands. An interesting and important feature of HPT-JT management is that parathyroid carcinoma may be present in as many as 15% of cases.

Parathyroid Cysts and Parathyroid Cancer

Parathyroid cysts are uncommon lesions of the neck and mediastinum and may present as a symptomatic neck mass or be identified during evaluation of PHPT. As many as 2.7% of patients with PHPT will have a cystic parathyroid gland. For patients with functional cysts, surgical excision should be performed in conjunction with intraoperative PTH monitoring to insure adequate resection and allow identification of concordant adenoma in another location or multiglandular disease.

Parathyroid carcinoma (PC) is a rare malignancy but warrants special mention here because the diagnosis can be made intraoperatively, histologically, or by both methods. Preoperative features which raise suspicion of malignancy include serum calcium level >14 mg/dL, PTH level >5 times normal, the presence of a palpable neck mass, or hoarseness (signifying involvement of the recurrent laryngeal nerve.) At exploration, features which suggest parathyroid carcinoma include: white color, firm consistency, gritty texture, and adherence to surrounding structures, and the alert surgeon needs to recognize these signs because en bloc resection of the lesion is then mandated. In the event of local recurrence, resection and neck dissection is best treatment. Palliative medical management with cinacalcet may be warranted; in a recent preliminary study examining 29 patients with inoperable parathyroid carcinoma, cinacalcet was able to modestly control hypercalcemia and reduce serum calcium by at least 1 mg/dL in 62% of patients, without significant decrease in serum PTH.

Treatment of SHPT and THPT

Medical Management of SHPT and THPT

Overall, it is easier to prevent SHPT than to treat it. The aim of medical management is to abate the progression of diffuse to nodular hyperplasia by reducing serum phosphorus and appropriately replacing activated vitamin D, which lowers serum PTH. Obsolete phosphate binding medications included aluminum (which was toxic) or calcium (which resulted in hypercalcemia). Currently, it is recommended that patients with hyperphosphatemia use calcium- and aluminum-free phosphate binders such as sevelamer HCl and lanthanum carbonate in addition to limiting dietary phosphate intake. Treatment with these agents is effective in lowering serum phosphorus but has little effect in reducing PTH levels. Calcitriol (1,25-dihydroxy vitamin D3) is used in SHPT to lower PTH levels but can induce hypercalcemia and hyperphosphatemia. Newer vitamin D analogs such as paricalcitol and doxercalciferol have been shown to reduce PTH levels with less toxicity. These analogs are designed to activate the vitamin D receptor selectively at the level of the parathyroid gland while minimizing the hypercalcemic and hyperphosphatemic effect seen with calcitriol.

The Kidney Disease Outcomes Quality Initiative (KDOQI) has issued guidelines with respect to the clinical practice goals of medical therapy for patients on renal replacement therapy. Serum calcium should be maintained between 8.4 and 9.5 mg/dL and serum phosphorus kept between 3.5 and 5.5 mg/dL. Together, the calcium phosphate product (Ca × P) should be less than 55. PTH levels should be controlled between 150 and 300 pg/mL. In general, standard treatment with phosphate binders and vitamin D sterols is only able to achieve target serum PTH levels in 26% of hemodialysis patients, and achieves all four KDOQI parameters for only 6% of patients.

The best treatment for SHPT is restoration of normal renal function by renal transplantation. When this is not possible or is delayed, the aforementioned strategies in addition to the calcimimetic agent cinacalcet can be used in treatment. Pooled data from phase III clinical studies of cinacalcet in combination with best standard care demonstrate a reduction in both serum PTH and phosphorus levels when compared to placebo. The addition of cinacalcet achieves a serum PTH target ≤300 pg/mL in 56% of patients compared with 10% of placebo-treated controls. Further, 41% of patients treated with cinacalcet are able to achieve both PTH and Ca × P parameters as opposed to 6% of those receiving placebo. The improved efficacy of cinacalcet in managing SHPT also translates into improved outcomes with a decreased risk of need for parathyroidectomy, bone fracture and cardiovascular hospitalization. Treatment with cinacalcet is generally well tolerated with the most common adverse side effects being nausea, vomiting, or diarrhea which may limit its use.
Surgery for SHPT and THPT

In SHPT, parathyroidectomy is indicated when medical management fails to meet the above stated goals. In addition, the KDOQI guidelines recommend parathyroidectomy for patients with PTH >800 pg/mL and hyperphosphatemia, extraosseous calcifications (calciphylaxis) with PTH >500 pg/mL, and progressive renal osteodystrophy or intractable pruritis.

The presence of hypercalcemia in patients with longstanding SHPT indicates autonomous progression to THPT, which can occur in patients who remain dialysis dependent as well as those who have received renal transplantation. For those who will remain dialysis dependent indefinitely, the development of THPT indicates medical failure and parathyroid surgery is indicated. The majority of patients (69–83%) who undergo renal transplantation with THPT, however, will demonstrate slow correction in serum calcium and PTH to near normal levels over the first year posttransplant with only 12.4% remaining hypercalcemic at 48 months posttransplant. Thus, when possible, THPT patients who have recently received or who anticipate renal transplantation should be managed nonoperatively for at least 12 months following transplantation, and surgery is usually reserved for patients with very severe disease.

There are two varieties of parathyroidectomy in the treatment of SHPT/THPT: total parathyroidectomy (with or without autotransplantation) and subtotal parathyroidectomy. These procedures have been equally validated and choice is largely left to the discretion and outcomes of the endocrine surgeon. In general, a balance needs to be struck between removing enough parathyroid tissue to achieve a normal PTH level, while guarding against hypoparathyroidism. All parathyroid glands should be identified and enough tissue resected to leave the patient with a tissue remnant weighing about 40–80 mg either in situ or at the site of autotransplantation.

Conclusion

Parathyroid disease is common and its complications are related to its chief hormone product, PTH. The biochemical diagnosis of hyperparathyroidism is determined by concurrent assessment of fasting serum calcium and PTH levels. The role of parathyroid imaging, by ultrasound and/or sestamibi nuclear scintigraphy, is to aid in the conduct of a planned exploration. Ultimately, exploration by an experienced endocrine surgeon is the most proven and effective treatment for patients with primary hyperparathyroidism or persisting tertiary hyperparathyroidism. The first parathyroid exploration is the safest and best chance for disease cure. Recently developed molecular therapies targeting the calcium sensing receptor offer promise in treating patients with secondary hyperparathyroidism.

References


Introduction

The parathyroid glands are involved in regulating serum calcium concentrations and bone metabolism by the secretion of parathyroid hormone (PTH). Low concentrations of serum calcium are sensed by a calcium-sensing receptor (CASR) on the surface of parathyroid cells and result in stimulation of PTH secretion, while high serum calcium concentrations inhibit PTH secretion. The parathyroid glands are derived from the endodermal third and fourth branchial pouches. The normal parathyroid glands each measures 4–6 mm in length and weighs 20–40 mg. Generally, a parathyroid gland greater than 50–60 mg is considered abnormal.

Parathyroid glands are composed of chief cells, transitional cells, oxyphil cells, and adipose tissue. Chief cells measure 8–10 μm have amphophilic to eosinophilic cytoplasm with intracytoplasmic glycogen and lipid. Oxyphilic cells are 12–20 μm and have prominent cytoplasmic mitochondria accounting for the granular eosinophilic cytoplasm. Oxyphilic cells are not usually identified in children. Transitional oxyphilic cells are smaller and have less cytoplasm. The cellularity of parathyroid glands is variable and distributed unevenly in the gland. Cellularity also differs with constitutional factors and age; cellularity is high in infants and children and decreases with age.

Sporadic Hyperparathyroidism

Clinical

Hyperparathyroidism is an increase in PTH which often results in increased serum calcium levels. Sporadic primary hyperparathyroidism is caused by parathyroid hyperplasia, adenoma, or carcinoma. The neoplastic conditions will be discussed in chapter 16. Parathyroid hyperplasia accounts for 15% of primary hyperparathyroidism.1 Parathyroid hyperplasia is more common in women than in men (2:1) and often occurs in the fifth decade, but may occur over a wide age range. The incidence of primary hyperparathyroidism increased in the early 1970s with the onset of screening serum calcium.

The clinical presentation has changed over time. While patients used to present with nephrocalcinosis and osteopenia, today they usually present with weakness and lethargy or, even more commonly, patients are asymptomatic with elevated screening serum calcium. One percent of women who undergo thyroidectomy have diffusely enlarged or nodular parathyroid glands, possibly representing an early normocalcemic subclinical form of hyperparathyroidism.2,3 Parathyroid hyperplasia has been associated with neck irradiation and drugs such as lithium.4,5

Patients with secondary hyperparathyroidism have increased PTH because of low serum calcium caused by disorders of vitamin D (Rickets, vitamin D deficiency or malabsorption), disorders of phosphate metabolism (malnutrition or malabsorption, renal disease, aluminum toxicity), tissue resistance to vitamin D, pseudohypoparathyroidism, hypomagnesemia, and calcium deficiency. Chronic renal failure is the most common cause of secondary hyperparathyroidism. Tertiary hyperparathyroidism is a rare condition, in which patients with secondary hyperparathyroidism develop an autonomously functioning parathyroid gland.

Histologic Features

Primary hyperparathyroidism caused by hyperplasia results in enlargement of multiple parathyroid glands. Sporadic and hereditary forms of primary parathyroid hyperplasia are histologically indistinguishable. Parathyroid glands usually show nodular hyperplastic areas but a diffuse increase in parenchymal cells can be seen (Figure 15.1). A study of sporadic primary hyperplasia showed diffuse hyperplasia was usually associated with moderately enlarged glands with little variability in gland size and morphologic patterns and was more prevalent in young patients with moderate hypercalcaemia.6 In nodular hyperplasia, the glands were more asymmetric in size, showed variable cellularity with more oxyphil cells, and were more frequent in elderly patients. Parathyroid glands in primary hyperplasia can show an increase in chief, clear, oxyphil, transitional cells, or a mixture of cell types. The cells can show sheet like growth, palisading, glandular formations, cribriforming,
papillary areas, microcystic formations, and cystic change. Surgeons and pathologists must be cautious in evaluating parathyroid glands in relation to size and cellularity as these parameters can vary greatly within a single patient with parathyroid hyperplasia. An asymmetrically enlarged gland can be misinterpreted as a parathyroid adenoma. Primary water-clear cell hyperplasia shows a diffuse increase in clear cells rather than nodular growth (Figure 15.2). Lipohyperplasia is rare and can occur both as a sporadic form and with familial benign hypocalciuric hypercalcemia (Figure 15.2).\textsuperscript{6,7}

The parathyroid glands in secondary hyperplasia usually show more diffusely hyperplastic changes than in primary hyperparathyroidism. The glands usually show diffuse hyperplasia of chief cells. Oxyphilic cells can form nodular areas, as can chief cells, and nodularity may increase with increasing renal failure making the glands indistinguishable from primary or tertiary hyperplasia.

Immunohistochemical Studies

Hyperplastic parathyroid glands show immunoreactivity for the same markers and normal parathyroid tissue: chromogranin A, synaptophysin, low molecular weight keratins, and PTH. The expression of p27, a cyclin-dependent kinase inhibitor that helps regulate the transition from the G1 to the S phase of the cell cycle, is the highest normal parathyroid, followed by hyperplasia, adenomas, and carcinomas.\textsuperscript{8} In situ hybridization showed no differences in p27 mRNA, indicating that the expression of the p27 gene is controlled at a posttranslational level in parathyroid tissues.\textsuperscript{8} Ki67 expression is higher in carcinomas than in hyperplasia and adenomas.\textsuperscript{8} Hyperplastic parathyroid glands overexpress fatty acid synthase.\textsuperscript{9} A study evaluating angiogenic factors endoglin, vascular endothelial growth factor (VEGF), and VEGF-R2 differentiated hyperplastic parathyroid tissues from neoplastic parathyroids.\textsuperscript{10}

Genetics

Unlike neoplastic causes of primary hyperparathyroidism, parathyroid hyperplasia is usually polyclonal although areas of monoclonality can be identified in nodular areas. Specific genetic abnormalities in idiopathic primary parathyroid hyperplasia have not been as well defined as in hereditary forms of hyperparathyroidism.

Hereditary Hyperparathyroidism

Clinical

Hereditary hyperparathyroidism is less common than primary sporadic hyperparathyroidism. The most common types of hereditary hyperparathyroidism are multiple endocrine neoplasia (MEN) type 1, MEN2A, familial hypocalciuric hypercalcemia, neonatal severe primary hyperparathyroidism,
hyperparathyroidism-jaw tumor syndrome (HP-JT), and familial isolated hyperparathyroidism (FIHP). MEN1 equally affects females and males and does not show ethnic or geographic differences. Parathyroid hyperplasia is the most common manifestation of MEN1, although parathyroid adenomas and carcinomas can occur. MEN1 associated hyperparathyroidism has an onset of 20–25 years of age which is four decades earlier than sporadic primary hyperparathyroidism. Other features of MEN1 include pituitary adenomas, neuroendocrine tumors of the pancreas, duodenum, thymus and lung, gastrinomas, adrenocortical adenomas and hyperplasia, facial angiofibromas, collagenomas, café-au-lait macules, lipomas, gingival papules, meningiomas, ependymomas, and leiomyomas.

MEN2A is associated with parathyroid hyperplasia and adenomas in 20–30% of cases. MEN2A is diagnosed clinically by the occurrence of at least two specific endocrine tumors (medullary thyroid carcinoma, pheochromocytoma, or parathyroid hyperplasia/adenoma).

Familial hypocalciuric hypercalcemia is caused by an autosomal dominant mutation in the CASR gene causing lifelong hypercalcemia without hypercalciuria. Neonatal primary hyperparathyroidism is a life-threatening disorder caused by homozygous inactivating mutation of the CASR gene. The parathyroid glands become hyperplasic, and infants are symptomatically hypercalcemic.

HP-JT is an autosomal dominant disorder of hyperparathyroidism, fibro-osseus jaw tumors, kidney cysts, hamartomas, and Wilms tumors. The parathyroids show hyperplasia or adenoma, and there is an increased risk of parathyroid carcinoma. FIHP, an autosomal dominant disorder, accounts for 1% of primary hyperparathyroidism. The cause of FIHP remains unknown in most families, but it has been thought to be a unique entity or possibly a subset may be variant of MEN1 or HP-JT syndrome. This disorder is also associated with an increased risk of parathyroid carcinoma.

Genetics

Although many genes associated with hereditary hyperparathyroidism have been identified, the exact mechanisms are still being elucidated (Table 15.1). MEN1 is a tumor suppressor gene on chromosome 11q13 which encodes menin, a 610 amino acid protein. Menin is truncated or absent with some sporadic parathyroid tumors. MEN1 germline mutation (11q13) MEN1 encodes menin, a 610 amino acid protein Autosomal dominant

Multiple endocrine neoplasia type 1

Multiple endocrine neoplasia type 2A

Familial hypocalciuric hypercalcemia

Casr (3q13.3–q21) mutation Autosomal dominant

Homozygous inactivating CASR mutation (3q13.3–q21) Autosomal recessive

Hyperparathyroidism-jaw tumor syndrome

Familial isolated hyperparathyroidism

Familial hypocalciuric hypercalcemia

Autosomal dominant

Autosomal dominant

Autosomal dominant

Autosomal dominant

Autosomal dominant

Autosomal dominant

Autosomal dominant

Autosomal dominant

Autosomal dominant

Autosomal dominant

Mutations in codon 634, with the other mutations identified mainly in codons 609, 611, 618, 612. Mutations in codon 634 are associated with high penetrance. Risk stratification by mutation status has been suggested as a basis for surgical decisions such that patients with the highest risk mutations would undergo prophylactic thyroidectomy at the youngest age.

The CASR is present in parathyroid, kidney, thyroid C-cells, intestine, and bone. CASRs sense extracellular calcium levels which inversely regulates the release of PTH. An inactivating mutation in the CASR gene (3q13.3–21) results in decreased calcium sensitivity of the cells and excess PTH secretion. Heterozygous inactivating CASR mutations occur in familial hypocalciuric hypercalcemia, and homozygous inactivating mutations occur in neonatal severe hyperparathyroidism (Table 15.2). Hypocalciuric hypercalcemia is caused autoantibodies directed at CASR and can simulate familial hypocalciuric hypercalcemia.

The HRPT2 gene mapped to 1q25–q32 encodes parafibromin. HRPT2 is a putative tumor-suppressor gene, the inactivation of which is involved in HP-JT syndrome and some sporadic parathyroid tumors.

Sporadic Hypoparathyroidism

Clinical

Hypoparathyroidism is characterized by hypocalcemia and hyperphosphatemia because of insufficient PTH secretion or when PTH is not able to function appropriately in target tissues.
GENETICS

A variety of genes are associated with hereditary hypoparathyroidism (Table 15.3). The TBC1 gene (tubulin-specific chaperone E) on chromosome 1q42-q43 encodes a chaperone protein required for folding of alpha-tubulin subunits and alpha-beta-tubulin heterodimers.46 Mutations in TBC1 cause Kenny–Caffey syndrome, which can have autosomal dominant or recessive transmission.46 Velocardiofacial syndrome, like DiGeorge syndrome, is caused by hemizygous deletion of 22q11.2 with most abnormalities due to haploinsufficiency of the TBX1 gene.45 Mutations in the APEXED (autoimmune polyendocrinopathy-candidiasis-ectodermal-dystrophy) or AIRE (autoimmune regulator) gene on chromosome 21q22.3, which codes for a putative transcription factor, have been identified in autoimmune polyglandular syndrome type 1 which has autoantigens against the CASR.49 Activating CASR mutations occur in familial isolated hypoparathyroidism.15,16 Familial isolated hypoparathyroidism, usually an autosomal dominant condition, can also be caused by mutation in PTH gene (11p15.3–p15.1)46, mutation or polymorphism in the GCM2 gene (6p24.2)45,52 or mutation in the GNCB gene (6p23–24),53 and there is an X-linked recessive (Xq26–q27)54 form. In addition, to familial isolated hypoparathyroidism, PTH mutations are identified in autosomal recessive hypoparathyroidism. Jansen chondrodysplasia is an autosomal dominant condition with activating mutations in PTH receptor type 1 (PTHr1) gene (3p22–p21.1), while Blomstrand chondrodysplasia is autosomal recessive with inactivating mutations in PTHr1.46 Pseudohypoparathyroidism is caused by defects in stimulatory guanine nucleotide binding protein because of GNAS1 mutation (Gsalpha1 protein of the adenyl cyclase complex). The GNAS1 gene (20q13.11) encodes a stimulatory guanine nucleotide binding protein which is acted on by PTH receptor type 1. Patients with pseudohypoparathyroidism type 1a have an autosomal dominant trait caused by an inactivating mutation in GNAS1, while those with pseudohypoparathyroidism type 1b are associated with defective methylation in GNAS1.55

Table 15.2. Calcium-sensing receptor (CASR) abnormalities in nonneoplastic parathyroid disease.

<table>
<thead>
<tr>
<th>Parathyroid disease</th>
<th>Genetic</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial hypocalciuric hypercalcemia</td>
<td>Inactivating (heterozygous loss-of-function) CASR mutation (3q13.3–q21)</td>
<td>Hyperparathyroidism</td>
</tr>
<tr>
<td>Neonatal severe hyperparathyroidism</td>
<td>Inactivating (homozygous loss-of-function) CASR mutation (3q13.3–q21)</td>
<td>Hyperparathyroidism</td>
</tr>
<tr>
<td>Familial isolated hypoparathyroidism</td>
<td>Various genes have reportedly been associated including CASR (3q13.3–q21)</td>
<td>Hypoparathyroidism</td>
</tr>
<tr>
<td>Sporadic idiopathic hypoparathyroidism</td>
<td>Unknown cause, but antibodies to calcium-sensing receptor in some cases</td>
<td>Hypoparathyroidism</td>
</tr>
<tr>
<td>Autoimmune polyglandular syndrome 1</td>
<td>Mutations in the APEXED (autoimmune polyendocrinopathy-candidiasis-ectodermal-dystrophy) or AIRE (autoimmune regulator) gene (21q22.3)</td>
<td>Hypoparathyroidism</td>
</tr>
</tbody>
</table>

Despite adequate regulating levels. Hypoparathyroidism can result from removal or damage to parathyroid glands during surgery, developmental disorders, autoimmune hypoparathyroidism, PTH defects, and defective regulation of PTH secretion.22 Sporadic idiopathic hypoparathyroidism is a common cause of hypoparathyroidism. Antibodies against the CASR have been identified in some cases, but the pathogenesis remains unclear.41 DiGeorge syndrome is characterized by congenital aplasia of the thymus resulting in deficiency of T-cells, parathyroid aplasia resulting in hypocalcemia, cardiac outflow tract defects, characteristic facies, and short stature.42-44 Kearns–Sayre syndrome is a rare neuromuscular disorder caused by abnormalities of mitochondrial DNA with an onset by young adulthood, hypoparathyroidism, eye movement limitation and pigmentation, weakness, cardiac conduction defects, hearing loss, ataxia, cognitive problems, and diabetes.44

Genetics

The genetic basis of sporadic idiopathic hypoparathyroidism is not known, although antibodies against the CASR have been identified in some cases.41 DiGeorge syndrome is caused by hemizygous deletion of 22q11.2 with most abnormalities due to haploinsufficiency of the TBX1 gene.45 Kearns–Sayre syndrome is caused by abnormalities of mitochondrial DNA.44

Hereditary Hypoparathyroidism

Clinical

Hereditary hypoparathyroidism is relatively rare and encompasses a variety of syndromes. Sanjad–Sakati syndrome includes congenital hypoparathyroidism, mental retardation, and growth failure, while patients with Kenny–Caffey syndrome also have osteosclerosis and recurrent bacterial infections.46 Both are chaperone diseases caused by a defect in tubulin physiology and parathyroid development.46 Velocardiofacial syndrome is autosomal dominant condition typified by neonatal hypocalcemia, cleft palate, cardiac and ocular anomalies, learning disabilities, and T-cell dysfunction. Autoimmune polyglandular syndrome type 1 is an autoimmune disorder affecting endocrine glands and skin.47 Familial isolated hypoparathyroidism is characterized by hypocalcemia and hyperphosphatemia because of inadequate PTH secretion. Jansen metaphyseal chondrodysplasia is a rare form of short-limbed dwarfism with hypercalcemia, hypophosphatemia, and normal to low PTH and PTH-related protein. Blomstrand chondrodysplasia is also associated with parathyroid disease.48 Patients with pseudohypoparathyroidism type 1a (Albright’s hereditary osteodystrophy) have short stature, short metacarpal and metatarsal bones, and round faces, while those with pseudohypoparathyroidism type 1b have hypocalcemia with PTH resistance confined to the kidney and lack other features of Albright’s hereditary osteodystrophy.
15. Nonneoplastic Parathyroid Diseases

Miscellaneous Nonneoplastic Parathyroid Disorders

Parathyroid Cysts

Clinically apparent parathyroid cysts are relatively uncommon and can be functioning with hyperparathyroidism or nonfunctioning and present as a mass or mimicking a cystic thyroid nodule. Functioning parathyroid cysts may represent cystic degeneration of a parathyroid adenoma. Parathyroid carcinoma has been reported in association with a parathyroid cyst. Small microcysts are not uncommon incidental findings at autopsy, while macrocysts are often investigated clinically. The cysts may also be intrathyroidal or mediastinal. Etiologies suggested for parathyroid cysts include developmental abnormalities, fluid accumulation in the parathyroid, coalescence of microcysts into clinical macrocysts, degeneration of a parathyroid neoplasm, and may from vestigial remnants of Kursteiner canals. A literature review of 93 patients with parathyroid cysts identified parathyroid hyperparathyroidism in 39 cases (42%). Over a 25-year period during which 22,009 thyroidectomies and 2,505 parathyroidectomies were performed, 38 nonfunctional parathyroid cysts were identified. The cysts presented as incidental findings on chest X-ray, compressive symptoms, or an asymptomatic palpable neck mass. Histologically, the cyst wall can contain a variety of elements including lymphoid, muscular, thymic, adipose, and mesenchymal tissues (Figure 15.3).

Amyloid

Amyloid can involve both normal and diseased parathyroid glands and can be identified in patients with and without systemic amyloidosis. Amyloid often shows an intracellular distribution in the parathyroid. Amyloid in parathyroid glands shows uniform reactions with Congo red and Thioflavin T, but the pathogenesis of this amyloid remains uncertain.

Parathyroiditis

Parathyroiditis can be autoimmune associated and may result in hypoparathyroidism or hyperparathyroidism.

Glycogen Storage Diseases

Pompe’s disease (type II glycogenosis) is a generalized glycogenosis caused deficiency of lysosomal acid alpha-1,4-glucosidase, which affects the central nervous system, heart, liver, muscle, and endocrine organs, including the parathyroids.
Conclusion

Nonneoplastic parathyroid disease encompasses a great variety of both sporadic and hereditary causes of hyperparathyroidism and hypoparathyroidism. The genetic basis for sporadic parathyroid disease is poorly understood. The genetic basis of hereditary parathyroid disorders is better understood, but the exact mechanisms still need to be further elucidated.

References

Introduction

The understanding of parathyroid diseases, particularly neoplastic processes, has advanced tremendously in the past century and a quarter since the original description of parathyroid glands by Sandström in 1880.1 Parathyroid diseases are classically stratified into two major groups: single gland (and perhaps more controversially, double gland) disease; and multigland disease. Both processes manifest as physiologically, macroscopically and microscopically abnormal parathyroid gland proliferations resulting in enlargement. However, single gland disease is considered neoplastic, consisting of adenomas and more uncommonly, carcinomas, while multigland disease is considered to be nonneoplastic, hence the term hyperplasia.

This grouping is of practical importance as the approach to clinical management differs based on categorization. However, as the understanding of the histologic spectrum of abnormal parathyroid morphology has grown, it has become readily apparent that when examining one gland alone, there are no histological features that reliably separate adenoma from hyperplasia. The distinction is contextual, based on the reference point of the findings of the other glands, clinical history, and more recently the intraoperative parathyroid hormone (PTH) levels. Conversely, molecular findings have suggested that in some cases of multigland disease, particularly in the multiple endocrine neoplasia setting and even in some cases of tertiary hyperparathyroidism, enlarged parathyroids are actually mono or oligoclonal rather than polyclonal as expected in nonneoplastic processes. Ultimately, pragmatism prevails and the current definition of parathyroid adenomas incorporates the functional status of the disease gland and other glands.

Parathyroid adenoma is defined as a benign neoplastic proliferation of a single parathyroid gland that upon removal results in long-term cure.2 Parathyroid carcinoma is a neoplastic parathyroid proliferation that fulfills clinical and/or histologic criteria for malignancy.3 These criteria are controversial and will be discussed in further detail subsequently. Advances in the understanding of molecular alterations in adenomas and carcinomas have resulted in an improved understanding of the similarities and differences from the hyperplasia categories. And from a practical standpoint, molecular testing shows promise particularly in the distinction between adenoma and carcinoma when traditional clinicopathologic parameters are inconclusive.

Sporadic Parathyroid Adenoma and Carcinoma

Clinical

Parathyroid adenomas are the most common cause of primary hyperparathyroidism, comprising 85% of this group. By comparison, parathyroid carcinomas contribute to less than 1% of all primary hyperparathyroidism.4 The annual incidence of adenoma in North America is 20–30 per 100,000 people. Parathyroid adenomas and carcinomas typically occur in the sixth to eighth decade with a female predilection of 2–3:1.5,6 The vast majority of adenomas are indeed sporadic without any identifiable etiologic factors. Adenomas have been epidemiologically linked with radiation exposure.7–9

Clinical manifestations of adenomas are the result of hypercalcemia in the setting of an elevated PTH. With the frequent testing of serum calcium as part of screening metabolic serum chemistry panels, many patients with adenomas have no complaints at initial discovery, though if a thorough clinical history is taken, signs and symptoms of hypercalcemia are found.10 Common clinical findings include nonspecific symptoms of fatigue, musculoskeletal or abdominal aches and pains, constipation, polydipsia, polyuria, and cognitive dysfunction. Other more severe complications rarely occur today, including osteitis fibrosa cystica, pathologic fractures, nephrolithiasis, nephrocalcinosis, renal insufficiency, peptic ulcer disease, hypertension, gout and pancreatitis. Parathyroid carcinomas are more likely to have extreme symptomatology and more often present with osteoporosis.
and nephrolithiasis. They are also more likely to present with a palpable neck mass, and are characteristically “adherent” to surrounding soft tissue and the thyroid because of their local infiltration. Parathyroid carcinoma patients tend to have much higher serum calcium and PTH levels than seen in adenomas. These clinical findings are described in more detail along with preoperative localization and surgical approach in Chap. 14. With current treatment modalities, durable normocalcemia can be attained in 98–100% of patients with parathyroid adenomas. Age, gender, and presence of metastatic disease are considered important prognosticators.

Gross and Histologic Features

Grossly, parathyroid adenomas are enlarged by size and by weight (on average 500 mg) and have a deeper red-brown appearance as compared to normal parathyroid tissue, which reflects a decrease in stromal fat and hyperemia. Cystic change may be present in up to 10% of sporadic adenomas. A rim of paler normal may be noted as well. Oxyphil adenomas (see below) may be mahogany brown, while lipoadenomas (see below) may be pale yellow. Carcinomas are typically larger (2,000–10,000 mg) and may be paler and more fibrotic.

Histologically, the prototypical parathyroid adenoma consists of an encapsulated solid, nested and corded proliferation of chief cells with minimal to no intervening fatty stroma surrounded by a rim of normal parathyroid tissue. However, these features may be occasionally seen in the glands of hyperplasia, and are thus not reliable alone. Practically speaking, any histology with increased parenchymal mass is acceptable as an adenoma if it is the only gland that is abnormal. Variant histologies include oxyphil rich adenomas, comprised of cells with mitochondria rich abundant granular eosinophilic modified chief cells (oxyphils), and lipoadenomas which show a paradoxic increase in fatty stroma along with cell mass. Parathyroid adenomas of all types generally have small round nuclei with only rare if any mitoses. The atypia present is often random, similar to that seen in most endocrine organ sites.

Minimal histologic criteria for defining parathyroid malignancy, and whether or not to incorporate clinical findings as criteria for malignancy (i.e., marked hypercalcemia and adherence to surrounding tissue) are up for debate. Histologically, parathyroid carcinomas tend to have broad fibrous bands and a trabecular growth pattern. Elevated mitotic counts are more frequent. Paradoxically, carcinomas may be more monomorphic than adenomas. However, these features may be seen in noninvasive parathyroid tumors that behave in a benign fashion, as well. Definitive criteria for malignancy that are generally accepted without argument are: vascular invasion, soft tissue/extracapsular invasion, thyroid invasion, perineural invasion, and nodal or distant metastatic disease.

Parathyroid neoplasms that are clinically worrisome, and/or have fibrosis, trabecular growth or elevated mitotic rates, but do not have definitive criteria for malignancy are labeled as “atypical adenomas.”

Histochemistry and Immunohistochemistry

Histochemical and immunohistochemical features of parathyroid neoplasms are summarized in Table 16.1. Histochemical stains for lipid such as Oil red O may prove the hyperfunctional status in adenomas and carcinomas by demonstrating depleted or absent intracytoplasmic lipid. Immunohistochemistry is usually of little value in parathyroid adenomas, except occasionally in the distinction between carcinoma. Here, a Ki-67 proliferative index greater than 6% is suggestive of a carcinoma, though many carcinomas have a proliferation index of less than 1%. Other immunostains used to distinguish adenoma from carcinoma are often surrogates for molecular markers. Losses of pRB1, p27, parafibromin protein immunoeexpression in carcinomas are reflective of alterations at the corresponding gene loci that are more frequently seen in carcinomas as compared to adenomas (see below). More recently, galectin-3 has been observed to be preferentially expressed in carcinomas. Cyclin D1, while frequently overexpressed in parathyroid neoplasia, does not discriminate between adenoma and carcinoma. Additionally, mechanisms (see below) for overexpression in these two entities may be different. Immunostains may also

| Table 16.1. Histochemical and immunohistochemical characteristics of parathyroid neoplasms. |
|-----------------------------------------------|----------------|----------------|-----------------
| Lipid stain (Oil Red O, Sudan IV)             | Normal parathyroid | Adenoma          | Carcinoma       |
| Vitamin D receptor                            | Abundant coarse globules | Decreased       | Decreased       |
| Calcium sensing receptor                      | Strong and diffuse | Decreased (2–30%) | Markedly decreased |
| Cyclin D1                                     | Rare cells positive | Positive (2–20%) | Positive (60%) |
| P27                                           | Diffusely positive | Decreased        | Markedly decreased |
| pRB                                           | Diffusely positive | Positive (but heterogeneous) | Absent (50–100%) |
| Parafibromin                                  | Diffusely positive | Diffusely positive (absent in FHJTS associated adenomas and <5% of sporadic adenomas) | Absent (70–100%) |
| Galectin-3                                    | Negative          | Negative (<5% positive) | Positive (>90%) |
| Ki-67                                         | Mean LI=1%        | Mean LI=2%       | Mean LI=8% (diagnostic if >6%) |
| **FHJTS** familial hyperparathyroidism jaw tumor syndrome, **LI** labeling index. |
be used to suggest hyperfunctioning status in a similar fashion to Oil red O stains. Calcium sensing receptor (CaSR) and vitamin D receptor (VDR) immunostains show a lower staining intensity in adenomas and an even lower intensity in carcinomas as compared to normal since these PTH modulators are decreased in response to the persistently elevated serum calcium levels.32,33

**Important Pathways**

In sporadic parathyroid neoplasms, adenomas and carcinomas, there are three different genes that define the important known pathways of tumorigenesis: CCND1/PRAD1, MEN1, and HRPT2. It is important to note that these alterations do not define separate carcinogenesis pathways, as more than one mutation can be seen in a parathyroid neoplasm.

**Genetics**

Commonly altered genes in parathyroid neoplasia, sporadic, and familial (see below) are summarized in Table 16.2.

**Oncogenes**

Among the first, and the major gene implicated in parathyroid adenoma development is CCND1/PRAD1 (chromosome 11q13) which encodes cyclin D1. Cyclin D1 functions by binding to the cyclin dependent kinases (Cdk4 and Cdk6) kinase complex that phosphorylates retinoblastoma protein (RB). RB represses the E2F transcription factor, but on phosphorylation, this repression is lost. Through a series of transcription events, this ultimately facilitates the transition of a cell from the G1 to the S-phase. Cyclin D1 thus promotes cell growth and can function as an oncogene. The most common known mechanism for overexpression of cyclin D1 is a pericentromeric gene rearrangement that brings the regulatory sequences of the PTH gene (on chromosome 11p15) in close proximity to the CCND1/PRAD1 gene.34,35 The simplest form of this would be a chromosomal inversion – inv(11) (p15; q13). However, breakpoints vary resulting in a distance between the PTH regulatory element and CCNR/PRAD1 that ranges from 15 to 200 kb.36 Additionally, not all cyclin D1 overexpression adenomas have this inversion – other mechanisms may exist for this overexpression. Nonetheless, cyclin D1 is overexpressed in 20–40% in sporadic adenomas, supporting an integral role in tumorigenesis.37 Furthermore, transgenic mouse models with this PTH directed cyclin D1 overexpression develop primary hyperparathyroidism.38

The original methodology used to assess for this rearrangement was restriction enzyme digestion and southern blot with a radiolabeled PTH probes.39,40 The ease and convenience of immunohistochemical staining of tissue sections has supplanted the approach to identify the gene rearrangement, particularly because of the variety and complexity of breakpoints.37 Another common mechanism of cyclin D1 overexpression seen in other tumors, namely via CCND/PRAD1 gene amplification has not been described in parathyroid adenomas. By immunohistochemistry, parathyroid carcinomas overexpress

<table>
<thead>
<tr>
<th>Oncogenes</th>
<th>Adenoma</th>
<th>Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CCND1/PRAD1</strong></td>
<td>Overexpression</td>
<td>Overexpression</td>
</tr>
<tr>
<td>Ch. 11q13</td>
<td>Pericentromeric inversion placing PTH promoter near CCND1/PRAD1 gene (5–10%)</td>
<td>Mechanism unknown possibly secondary</td>
</tr>
<tr>
<td>Encodes cyclin D1</td>
<td>Other mechanisms unknown</td>
<td>Not described</td>
</tr>
<tr>
<td><strong>RET</strong></td>
<td>Not seen in sporadic cases</td>
<td>Not described</td>
</tr>
<tr>
<td>Ch. 10q11</td>
<td>Seen only in familial syndromes (MEN-2A) (20–30%)</td>
<td>Germline mutation typically in codon 634 resulting in constitutive activation</td>
</tr>
<tr>
<td>Encodes receptor tyrosine kinase</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tumor suppressor genes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MEN</strong></td>
<td>Somatic mutations (sporadic cases ~20%) leading to loss of function</td>
<td>Somatic mutations in almost 15% (sporadic)</td>
</tr>
<tr>
<td>Ch. 11q13</td>
<td>LOH inactivation of second allele (20–30%)</td>
<td>&lt;Carcinomas in MEN-1 syndrome are exceptionally rare&gt;</td>
</tr>
<tr>
<td>Encodes Menin</td>
<td>Biallelic mutations rare</td>
<td>Somatic mutations common (sporadic cases 66–100%)</td>
</tr>
<tr>
<td><strong>HRPT2</strong></td>
<td>Somatic mutations leading to loss of function uncommon (sporadic cases &lt;10%, sporadic cystic tumors 20%)</td>
<td>Germline mutations characteristic of carcinomas in FHJTS</td>
</tr>
<tr>
<td>Ch. 1q21-23</td>
<td>Germline mutations characteristic of adenomas in FHJTS</td>
<td>LOH inactivation of second allele</td>
</tr>
<tr>
<td>Encodes parafibromin</td>
<td>LOH inactivation of second allele</td>
<td>Rare cases with hypermethylation</td>
</tr>
<tr>
<td><strong>RB1</strong></td>
<td>LOH in up to 30%</td>
<td>LOH in up to 85%</td>
</tr>
<tr>
<td>Ch. 13q14</td>
<td>Other mutations not described</td>
<td>Other mutations not described</td>
</tr>
<tr>
<td>Encodes RB protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BRCA2</strong></td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Ch. 13q12-14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Encodes Brca2 protein</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
cyclin D1 in up to 60% of cases.\textsuperscript{32} The molecular mechanism for this is not well studied, but may be related to alterations of parafibromin (see below).

While the germline mutations in the \textit{RET} proto-oncogene define the multiple endocrine neoplasia syndrome 2 (MEN-2), somatic mutations have not been seen in sporadic parathyroid adenomas or carcinomas.\textsuperscript{41}

\textbf{Tumor Suppressor Genes}

The \textit{MEN1} gene is also located on chromosome 11q13, but functions as a putative tumor suppressor gene. While this gene is typically associated, as its name implies, with multiple endocrine neoplasia 1, this gene is commonly altered in sporadic adenomas. This gene encodes menin, a protein that interacts with the TGF-\textbeta/activin signaling pathway.\textsuperscript{42} TGF-\textbeta inhibits the growth of most cells via interaction with type I and II serine–threonine kinase receptors. TGF-\textbeta binds to the constitutively phosphorylated type II receptor which in turn transphosphorylates a type I receptor. This results in the phosphorylation of the SMAD complex which then translocates to the nucleus to initiate transcription. Menin normally binds to SMAD-3 in the nucleus and facilitates transcription. Thus, when menin is altered, the TGF-\textbeta mediated inhibition is weakened facilitating cell growth. Loss of heterozygosity (LOH) at the \textit{MEN1} gene locus can be observed in 20–30% of parathyroid adenomas, by FISH and or PCR based microsatellite analysis.\textsuperscript{43,45} A fraction of these cases will have demonstrable mutations of the other allele.\textsuperscript{44,46} These mutations span exons 2–10 of the \textit{MEN1} gene.\textsuperscript{57} To date, 203 of the 1,336 mutations noted to date are described in somatic disease.\textsuperscript{48} There do not appear to be any mutational hotspots in sporadic or familial disease.\textsuperscript{49} But somatic mutations are more likely to involve exon 2, though the significance of this finding is unclear.\textsuperscript{50}

The standard method for detection of these mutations include direct sequencing of the gene, occasionally preceded by screening for mutations by single strand conformation polymorphism (SSCP) or dideoxy fingerprinting (ddF).\textsuperscript{19} Since several different mutations have been described, a directed test such as restriction site based analysis or amplification of a limited portion of the gene will not reliably detect a large percentage of mutations. In rare instances, instead of LOH and a mutation of the remaining allele, parathyroid adenomas may show biallelic inactivating mutations.\textsuperscript{50} While initially only reported rarely, somatic \textit{MEN1} mutations may be seen in almost 15% of parathyroid carcinomas based on recent studies.\textsuperscript{32,45}

Another relatively more recently characterized gene is \textit{HRPT2}, which is mapped to chromosome 1q21–23 and is also an important gene in parathyroid tumorigenesis, particularly in carcinomas. \textit{HRPT2} mutations may be noted in 66–100% of sporadic parathyroid carcinomas, but are only detected in about 8% of sporadic adenomas.\textsuperscript{3,32,45,51} Tumors with this alteration are often cystic, and in sporadic cystic adenomas the prevalence of LOH in the 1q region is as high as 20%.\textsuperscript{53} \textit{HRPT2} is composed of 17 exons and encodes a 531 amino acid protein parafibromin which is involved in the RNA polymerase/Paf-1 complex and thus has a presumed histone deacetylation role.\textsuperscript{32} This tumor has a presumed tumor suppressor function in parathyroid glands, since the described mutations in \textit{HRPT2} lead to impaired parafibromin function. But the specific mechanism of growth inhibition by wild-type parafibromin is unclear. One study indicates that parafibromin normally inhibits cyclin D1 expression.\textsuperscript{53} Thus, a potential model of neoplasia is the loss of this repression with \textit{HRPT2} mutations. But whether the effect of parafibromin on cyclin D1 is direct or mediated through other steps has not been resolved.

Similar to \textit{MEN1} mutations of \textit{HRPT2} are detected by direct sequencing, often preceded by a screening procedure such as SSCP. Mutations that have been described span all 17 exons, thus detection strategies would have to be fairly comprehensive. The mechanism for inactivation of the second allele is usually LOH, which can be assessed via amplification of microsatellite markers that span this gene locus.\textsuperscript{49} Loss of parafibromin protein expression by immunohistochemistry may serve as a surrogate for gene alterations.\textsuperscript{54} Interestingly, parathyroid carcinomas arising in the background of secondary/tertiary hyperparathyroidism (which are exceptionally rare) do not show loss of parafibromin stain suggesting a different pathogenesis.\textsuperscript{55}

The gene \textit{RB1} on chromosome 13q14 encodes RB protein which as mentioned earlier interacts with the cyclin D1/Cdk 4/6 complex to direct the transition from the G1 to S phase. Inactivation of this gene has been linked not only to retinoblastoma, but to several other malignancies as well. Up to 100% of parathyroid carcinomas show LOH at \textit{RB1}, but this alteration is not specific for malignancy as it may be seen in up to 30% of adenomas as well. The role of \textit{RB1} in parathyroid carcinogenesis is debated.\textsuperscript{50,56} Despite the high frequency of LOH, \textit{RB1} point mutations, deletions, and insertions have not been described suggesting the possibility that another gene in this region may be responsible for tumorigenesis with \textit{RB1} loss being an epiphenomenon.

The gene \textit{BRCA2}, on chromosome 13q12–14 has also been implicated in parathyroid carcinogenesis. LOH at this gene locus has been reported in up to 85% of carcinomas in one small set of cases. It is often found in conjunction with loss of \textit{RB1}, and similarly, point mutations, insertions, and deletions at this gene locus have not been described in parathyroid tumors raising doubts about its contribution to tumorigenesis.\textsuperscript{56}

\textbf{Epigenetics}

Understanding of epigenetic alterations contributing to parathyroid tumorigenesis is sparse. One study has indicated that in a few cases, hypermethylation of \textit{HRPT2} as assessed by methylation specific PCR is also a mechanism for inactivation in parathyroid carcinoma.\textsuperscript{57}
**Special Techniques**

One fundamental biologically relevant question is the validity of clinicopathologic classification of parathyroid disease – is single gland disease truly monoclonal with multigland disease (hyperplasia) being a reactive polyclonal proliferation? Studies using various X-linked inactivation based methodologies generally support the notion that single gland disease is indeed monoclonal with at least 75% of adenomas showing a monoclonal pattern of hybridization. However, clonal patterns of X-linked inactivation may be demonstrated in hyperplasias as well particularly in the nodular form.46,58,59 One caveat is that the X-inactivation patch size in parathyroid is not well described, and if similar to thyroid,60 the apparent clonality in multigland disease may simply reflect a large patch size.

Only a handful of genes involved in parathyroid tumorigenesis are well characterized (see above). However, genomic profiling has been performed on these tumors with the potential to identify additional candidate genes. In addition to losses at chromosome 11q13 (where MEN1 resides), losses at chromosomes 1, 6q, 11q23, 13q and 15q are frequent as noted by comparative genomic hybridization (CGH) suggesting the presence of additional candidate tumor suppressor genes in this region.61–63

Emerging gene array data has suggested the capability of distinguishing adenoma from hyperplasia. One custom designed array demonstrated differential kinase expression profiles between adenomas and hyperplasia.64 In a comprehensive survey by Haven et al,65 parathyroid disease was resolved into three basic categories based on gene expression profile: (1) hyperplasia, (2) adenomas, MEN associated hyperplasias, and tertiary hyperplasias, and (3) carcinomas and HRPT associated adenomas. The implications are that MEN associated hyperplasias may be more accurately considered “adenomatoses,” and that most adenomas are not precursors to carcinoma, which requires a specialized pathogenesis. Adenoma size may also be a determinant of gene expression, with a variety of calcium ion binding proteins being overexpressed in larger adenomas.66

Panel based microsatellite assessment at several known tumor suppressor gene loci for LOH is a popular, clinically available methodology that has been applied to several tumor types. Since the known biology of parathyroid disease is replete with tumor suppressor genes, some of which are selectively expressed in carcinomas, an LOH panel can help distinguish between adenoma and carcinoma in clinicopathologically challenging cases. In addition to specific gene loci, a panel can generate a fractional allelic loss (FAL) value that is to some extent an estimate of genetic abnormalities. Hunt et al67 demonstrated that the FAL for a small set of parathyroid carcinomas was significantly higher than for adenomas and hyperplasias.

**Hereditary**

**Background**

**Clinical**

Single gland disease in the setting of familial hyperparathyroidism is less common than multigland disease. As such, the MEN syndromes are discussed in more detail in Chap. 11. However, as noted previously, by gene expression profile, MEN associated multigland disease may be more aptly considered an adenomatosis rather than a hyperplasia.65 Parathyroid disease in MEN-1 syndrome is invariably multigland disease. MEN-2 syndrome on the other hand may present with less than four gland involvement or even with only one enlarged gland, but should be treated as a disease state with potential multigland involvement.66 FHJTS is one familial hyperparathyroidism syndrome, in which single gland disease, i.e., adenomas are fairly common. With this syndrome, up to 15% of patients develop parathyroid carcinoma. Up to 30% will also develop ossifying fibromas of the mandible.66 Other extra parathyroid manifestations include renal lesions including simple cysts, mixed epithelial stromal tumors, and Wilms tumors, and more recently uterine mesenchymal neoplasms including leiomyomas and adenomyomas.66

**Gross and Histologic Features, Histochemistry and Immunohistochemistry**

Grossly, parathyroid neoplasms in MEN syndromes are similar in appearance to their sporadic counterparts. Parathyroid tumors in FHJTS on the other hand are notoriously cystic.19 The histologic spectrum and immunophenotype of familial parathyroid tumors is similar to sporadic tumors. One caveat is that even histologically normal appearing parathyroid tissue in MEN patients may be physiologically abnormal.

**Important Pathways**

As with sporadic parathyroid disease, MEN1 and HRPT2 are important genes in the pathogenesis of familial hyperparathyroidism. Here, however, mutations in the RET proto-oncogene also play a role in pathogenesis.

**Genetics**

**Oncogenes**

**RET** is a 20 exon gene located on chromosome 10q11 and encodes a receptor tyrosine kinase required for cell growth and differentiation. Common mutations in MEN-2A result in the disruption of cysteine residues that are normally involved disulfide bonding. This disruption leads to homodimerization with another unpaired mutant RET molecule resulting in constitutive activation. Parathyroid involvement in MEN-2A
is more variable than MEN-1 with only 20–30% of patients manifesting with parathyroid disease. Mutations tend to cluster around exons 10–16, and point mutations of codon 634 result in a higher likelihood of parathyroid disease involvement.71

**Tumor Suppressor Genes**

HRPT2 shows germline mutations in FHJTS as opposed to the somatic mutations seen in sporadic adenomas and carcinomas (see detailed discussion above). The relatively high risk of carcinoma in FHJTS kindreds supports its role in malignant transformation.52,65,66 In contrast, MEN-1 syndrome does not appear to have an increased risk of carcinomatosis,45 even though somatic mutations of the MEN gene are not uncommon in sporadic carcinomas.

**Epigenetics**

While genomic imprinting has been known to effect certain phenotypes of familial hypoparathyroidism/pseudohypoparathyroidism, such phenomena have not been described in familial hyperparathyroidism states.

**Special Techniques**

As noted earlier, parathyroid proliferations in the setting of MEN are mono or oligoclonal rather than polyclonal as expected in a truly hyperplastic process. It is also interesting to note that parathyroid disease in the setting of MEN syndromes, whether single or multigland disease, clusters by gene array with adenomas (see above). Gene expression profiling lends credence to the notion that HRPT2 related parathyroid neoplasms represent a distinct tumorigenesis pathway. All tumors that harbor these mutations, including FHJTS adenomas, parathyroid carcinomas, and tumors arising in familial isolated hyperparathyroidism appear to form one main cluster.

**Summary**

Parathyroid neoplasms are traditionally considered to be represented by single (and rarely double) gland disease, namely adenomas and carcinomas. For sporadic adenomas, key gene alterations include: cyclin D1 overexpression, prototypically secondary to a pericentromeric inversion of chromosome 11 that places the PTH gene promoter adjacent to CCND1/PRAD1 gene; and somatic MEN mutations. For sporadic carcinomas, mutations on HRPT2 are the most common and significant, though they may be seen a subset of adenomas as well. LOH at the RB1 and BRCA2 loci are common, but not restricted to carcinoma, but other mutations (insertions, deletions, etc.) have not been described raising doubts about the primary significance of these particular alterations. Chromosomal losses at 1, 6q, 11q23, 13q, and 15q suggest the presence of additional tumor suppressor genes at these loci. Evaluation of LOH across multiple loci may be clinically useful in cases where the distinction between adenoma and carcinoma is particularly challenging.

Familial parathyroid proliferations are generally multigland disease and grouped with hyperplasias. However, clonality studies and gene expression profiling suggest that these hyperplasias are, from a biologic standpoint, “adenomatoses.” Unlike sporadic parathyroid neoplasms, activating mutations of the RET proto-oncogene do have a role in familial hyperparathyroidism with mutations of the codon 634 being most commonly associated with hyperparathyroidism. FHJTS is defined by germline mutations on HRPT2, and predispose affected patients to a relatively high risk of carcinoma, while MEN syndrome patients do not. Genotypically, all HRPT2 associated parathyroid neoplasms (both benign and malignant) cluster separately from other parathyroid disease, suggesting a fundamentally unique pathogenesis.

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Section 4
Pituitary Diseases
Clinical Detection and Treatment of Benign and Malignant Pituitary Diseases

Dima L. Diab and Amir H. Hamrahian

Introduction

Anatomy and Physiology of the Pituitary Gland

The pituitary gland weighs about 0.5–1 g and is divided into an anterior and a posterior lobe. The pituitary gland sits in the sella turcica immediately behind and superior to the sphenoid sinus. Cavernous sinuses are located laterally on each side of the sella and include the internal carotid artery and cranial nerves III, IV, V1, V2, and VI. Magnetic resonance imaging (MRI) is the best method for visualization of hypothalamic–pituitary anatomy (Figure 17.1).

Anterior pituitary hormones are regulated by hypothalamic releasing and inhibitory hormones as well as negative feedback action of the target glandular hormones at both the pituitary and hypothalamic levels (Table 17.1). Among the pituitary hormones, only secretion of prolactin is increased in the absence of hypothalamic influence since it is mainly under tonic suppression by dopamine, the main prolactin inhibitory factor.1 Antidiuretic hormone (ADH, Vasopressin) is produced by the supraoptic and paraventricular nuclei of the hypothalamus and travels in axons through the pituitary stalk to the posterior pituitary gland.

Benign Diseases: Pituitary Adenomas

Definition and Prevalence

Pituitary adenomas arise from adenohypophyseal cells and are almost always benign (Table 17.2). They are arbitrarily designated as microadenomas (<10 mm in diameter) and macroadenomas (≥10 mm in diameter) (Figure 17.2). The true incidence and prevalence of pituitary adenomas is difficult to establish; however, autopsy studies suggest that up to 20% of normal individuals may harbor pituitary microadenomas.2 Those that are discovered by CT or MRI examination, in the absence of any symptoms or clinical findings suggestive of a pituitary disease, are referred to as pituitary incidentalomas.3 Recent advances in molecular biology have confirmed that most pituitary adenomas are monoclonal in origin. Some possible underlying mechanisms include overexpression of pituitary oncogenes, inactivation of tumor suppressor genes, hypersecretion of hypothalamic-releasing hormones and/or hyposecretion of inhibitory hormones.4,5

Pituitary adenomas are rarely associated with parathyroid and neuroendocrine hyperplasia or neoplasia as part of the multiple endocrine neoplasia type I (MEN I) syndrome. Other rare genetic disorders associated with pituitary tumors include Carney syndrome, MacCune–Albright syndrome, and familial pituitary disorders such as familial acromegaly.

Signs and Symptoms

Pituitary tumors may present with signs and symptoms related to mass effect (Table 17.3), including pituitary insufficiency and/or hormonal hypersecretion. Pituitary adenomas are the most common cause of hypopituitarism, but other causes include parasellar diseases, pituitary surgery, radiation therapy, inflammatory and granulomatous diseases, and head injury. The sequential loss of pituitary hormones deficiency secondary to a mass effect is in the following order: GH, LH/FSH, TSH, ACTH, and prolactin.1 Isolated deficiency of various anterior pituitary hormones may occur. In general, pituitary microadenomas are rarely associated with hypopituitarism. Diabetes insipidus is almost never seen in patients with pituitary adenomas during initial presentation, except in those with very large tumors extending superiorly, affecting the hypothalamus.

Impingement on the chiasm or its branches by a pituitary tumor may result in visual field defects, most commonly as bitemporal hemianopsia. Lateral extension of the pituitary mass to the cavernous sinuses may result in diplopia, ptosis, or altered facial sensation. Among the cranial nerves, the third nerve is the most commonly affected. There is no specific headache pattern associated with pituitary tumors except SUNCT (short lasting unilateral neuralgiform headache with conjunctival injection and tearing). In some patients, the headache is unrelated to the pituitary adenoma.
Diagnosis

Pituitary MRI is the preferred diagnostic imaging technique in patients suspected to have pituitary pathology including pituitary adenomas (Figure 17.2). Once a pituitary adenoma is found, it is necessary to assess its type (functional vs. nonfunctional), pituitary function, and the presence of mass effect such as visual field defects, especially in those with macroadenomas. Hormonal evaluation can be usually performed on an outpatient basis.

Table 17.1: Pituitary: relationship among hypothalamic, pituitary, target glands, and feedback hormones.

<table>
<thead>
<tr>
<th>Hypothalamic regulatory hormone</th>
<th>Pituitary hormone</th>
<th>Target gland</th>
<th>Feedback hormone</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRH</td>
<td>TSH</td>
<td>Thyroid gland</td>
<td>T4, T3</td>
</tr>
<tr>
<td>LHRH</td>
<td>LH</td>
<td>Gonad</td>
<td>E2, T</td>
</tr>
<tr>
<td>LHRH</td>
<td>FSH</td>
<td>Gonad</td>
<td>Inhibin, E2, T</td>
</tr>
<tr>
<td>GHRH, SMS</td>
<td>GH</td>
<td>Multiorgans</td>
<td>IGF-1</td>
</tr>
<tr>
<td>PIF</td>
<td>Prolactin</td>
<td>Breast</td>
<td>?</td>
</tr>
<tr>
<td>CRH, ADH</td>
<td>ACTH</td>
<td>Adrenal</td>
<td>Cortisol</td>
</tr>
</tbody>
</table>

ADH antidiuretic hormone; CRH corticotropin-releasing hormone; E2 estradiol; GHRH growth hormone-releasing hormone; LHRH luteinizing hormone-releasing hormone; PIF prolactin release-inhibitory factor; SMS somatostatin; T testosterone; TRH thyrotropin-releasing hormone.

Table 17.2: Pituitary: prevalence of pituitary adenomas.

<table>
<thead>
<tr>
<th>Adenoma type</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH cell adenoma</td>
<td>15</td>
</tr>
<tr>
<td>PRL cell adenoma</td>
<td>30</td>
</tr>
<tr>
<td>GH and PRL cell adenoma</td>
<td>7</td>
</tr>
<tr>
<td>ACTH cell adenoma</td>
<td>10</td>
</tr>
<tr>
<td>Gonadotroph cell adenoma</td>
<td>10</td>
</tr>
<tr>
<td>Nonfunctioning adenoma</td>
<td>25</td>
</tr>
<tr>
<td>TSH cell adenoma</td>
<td>1</td>
</tr>
<tr>
<td>Unclassified adenoma</td>
<td>2</td>
</tr>
</tbody>
</table>

GH growth hormone; PRL prolactin; ACTH adrenocorticotropic hormone; TSH thyroid-stimulating hormone.

Table 17.3: Pituitary: clinical manifestations of pituitary tumors secondary to mass effect.

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td></td>
</tr>
<tr>
<td>Chiasmal syndrome</td>
<td></td>
</tr>
<tr>
<td>Cranial nerves III, IV, V1, V2 and VI dysfunction</td>
<td></td>
</tr>
<tr>
<td>Hypothalamic syndrome*</td>
<td></td>
</tr>
<tr>
<td>Disturbances of thirst, appetite, satiety, sleep, and temperature</td>
<td></td>
</tr>
<tr>
<td>Diabetes insipidus</td>
<td></td>
</tr>
<tr>
<td>SIADH (syndrome of inappropriate ADH secretion)</td>
<td></td>
</tr>
<tr>
<td>Obstructive hydrocephalus*</td>
<td></td>
</tr>
<tr>
<td>Frontal and temporal lobe syndromes*</td>
<td></td>
</tr>
<tr>
<td>CSF rhinorrhea</td>
<td></td>
</tr>
</tbody>
</table>

*Very rare and may only be associated with giant pituitary tumors with significant superior extension.

Treatment

The goals for treatment of a pituitary tumor include reduction or complete removal of the tumor, elimination of mass effect if present, normalization of hormonal hypersecretion, and restoration of normal pituitary function. Some patients, especially those with large tumors, may require several therapeutic modalities including medical, surgical, and radiation therapy. The most important factor in pituitary surgery is availability of a good neurosurgeon. In general, once a patient has been diagnosed with a pituitary tumor, lifelong
medical follow-up is necessary to detect early recurrence, to monitor hormone replacement, and to treat any complication related to the tumor.

**Prolactinoma**

Clinical features of prolactinomas may be related to excess prolactin and associated secondary hypogonadism or mass effect. All patients with macroprolactinomas and most patients with microprolactinomas require treatment. Some indications for treatment of patients with microprolactinomas include bothersome galactorrhea, oligomenorrhea/amenorrhea, infertility, and sexual dysfunction. Medical therapy with dopamine agonists is the therapy of choice for most patients, since medical therapy is effective in decreasing adenoma size and restoration of normal prolactin level in majority of patients. Dopamine agonists usually restore visual field defects to an extent similar to surgery. Therefore, visual field defects associated with prolactinomas are not a neurosurgical emergency. Cabergoline and bromocriptine are potent inhibitors of PRL secretion and often result in tumor shrinkage. Dopamine agonists should be initiated slowly, since side effects often occur at the beginning of treatment. The most common side effects of dopamine agonists include nausea, headache, dizziness, nasal congestion, and constipation. Bromocriptine is the drug of choice in women who want to become pregnant due to considerable worldwide experience with the drug. Cabergoline is more potent, may be taken only twice a week, and is better tolerated by most patients. There have been some reports about association of high dose ergot-derived dopamine agonists, such as cabergoline and pergolide, with valvular heart disease, especially in patients with Parkinson’s disease. Considering the superior efficacy and tolerability of cabergoline, routine switching of patients on cabergoline to bromocriptine is not indicated at this point. However, periodic echocardiogram surveillance in patients on long-term therapy with cabergoline is recommended until further studies involving patients on low dose therapy are available.

Surgery is reserved for patients who are intolerant of or refractory to medical therapy. Radiation therapy may be considered for patients who poorly tolerate dopamine agonists and can not be cured by surgery (e.g., tumor invasion of cavernous sinuses).

**Acromegaly**

Clinical features of acromegaly may be related to excess GH/IGF-1 or associated mass effect including hypopituitarism, since most patients present with pituitary macroadenomas (Table 17.4). Measurement of IGF-1 is the initial screening test of choice (Figure 17.3). Acromegaly is associated with increased morbidity and mortality if untreated. The goal of therapy for most patients is to achieve a normal sex and age-adjusted IGF-1 and GH < 1 ng/mL. Surgery is the treatment of choice for most patients presenting with acromegaly even if a cure cannot be achieved. Even a subtotal resection of the tumor will improve the efficacy of subsequent adjuvant therapy. Conventional fractionated radiotherapy is very effective for tumor size control but multiple studies have shown poor long-term results in regard to normalization of IGF-1 level. Radiosurgery (Gamma knife) achieves remission faster and is more effective in normalization of IGF-1 compared to fractionated radiotherapy, but the rate of hypopituitarism seems to be similar.

Medical treatment of acromegaly has gained significance since the limitations of radiations and surgical therapy have become evident. Somatostatin analogues inhibit GH secretion mainly by binding to somatostatin receptors type 2 and 5 and result in normalization of IGF-1 in up to 65% of patients. The most common side effects are gastrointestinal, including diarrhea, abdominal pain, and nausea. Gallbladder sludge and cholelithiasis have been reported in up to 25% of patients on long-term therapy with somatostatin analogues but the majority are asymptomatic. Dopamine agonists have been reported to have variable efficacy in patients with acromegaly but may be an attractive first line medical therapy especially in those with cosecretion of prolactin and GH. Pegvisomant has increased affinity to GH receptors as compared to native GH but inhibits its dimerization, which is necessary for the action of GH. It is administered once daily and is usually reserved for those not responding to other medical therapies. It is very effective, with normalization of IGF-1 seen in up to 95% of patients. Due to its mechanism of action and associated increase in GH during therapy, the tumor size needs to be monitored during therapy. Liver function test needs to be monitored during therapy and the drug to be discontinued if liver enzymes increase to more than three times the upper limit of normal. During therapy with Pegvisomant, IGF-1 should be used to monitor therapy. Recently, it was shown that activation of the Ret receptor blocked somatotroph tumor growth in mice, providing a novel potential therapeutic target for treating GH-cell adenomas.

**Table 17.4. Pituitary clinical features in patients with Acromegaly.**

<table>
<thead>
<tr>
<th>Symptom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acral enlargement</td>
</tr>
<tr>
<td>Arthralgias, neuropathic joints</td>
</tr>
<tr>
<td>Carpal tunnel syndrome</td>
</tr>
<tr>
<td>Coarsening of facial features</td>
</tr>
<tr>
<td>Excessive sweating</td>
</tr>
<tr>
<td>Goiter</td>
</tr>
<tr>
<td>Hypertension, congestive heart failure</td>
</tr>
<tr>
<td>Impaired glucose tolerance, diabetes mellitus</td>
</tr>
<tr>
<td>Macroglossia</td>
</tr>
<tr>
<td>Malocclusion and tooth gaps</td>
</tr>
<tr>
<td>Pituitary mass effect including headache and visual field defect</td>
</tr>
<tr>
<td>Pituitary insufficiency</td>
</tr>
<tr>
<td>Sensory and motor peripheral neuropathy</td>
</tr>
<tr>
<td>Snoring, sleep apnea</td>
</tr>
<tr>
<td>Symptoms associated with hyperprolactinemia</td>
</tr>
<tr>
<td>Thick and course skin, skin tags</td>
</tr>
</tbody>
</table>
Cushing’s Disease

Clinical features of Cushing’s syndrome is shown in Table 17.5. Once the diagnosis is established (Figure 17.4), surgical removal of the ACTH-secreting pituitary tumor is the treatment of choice. Availability of an experienced neurosurgeon is crucial with up to 80% long-term remission rate following surgery. A low (<3 µg/dL) or undetectable cortisol level postoperatively off glucocorticoids is considered to be a good marker for long-term cure. For those not cured by the surgery, other options include reoperation and radiotherapy. Bilateral adrenalectomy is reserved for those who continue to be hypercortisolemic. Medical therapy for Cushing’s syndrome has limited value because of the associated toxicity and gradual decrease in efficacy. It is mainly used for short-term symptomatic control or preparation of patients prior to surgery. Among the available agents, ketoconazole and metyrapone are the most commonly used. During therapy, liver function tests need to be closely monitored. Some reports indicate that the use of cabergoline may be effective in at least a subpopulation of patients with CS, which may be worth a trial before initiating ketoconazole. Mifepristone, a cortisol receptor antagonist is currently undergoing clinical trials with promising results. Finally, a novel target for Cushing’s disease therapy has been proposed, whereby bone morphogenetic protein-4 (BMP-4), which is induced by the retinoic acid pathway, was shown to inhibit corticotroph cell tumor growth in mice.

TSH-Secreting Adenoma (TSHoma)

Patients usually present with clinical and biochemical evidence of hyperthyroidism without suppressed TSH. In patients with TSH-secreting adenomas, surgery is the primary therapeutic approach. Radiation is generally used for those with residual tumor. Somatostatin analogues are effective in most patients for control of excess TSH production leading to improvement in hyperthyroidism and possibly to a decrease in tumor size. Beta blockers should be initiated in patients with uncontrolled hyperthyroidism and antithyroid medications may be used only for a short period prior to surgery (if somatostatin analogs cannot be used) since long-term usage may result in stimulation of tumor growth.

Nonfunctional/Glycoprotein-Secreting Pituitary Adenoma

Patients with small nonfunctional pituitary adenomas are usually observed; however, the standard treatment for those with mass effect is surgery mainly through the transsphenoidal approach. Radiotherapy is indicated in those with

<table>
<thead>
<tr>
<th>Table 17.5. Pituitary: clinical features suggestive of Cushing’s syndrome.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central obesity</td>
</tr>
<tr>
<td>Unexplained osteoporosis</td>
</tr>
<tr>
<td>Proximal myopathy</td>
</tr>
<tr>
<td>Wide purplish striae (&gt;1 cm)</td>
</tr>
<tr>
<td>Facial plethora</td>
</tr>
<tr>
<td>Spontaneous bruising</td>
</tr>
<tr>
<td>Hypokalemia</td>
</tr>
<tr>
<td>Serial photographs</td>
</tr>
</tbody>
</table>

Fig. 17.3. Diagnostic work up of acromegaly.
residual pituitary tumor following surgical debulking or in those who may not be surgical candidates. The use of high-dose dopamine agonists has been associated with a decrease in tumor size in about 10% of such patients.\textsuperscript{16}

Malignant Diseases

Pituitary carcinomas are extremely rare,\textsuperscript{17} comprising about 0.2% of all pituitary tumors. These usually arise from invasive pituitary adenomas. Current therapeutic modalities are mainly palliative and the prognosis of these tumors is relatively poor. Additional research delineating the underlying molecular mechanisms in the pathogenesis of these tumors would hopefully lead to the development of targeted molecular therapies, including drugs based on growth factor and kinase inhibitors, potentially improving the outcome of these patients.\textsuperscript{18}

Pituitary metastases from other malignancies can occur. These are found in up to 3.5% of patients with cancer.\textsuperscript{19} The posterior pituitary is the preferred site for metastatic spread, since its vascular supply is derived directly from the carotid arterial circulation, as opposed to the anterior lobe, which has an indirect supply from the portal circulation.\textsuperscript{15} Carcinomas that metastasize to the pituitary include breast, lung, prostate, renal cell, and gastrointestinal tract cancers.\textsuperscript{3,20} Hemopoietic malignancies that may present with posterior lobe metastases include plasmacytomas, lymphomas, and leukemias. Although the majority of metastases occur in the context of advanced malignancy, symptomatic posterior lobe metastases, mainly diabetes insipidus, may be the presenting sign of occult malignancy. Pituitary imaging may not clearly distinguish metastatic deposits from a pituitary adenoma, and diagnosis is mostly made by histologic study of the resected specimen.\textsuperscript{21} When the diagnosis is clear-cut in the presence of a primary cancer, low-dose pituitary radiation may be sufficient to shrink the metastasis and decrease morbidity. Depending on the responsiveness of the primary tumor and the clinical status of the patient, chemotherapy may be considered.

Summary

In summary, pituitary adenomas are essentially benign tumors, which may cause signs and symptoms of space occupation, hypopituitarism, or hormone excess. With the exception of prolactinomas, surgery remains the most appropriate initial treatment to relieve optic chiasmal compression and to reduce hormone hypersecretion. Persistent hormone elevation may be successfully treated with medical therapy and/or radiotherapy. Regular surveillance is necessary to monitor for recurrence as well as to detect development of hypopituitarism. Pituitary carcinomas are very rare and are associated with a poor outcome. It is hoped that further studies of the molecular pathogenesis of these tumors will allow for precise molecular targeting and improve the overall response rates in such cases.

References


Introduction

The role of molecular testing in the routine evaluation of pituitary disease is in its infancy. There is currently a very limited role for such testing in the routine clinical evaluation of pituitary lesions. Research studies using molecular techniques is augmenting our understanding of pituitary disease and particularly neoplastic lesions. This chapter provides an overview of the interface between molecular pathology and both nonneoplastic and neoplastic conditions of the pituitary gland.

Inflammatory Lesions

A variety of inflammatory lesions can affect the pituitary gland. Infectious diseases in the form of abscess can rarely involve the gland itself (Figures 18.1–18.3). Special stains for microorganisms can be useful sometimes in identifying the etiologic agent. The abscess may be associated with granulomatous inflammation (granulomatous hypophysitis). The most common etiologic agents associated with granulomatous inflammation include tuberculosis, fungal infections, and syphilis. The diagnostic yield in tissue sections is relatively low for some of these agents, particularly tuberculosis; use of Ziehl–Neelsen or FITE stains may allow for early identification of the organism and a more directed treatment. Occasionally, sarcoidosis may also affect the pituitary gland; typically, the granulomatous inflammation in the setting of sarcoidosis is not associated with necrosis (Figure 18.4). Rare cases of inflammatory pseudotumor marked by prominent chronic inflammation and fibrosis have also been reported to involve the sellar and parasellar region. Patients may present with hypopituitarism and imaging studies can show a lesion which mimics a tumor.

Perhaps, the most well-known inflammatory condition to involve the pituitary gland is lymphocytic hypophysitis. The disorder is thought to have an immunologic basis and is associated with other autoimmune disorders including thyroiditis, adrenalitis, atrophic gastritis, and parathyroiditis. The vast majority of patients are female, and commonly, this disorder presents during late pregnancy or in the postpartum period. The affected pituitary gland is typically enlarged, and patients may present with symptoms related to mass effect including headaches and visual field problems. Most patients also manifest with endocrine abnormalities related to hypofunctioning of the adenohypophysis, secondary to destruction of the gland by the inflammation. An enlargement of the gland can be observed on imaging studies, and occasionally, the imaging findings may resemble that of a pituitary adenoma, often resulting in the patient being biopsied for evaluation.

Microscopically, lymphocytic hypophysitis is marked by a prominent lymphoplasmacytic infiltrate (Figure 18.5). Occasional lymphoid aggregates and variable numbers of neutrophils and eosinophils may be observed. Destruction of the adenohypophysis accompanied by variable degrees of fibrosis are also common (Figure 18.6). Immunohistochemistry confirms that the inflammatory infiltrate consists of a mixture of benign appearing B and T lymphocytes. Ancillary molecular testing is usually not required to arrive at the proper diagnosis.

The clinical course of the disease is variable. Some patients develop progressive and permanent hypopituitarism; whereas, others show a partial or total return of function to the pituitary gland in their recovery process. Most patients require some treatment, particularly during the course of the disease, in order to quell the inflammatory response and prevent further destruction. A frozen section diagnosis is useful in patients who undergo surgical intervention. Identification of the pathology may prevent a more radical resection of the gland and help preserve future residual pituitary function.

Sheehan’s Syndrome

Sheehan’s syndrome is a rare condition resulting in pituitary infarction; it may be associated with postpartum uterine hemorrhage. The syndrome usually manifests as hypopituitarism and primarily affects the adenohypophysis with relative
Fig. 18.1. Acute inflammation associated with an abscess in the adenohypophysis (hematoxylin and eosin, original magnification 200×).

Fig. 18.2. Organizing abscess in the pituitary gland (hematoxylin and eosin, original magnification 200×).

Fig. 18.3. Toxoplasma gondii organisms in a pituitary abscess; the patient was severely immunocompromised (hematoxylin and eosin, original magnification 400×).

Fig. 18.4. Sarcoidosis with non-necrotizing granulomatous inflammation (hematoxylin and eosin, original magnification 200×).

Fig. 18.5. Prominent lymphoplasmacytic infiltrate in lymphocytic hypophysitis (hematoxylin and eosin, original magnification 200×).

Fig. 18.6. Fibrosis and chronic inflammation in lymphocytic hypophysitis (hematoxylin and eosin, original magnification 200×).
spare of the neurohypophysis. Necrotic tissue is eventually replaced by scar. Patients typically require replacement hormonal therapy.

**Empty Sella Syndrome**

Empty sella syndrome is marked by a decrease in the volume of the sellar contents because of extension of the subarachnoid space into the sella turcica. The condition may present in a primary form because of an incomplete or incompetent sellar diaphragm with downward herniation of the arachnoid membrane, resulting in compression of the pituitary gland. This form is more commonly encountered in women. Secondary forms of empty sella syndrome may be associated with a variety of causes including radiation, hemorrhagic necrosis, and previous surgery. Middle-aged women with histories of hypertension and obesity are at higher risk. Occasionally, an adenoma may be observed in association with secondary forms of the disease.

**Cysts of Pituitary Gland**

Four major types of cystic lesions may be encountered in the region of the pituitary gland: Rathke’s cleft cysts, dermoid cysts, epidermoid cysts, and cystic craniopharyngiomas. Craniopharyngiomas will be discussed later in this chapter.

Rathke’s cleft cysts are derived from remnants of the Rathke’s pouch and typically are situated between the anterior and intermediate lobes of the pituitary gland. A commonly encountered finding, it may be seen in up to 20% of patients at the time of autopsy. There is no gender predilection. Lesions are most commonly observed in adults. Large cysts may be symptomatic because of mass effect, resulting in compression of the optic chiasm or pituitary gland. Pathologically, the cysts are typically lined by a single layer of ciliated cuboidal or columnar epithelium with scattered mucous secretory type cells (Figure 18.7). Focal squamous metaplasia may be observed. The cyst lining will stain with antibodies to cytokeratin and epithelial membrane antigen. When the squamous metaplasia component is prominent, confusion of this lesion with other squamous-lined cysts may occur.

Dermoid cysts arising in the sellar region may develop from inclusion of epithelial elements during the closure of the neural tube. Cysts usually present in childhood with no gender predilection. A CT scan may show a low density mass; whereas on MRI imaging, heterogeneous signal intensity due to the presence of hair or sebaceous material may be present. Microscopically, the cysts resemble their counterparts elsewhere in the body. The cysts are lined by squamous epithelium with associated adnexal structures including hair follicles, sebaceous glands, eccrine glands, and apocrine glands (Figure 18.8). Rarely, mature bone may be seen.

Epidermoid cysts can also arise in the sellar or parasellar region. In contrast to the dermoid cysts, epidermoid cysts lacks adnexal structures. Rupture and leakage of cysts may elicit an inflammatory giant cell reaction. The squamous cell epithelial lining often demonstrates evidence of maturation with a surface granular layer (Figure 18.9), a feature that is typically not observed in the squamous metaplasia of the Rathke’s cleft cyst and in cystic craniopharyngiomas. Molecular genetic studies currently play no role in the differentiation of cystic lesions in the sellar and pituitary gland region.
Craniopharyngioma

Craniopharyngiomas are low grade (WHO grade I) tumors which comprise between 1 and 5% of all intracranial neoplasms.\(^{14,15}\) They have a bimodal age presentation including one peak in the first two decades of life and a second peak in adults during the fifth and sixth decades of life. The papillary variant of craniopharyngioma is almost exclusively seen in adults.\(^{16,17}\) There is no gender predilection. The vast majority of these tumors arise in the suprasellar compartment, although rare cases of tumors situated in other locations, such as the sphenoid sinus, have been reported. The clinical presentation most commonly includes visual disturbances, endocrine abnormalities related to pituitary dysfunction, diabetes insipidus, and cognitive impairment. On CT imaging, these tumors frequently demonstrate contrast enhancement and often have a cystic component. Calcifications may be identifiable. On MRI studies, T1-weighted imaging shows homogeneously intense areas corresponding to cysts and the solid component is isointense.

Grossly, these tumors may be variably solid and cystic. On sectioning, the cysts contain a green-brown fluid that has been likened to motor oil. The tumor and its surroundings frequently show changes of fibrosis, calcification, ossification, and cholesterol cleft formations. The papillary variant of craniopharyngioma often is well-circumscribed and less commonly cystic and calcified.

Microscopically, there are two variants of craniopharyngioma that are well-recognized: adamantinomatous and papillary. The adamantinomatous variant is marked by the presence of squamoid epithelium arranged in lobules and trabeculae separated by dense connective tissue. The perimeter of the squamoid islands is often lined by palisaded columnar epithelium. The centers of the epithelial islands often have a loosely cohesive appearance and has been referred to as stellate reticulum (Figure 18.10). Nodules of keratin material and calcification are commonly seen (Figure 18.11). Cystic cavities may be lined by a flattened squamoid-type epithelium which lacks a granular layer (Figure 18.12). The adjacent tissue frequently shows a granulomatous reaction with cholesterol cleft formations (Figure 18.13). Prominent gliosis, sometimes accompanied by Rosenthal fiber formations (Figure 18.14), is a fairly common finding around the perimeter of the lesion.

The papillary craniopharyngioma is characterized by squamous-type epithelium lining fibrovascular cores (Figure 18.15). In contrast to epidermoid cysts, there is no obvious maturation of the squamous epithelium as one progresses...
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...from the base to the surface. Occasionally, ciliated cells or goblet cells may be intermixed with the squamous epithelium. Microcalcifications and abundant cholesterol cleft formations and granulomatous responses are unusual in this variant. The epithelial component of both variants will stain with a variety of cytokeratin markers.

Examination of these tumors with cell proliferation markers, such as Ki-67 or MIB-1, shows immunoreactivity primarily located around the periphery of the squamoid nests in the adamantinomatous variant. Cell proliferation marker immunoreactivity is more homogeneously distributed in the papillary variant. No relationship between labeling indices and tumor recurrence or behavior has been documented reliably in the literature.

There is currently no role for the routine evaluation of these tumors from a molecular or genetic perspective. Multiple chromosomal abnormalities have been documented in the few cases that have been studied. Mutation of the beta-catenin gene has been noted in the majority of adamantinomatous craniopharyngiomas. This results in an accumulation of nuclear beta-catenin protein which can be demonstrated by immunohistochemistry. In a few cases, similar beta-catenin mutations were identified in mesenchymal cells situated between the squamous epithelioid cells, suggesting a biphasic nature for this neoplasm. Interestingly, beta-catenin mutations have not been demonstrated in papillary craniopharyngiomas. Although comparative genomic hybridization studies have demonstrated genomic alterations...
in the majority of adamantinomatous craniopharyngiomas, significant chromosomal imbalances have not been identified in these tumors.

A significant factor associated with tumor recurrence is the extent of surgical excision. Generally, tumors greater than 5 cm in diameter are associated with a worse prognosis, as are incompletely resected neoplasms. The literature is mixed with regard to prognosis associated with histologic subtype with some papers reporting a better prognosis for the papillary variant, while other papers failing to demonstrate a significant difference. Rare cases of malignant transformation to squamous cell carcinoma, particularly following radiation therapy, have been documented.

Pituitary Hyperplasia

Pituitary hyperplasia can be physiological or pathological and clinically indistinguishable from an adenoma. Radiological imaging can sometimes distinguish hyperplasia from normal: in hyperplasia, the proliferation is diffuse, and there is no normal rim that enhances with gadolinium. A reticulin stain can also help differentiate these entities. In hyperplasia, the acinar architecture is maintained and the reticulin network is preserved, but the acini are increased in size. In contrast, pituitary adenomas are characterized by complete disruption of the reticulin fiber network. Immunohistochemical stains for hormones are required to determine the secretory nature of the hyperplastic cell population.

Pituitary hyperplasia can be diffuse or nodular, unifocal or multifocal. In the diffuse type, the number of cells increases without a major change in the morphology or acinar architecture. In the focal type, there is a small circumscribed increase of cell type without clinical significance.

Cell proliferation indices, though higher than the normal adenohypophysis, are not high enough to be used as a reliable marker. Similarly, molecular techniques, such as in situ hybridization, PCR, and blotting studies have contributed little to the diagnosis of pituitary hyperplasia. Due to the difficulty in establishing the diagnosis, the incidence of pituitary hyperplasia in general and the frequency of its various patterns are not known.

Pituitary Adenoma

Recognizing molecular determinants in normal pituitary gland cytodifferentiation is important in determining the cytogenesis of pituitary adenomas, as well as providing a framework for classification. Three main pathways of cell differentiation have been identified in the anterior pituitary. Adenohypophysial cells arise from Rathke’s pouch stem cells, and the corticotrophs are the first cells to differentiate. This process is determined by the Tpit transcription factor that works in concert with Ptx1 and neuroD1, previously called corticotroph upstream transcription element (CUTET) binding proteins. The second line of differentiation is via Pit-1 expression, activating growth hormone (GH), prolactin (PRL), and beta-thyrotropin (b-TSH) genes. The somatotroph phenotype is determined, and the expression of the estrogen receptor allows PRL and GH to be made in a bihormonal population of mammamotrophs. Mature lactotrophs develop in the presence of a putative GH repressor, which has not yet been identified. Some of the Pit-1-expressing cells also make thyrotroph embryonic factor (TEF) and develop into thyrotrophs when GH repressor and GATA-2 are present. Somatotrophs, mammamotrophs and lactotrophs transdifferentiate in a reversible fashion, and all three cell types are Pit-1 dependent. The third line of differentiation is that of the gonadotrophs, which are determined by steroidogenic factor-1 (SF-1) and GATA-2 in the presence of estrogen receptor. Commerically available antibodies exist for the nuclear transcription factors, Pit-1, and SF-1.

The 2004 WHO Classification of Tumors report contains three accepted types of primary adenohypophysial lesions: typical pituitary adenoma, atypical pituitary adenoma, and pituitary carcinoma. An atypical adenoma is an invasive tumor marked by an elevated mitotic index, a MIB-1 labeling index >3%, and an extensive nuclear immunostaining for p53. Pituitary carcinoma is defined by metastasis or noncontiguous spread. The most important clinical and prognostic features of pituitary adenomas remain the hormonal profile and subtype classification, because specific subtypes (such as the sparsely granulated growth hormone adenoma) have a greater propensity for aggressive clinical behavior.

Pituitary adenomas are classified clinically into functioning or nonfunctioning adenomas, according to whether or not an endocrine syndrome is present. The majority of adenomas are functioning; however, about a third of all pituitary adenomas lack clinical or biochemical evidence of hormone excess. According to tumor size, adenomas are divided into microadenomas (<1 cm in diameter) and macroadenomas (>1 cm diameter). Macroadenomas possess a tendency for suprasellar extension, gross invasion, and recurrence. Staining characteristics of the tumor cells, such as acidophilic, basophilic, or chromophobe, are outdated as a primary means of classification, because they do not identify specific adenoma subtypes. Adenomas are histologically marked by a disruption of the normal nested pattern of the adenohypophysis (Figure 18.16). Growth patterns (diffuse, pseudopapillary, trabecular, etc.), are of no prognostic significance, but they may be helpful in the differential diagnosis of adenoma (Figures 18.17–18.19). Hormone content of the tumor can be assessed by immunohistochemistry; however, it does not always discriminate between specific subtypes of adenomas that have prognostic clinical significance.

Recently, a number of novel molecular techniques, such as in situ hybridization, have been applied to the experimental study of pituitary tumors. These methods are primarily regarded as...
18. Nonneoplastic and Neoplastic Pituitary Diseases

Prolactinomas comprise nearly 80% of functioning pituitary tumors and about 40–50% of all pituitary adenomas. Most are microadenomas, occurring in reproductive-age women who present with amenorrhea, galactorrhea, and infertility. In men and elderly women, prolactinomas are usually macroadenomas and are commonly associated with headaches, neurological deficits, and visual loss. Impotence and decreased libido are common symptoms in men with hyperprolactinemia.

By light microscopy, tumor cells are medium-sized with chromophobic or slightly acidophilic cytoplasm and central, oval nucleus. Small nucleoli can be present (Figure 18.20). Approximately 10–20% of cases show variably prominent research tools, but in the near future they may be used in routine diagnosis.

Fig. 18.16. Normal nested architecture of the adenohypophysis. Each nest shows a mixture of acidophilic, basophilic and chromophobe cells (hematoxylin and eosin, original magnification 200×).

Fig. 18.17. Diffuse growth pattern in a pituitary adenoma; this is the most common pattern seen (hematoxylin and eosin, original magnification 200×).

Fig. 18.18. A pseudopapillary architecture in a pituitary adenoma (hematoxylin and eosin, original magnification 200×).

Fig. 18.19. A trabecular growth pattern in a pituitary adenoma (hematoxylin and eosin, original magnification 100×).

Fig. 18.20. Prolactin secreting adenoma with nucleolated tumor cells (hematoxylin and eosin, original magnification 400×).
microcalcifications. Nuclear pleomorphism may be evident but is not of any clinical significance (Figure 18.21). Immunohistochemistry shows nuclear prolactin (PRL) positivity in a “dot like” pattern, reflecting hormone localization in the Golgi complex (Figure 18.22). If previously treated with dopamine agonists, there is atrophy of lactotrophs with resultant tumor shrinkage and hyperchromasia of the nuclei. Strong nuclear positivity for Pit-1 is seen. Ultrastructurally, lactotroph adenomas can be subclassified into sparsely and densely granulated variants. Sparsely granulated tumors are the most common and resemble normal, actively secreting lactotrophs. Adenoma cells show a prominent rough endoplasmic reticulin (RER) network, conspicuous Golgi complex, and a sparse number of small (150–300 nm) secretory granules. The densely granulated variant is very rare and contains elongated acidophilic cells with elongated nuclei and increased chromatin. Reactivity for PRL is strong and diffuse. Electron microscopy reveals large, somewhat densely arranged secretory granules with exocytoses and a well developed RER. Amyloid derived from PRL is seen in up to 48% of adenomas. Cell proliferation indices are typically low.

About 20% of pituitary adenomas are associated with evidence of growth hormone (GH) secretion, which can be accompanied by high serum GH and insulin-like growth factor 1 (IGF-I) levels. Patients presenting with acromegaly or gigantism may rarely have somatotroph hyperplasia owing to ectopic production of growth hormone releasing hormone; therefore, the reticulin stain may be critical to exclude this possibility. Both genders are affected with a mean age of presentation of 40–45 years. Most acromegalic patients have macroadenomas when first diagnosed, often with suprasellar expansion and parasellar invasion.

GH secreting adenomas are either eosinophilic or chromophobic. In eosinophilic adenomas, the cytoplasm shows considerable granularity. The oval nucleus tends to be central with a prominent nucleolus. Nuclear pleomorphism and multinucleation are common. GH reactivity is marked by diffuse cytoplasmic staining throughout the tumor (Figure 18.23). By electron microscopy, the densely granulated variant has adenomatous cells that resemble normal somatotrophs, having a well developed RER network, prominent Golgi complexes, and numerous large (300–600 nm) secretory granules. In sparsely granulated GH tumors, the cytoplasm is more chromophobic, the nucleus tends to be eccentric, and the cytoplasm contains a few small (100–250 nm) secretory and paranuclear eosinophilic structures called “fibrous bodies.” These bodies are strongly positive for cytokeratin and ultrastructurally, represent accumulations of intermediate filaments and tubular formations. GH immunostaining is focal.

Fig. 18.21. Scattered pleomorphic nuclei in a prolactinoma (hematoxylin and eosin, original magnification 400×).

Fig. 18.22. Dot-like immunoreactivity in a prolactin positive adenoma – same tumor as Figure 18.20 (prolactin, original magnification 400×).

Fig. 18.23. Diffuse positive staining of an adenoma with growth hormone antibody (growth hormone, original magnification 400×).
within the tumor and tends to be localized in a paranuclear distribution. GH reactivity is weaker in the sparsely granulated variant, and may even be negative in some cases. In situ hybridization may be used to detect GH mRNA, which will be positive in sparsely granulated GH cell adenomas. CAM 5.2 identifies perinuclear keratin in densely granulated adenomas and fibrous bodies in the sparsely granulated adenomas. Long-acting somatostatin analog may change the morphology, resulting in varying degrees of perivascular and interstitial fibrosis. The clinical importance of distinguishing these two subtypes of GH cell adenomas is controversial, but the sparsely granulated GH cell adenomas appear to exhibit more aggressive biological behavior than the densely granulated tumors.

Adrenocorticotropic hormone (ACTH) dependent Cushing’s syndrome is caused by pituitary ACTH secreting adenomas in 80% of cases. ACTH secreting adenomas constitute two major groups: endocrinologically active tumors associated with either Cushing’s disease or Nelson’s syndrome, and tumors that are clinically nonfunctioning, also known as silent corticotroph adenomas. Cushing’s disease affects patients of varying ages, with a peak at 30–40 years, and it shows a female predominance. On rare occasions, corticotroph cell hyperplasia may be the source of Cushing’s disease (Figure 18.24).

Microadenomas associated with Cushing’s disease are densely granulated with strongly basophilic cells and strong PAS positivity. Immunohistochemically, they express ACTH and usually react strongly with the CAM 5.2 antibody and cytokeratins 7 and 8. Crooke’s hyaline change may also be present. In addition, other peptides related to the proopiomelanocortin (POMC) precursor molecule, including beta-lipotropin, beta-endorphin, and alpha-melanocyte-stimulating hormone, can be present. The ultrastructural features are characterized by well-differentiated cells that resemble normal corticotrophs. Well developed organelles, including RER, smooth endoplasmic reticulum, and conspicuous Golgi are associated with numerous large (250–700 nm) secretory granules, which often are different shapes and vary in electron density. Sparsely granulated ACTH cell adenomas are only weakly basophilic and weakly PAS-positive or chromophobic. Cellular pleomorphism is more frequent. Immunostaining reactions, especially for ACTH, are weaker than the densely granulated variant, and may be negative (Figure 18.25). EM reveals less numerous and often smaller secretory granules than in the densely granulated ACTH cell adenomas, whereas the organelles structures are similar or slightly irregular. In adenomas from patients with Cushing’s disease, bundles of intermediate filaments adjacent to the nucleus and/or forming large circles are easily identified. Histologic and immunohistochemical studies are sufficient for the right diagnosis.

Crooke’s cell adenomas are very rare and the morphology can be quite atypical, with prominent nuclear pleomorphism and large cells that can resemble gangliocytoma or metastatic carcinoma. It is defined by PAS positivity and immunoreactivity for ACTH, as well as the dense ring of keratin that fills the tumor cell cytoplasm, and is identified with CAM 5.2 staining. The clinical course is variable, with some tumors demonstrating an aggressive behavior, and others only being discovered as small incidental inactive adenomas in postmortem pituitaries. In a recent study, invasiveness was found in 72% of these adenomas. The rare silent corticotroph cell adenoma demonstrates immunoreactivity for ACTH without clinical signs of Cushing’s disease or serum levels reflecting excess. The majority are macroadenomas and present with signs of a mass lesion and show a high tendency to hemorrhage and necrosis (apoplexy) (Figure 18.26), which may be the presenting symptom in about a third of patients.

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Fig. 18.24. Normal adenohypophysis stained with ACTH antibody. Regional variability of staining should not be misinterpreted as representing a hyperplasia (ACTH, original magnification 200x).

Fig. 18.25. Diffuse but weak staining of a ACTH secreting adenoma (ACTH, original magnification 200x).
Thyroid stimulating hormone (TSH) cell adenomas are the least frequent of pituitary adenomas. Most are invasive macroadenomas (Figure 18.27). Light microscopy reveals medium-sized chromophobic cells. Globular PAS-positive inclusions representing lysosomes may be found. Immunostaining for TSH is variable and may be negative. The ultrastructure is characterized by similarities to normal TSH cells with often prominent RER, globoid Golgi complexes, and sparse small (150–250 nm), rod-shaped or spherical or irregular secretory granules.

Gonadotropin-secreting adenomas secrete follicle stimulating hormone (FSH) and/or leutinizing hormone (LH), and do not usually have a clinical syndrome related to hormone overproduction. They may present with symptoms related to mass effect. With modern lab techniques, a large number of pituitary adenomas previously classified as non-functioning adenomas have been found to produce gonadotropins or their subunits. Hence, gonadotroph adenomas may account for a large proportion of clinically nonfunctioning adenomas and about 20% of all adenomas. They occur most frequently in the sixth decade or older, showing a slight male predominance. By light microscopy, most cells have chromophobic cytoplasm and a nucleus with a fine chromatin pattern. The cells may be arranged in a diffuse pattern, but a distinct papillary arrangement is commonly seen. Immunohistochemistry demonstrates varying levels of reactivity for beta-FSH, beta-LH, alpha-SU, or a combination. Ultrastructurally, these adenomas are characterized by elongated polar cells containing scant numbers of small (50–200 nm) secretory granules. The secretory granules are distributed unevenly within the cytoplasm or along the cytoplasmic membrane.

Plurihormonal adenomas have unusual immunoreactivity for more than one type of pituitary hormone, which are unrelated by normal cytogenesis and development of anterior pituitary. Clinically, these patients present with symptoms attributable to mass effect. With improved methods of immunostaining and the increased use of monoclonal antibodies, the incidence of plurihormonal adenomas is lower than expected from former studies with polyclonal antibodies. The most common combination is an adenoma secreting PRL and GH. Reports of adenomas expressing GH or PRL with gonadotropins are now recognized to reflect an aberration that was identified as non-specific cross-reactivity. Plurihormonal adenomas can be monomorphous or plurimorphous in nature.

Approximately 20% of adenomas show neither clinical nor immunohistochemical evidence of hormone production and are labeled as null cell adenomas. Most commonly, they arise in postmenopausal females and elderly males, presenting with signs and symptoms of mass effect. By light microscopy, they are mostly chromophobic and can be arranged in a diffuse pattern and/or in well-defined papillary arrangements. Oncocytic degeneration can be seen.

Pituitary adenomas without clinically active hypersecretion are characterized as non-functioning pituitary adenomas (NFPA). They represent about one quarter of all pituitary tumors and are often asymptomatic and manifest themselves only after they have grown to sufficient size to produce mass effect. Symptoms referable to hypopituitarism can often be elicited and verified by endocrine testing. Moderate prolactinemia from stalk compression may be present; sometimes these adenomas are erroneously diagnosed as prolactinomas. A subset of clinically inactive adenomas are silent adenomas, which are characterized by the insufficient secretion of active hormones into the circulation, despite their expression. Most of these are ACTH cell adenomas.

Pituitary apoplexy is the rapid enlargement of an adenoma due to tumoral infarction and hemorrhage, presenting usually as an acute event and often neurosurgical emergency. All immunotypes of adenoma may present with apoplexy, but large nonfunctioning adenomas are particularly prone.
The majority of pituitary adenomas are sporadic, although some arise as a component of familial syndromes. Pituitary adenomas can occur in a familial setting in multiple endocrine neoplasia type I (MEN 1), Carney complex (CNC) and in the context of isolated, autosomal dominant acromegaly or gigantism. Less frequently, they can occur in the context of a familial predisposition to the development of pituitary tumors. Another rare genetic disorder associated with GH and PRL producing tumors is McCune–Albright syndrome (MAS).

So far, four genes have been identified that predispose to familial pituitary tumorigenesis. Multiple endocrine neoplasia type I (MEN 1) (11q13) and protein kinase A regulatory subunit-I-alpha (PRKAR1A)(17q24) genes have been associated in multiple endocrine neoplasia type 1 (MEN1) and Carney complex (CNC), respectively. More recently, pituitary adenoma predisposition (PAP) genes, cyclin-dependent kinase inhibitor 1B (CDKN1B) (12p13), and aryl hydrocarbon receptor (AHR) interacting protein (AIP) (11q13) have been identified. In addition, familial pituitary adenoma is seen in the isolated familial somatotropinomas (IFS) (linkage to 11q13) and familial isolated pituitary adenomas (FIPA), the former probably in part allelic to PAP and caused by mutations in AIP gene, and in the FIPA phenotype. MEN1 is a tumor suppressor gene; the syndrome is inherited as an autosomal dominant trait. Pituitary adenomas affect 23–30% of MEN1 patients. MEN1 mutations predispose to all major pituitary tumor subtypes, but the most common subtypes seen are those secreting PRL (60%) and GH (20%); ACTH secreting and nonfunctional adenomas represent less than 15%.

Pituitary Carcinoma

Pituitary carcinomas are very rare neoplasms, representing fewer than 0.2% of all anterior pituitary tumors. The definition of carcinoma depends on the demonstration of craniospinal and systemic metastases. The most common sites of metastases are subarachnoid space, cervical lymph nodes, bone, liver, and lungs. There appears to be an equal gender distribution and tumors present with a peak incidence in the fifth decade. The diagnosis of pituitary carcinoma cannot be based solely on histologic appearance, as invasion, cellular pleomorphism, nuclear abnormalities, mitotic activity, and necrosis can all be seen in adenomas. Tumors arise in the anterior lobe and depending on growth rate, spread to neighboring structures. Craniospinal space spread and systemic spread follows. The majority of cases are endocrinologically functioning tumors, most commonly secreting PRL or ACTH. Carcinomas show a higher Ki-67 labeling index and increased p53 protein expression as compared with adenomas. Ras mutations can be found in PRL cell carcinomas. Nuclear p27 protein expression is significantly decreased in adenomas as compared with normal pituitary, and is much lower in pituitary carcinoma as compared to both normal and adenomas. Using comparative genomic hybridization (CGH), chromosomal imbalances are common, with gains being more common than losses; the most frequent changes are gains of chromosomes 5, 7p, and 14q. The prognosis of pituitary carcinomas is generally poor, although patients with long-term survival have been described.

Tumor Proliferation Markers and Invasiveness in Adenomas and Carcinomas

The molecular mechanisms controlling cell proliferation and invasion in pituitary tumors are largely unknown. Studies of proliferative activity have been used as an adjuvant tool for distinguishing between aggressive and indolent, benign pituitary adenomas. Clinically functioning adenomas have a significantly higher growth fraction than nonfunctioning tumors. Growth fractions are also significantly higher among invasive adenomas and pituitary carcinomas when compared with noninvasive adenomas.

Invasive adenomas may demonstrate p53-positive nuclei, which are found to be absent in regular adenomas. p53 expression has been correlated significantly with the numbers of MIB-1 positive nuclei and PCNA positive nuclei. Investigators have found that cyclooxygenase-2 (COX-2) expression correlates with patient age, but not with tumor size or invasiveness. Galectin-3, a beta galactosidase binding protein implicated in cellular differentiation and proliferation as well as angiogenesis, tumor progression, and metastasis, may play a role in pituitary tumor progression. The polypeptide insulin-like growth factor-1 (IGF-I) is demonstrable in most pituitary adenomas, but a distinct correlation to adenoma growth could not be found. Parathormone (PTH)-related protein was demonstrated in normal pituitary and in pituitary adenomas, as well as overexpressed in a metastasis of GH secreting carcinoma.

Oncogenes and Tumor Suppressor Genes in Pituitary Tumorigenesis

The mechanisms underlying human pituitary tumorigenesis and progression are largely unknown. X-chromosomal inactivation analyses in a large number of pituitary adenomas demonstrated that a majority of the tumors are monoclonal in origin. Sporadic and low frequency mutations in oncogenes and tumor suppressor genes are observed in pituitary adenomas. Two well-characterized genetic abnormalities have been identified in pituitary adenomas. The first one is a putative tumor suppressor gene at 11q13, the genetic defect in multiple endocrine neoplasia 1 (MEN1). In patients with MEN1 syndrome, loss of 11q13 is present in pituitary adenomas, but only 3% of pituitary adenomas occur in the
context of MEN1. Loss of heterozygosity (LOH) at 11q13 has been demonstrated in 10–20% of sporadic pituitary adenomas, suggesting the presence of a tumor suppressor gene at this location, but the somatic MEN1 mutation rate is very low (approximately 2%). Therefore, the MEN1 gene does not appear to play a major role in the pathogenesis of sporadic pituitary adenomas.

The second gene mutation described in pituitary adenomas is the gsp oncogene mutation of the alpha subunit of the G-protein. The gsp mutation has been identified in about 40% of GH secreting adenomas, 10% of nonfunctioning pituitary adenomas, and 5% of corticotroph adenomas. Although some clinical and biochemical differences are observed, these tumors do not differ morphologically from tumors without gsp. The activating mutation of gsp is demonstrated in McCune–Albright syndrome, which is characterized by somatotroph hyperplasia and polyostotic fibrous dysplasia of the bones.

A number of other oncogenes and tumor suppressor genes have been identified as possible players in the pituitary tumorigenesis. The protein expressed by the pituitary transforming gene (PTTG) has roles in mitosis control, cell transformation, and DNA repair. Three homologue genes have been identified: (1) PTHL1 located on chromosome 5q33, (2) PTTG2 on chromosome 4p12; and (3) PTTG3 on chromosome 8q22. PTTG protein is expressed at low levels in the normal human pituitary, but at high levels in a variety of solid tumors, including pituitary adenomas. Approximately 90% of pituitary adenomas overexpress PTTG, with the highest levels seen in ACTH secreting and nonfunctioning pituitary tumors. One study reported higher expression levels of PTTG, PTTG binding factor, and fibroblast growth factor 2 (FGF-2) and its FGF receptor FGFR1 mRNAs in pituitary adenomas as compared to normal tissue.

In the clinical setting, where other markers of tumor aggressiveness (such as expression of PCNA, Ki-67, etc.) have failed, PTTG and possibly FGF-2 and FGFR1 may be the best available markers of pituitary tumors. In addition, FGF-2 and -4 have been implicated in prolactinoma development. Expression of the pituitary tumor derived (ptd)-FGFR4 protein is more frequently found in macroadenomas than in microadenomas and correlated with the Ki-67 labeling index. Cyclins A, B, and E are expressed in all adenoma types and are significantly higher in macroadenomas when compared to microadenomas. Screening of numerous adenomas failed to reveal mutations of the tumor suppressor gene RB; however, allelic loss of RB has been demonstrated in some highly invasive adenomas and pituitary carcinomas. Point mutations of the H-ras proto-oncogene have been identified in rare cases of distant metastases of pituitary carcinoma.

A number of growth factors and hypothalamic trophic factors are also believed to participate in the maintenance of pituitary tumors by dysregulated autocrine and paracrine mechanisms.

The vast majority of genetic alterations in pituitary adenomas seem to be due to promoter methylation or acetylation with silencing of tumor suppressors. The mRNA expression of GADD45 gamma gene (growth arrest and DNA damage inducible gene family) is significantly reduced in clinically nonfunctioning pituitary adenomas using cDNA representational difference analysis. This is believed to be due to promoter methylation.

Granular Cell Tumor

Granular cell tumors arising in the neurohypophysis are relatively rare lesions. These tumors show a female predominance and most commonly present with symptoms related to visual field deficits secondary to compression of the optic nerve and chiasm. Occasionally, endocrine abnormalities may also be observed. Most of these tumors are slow growing and symptoms may develop gradually over a long period of time. On imaging, the tumor presents as a well circumscribed, suprasellar mass which demonstrates areas of contrast enhancement. In contrast to craniopharyngiomas which may arise in the region, calcification is unusual in these lesions. Necrosis, cystic degeneration, and hemorrhage are uncommon.

Morphologically, these tumors resemble their extra-pituitary counterparts in that they are marked by a proliferation of polygonal cells with abundant granular, eosinophilic cytoplasm (Figure 18.28). Ultrastructurally, the granular appearance of the cytoplasm correlates with an increased number of lysosomes. Cells may be arranged in nodules and focal spindling of cells may be evident. Perivascular chronic inflammation is relatively common. Mitotic activity is usually not observable. Tumors which show evidence of increased nuclear pleomorphism, prominent nucleolation, and increased mitotic activity are designated as atypical granular cell tumors by some; the significance of these lesions is uncertain. By immunohistochemistry, the tumor demonstrates positivity with antibodies to CD68, S-100 protein, cathepsin B, and alpha-1-antitrypsin. Tumors do not stain with antibodies to neurofilaments, cytokeratins, synaptophysin, or chromogranin.

Anecdotal studies examining genetic alterations in these tumors have been performed. Utilizing comparative genomic
hybridization, one tumor studied did not show any evidence of chromosomal imbalances. In another case of atypical granular cell tumor, the majority of tumor cells demonstrated p53 accumulation.

The majority of these tumors behave in a benign fashion (WHO grade I). Because of their nodular architecture and their epithelioid appearance, they may be confused with pituitary adenoma, if one is not careful.

Pituicytoma

Pituicytomas are rare tumors arising in adults in the neurohypophysis. In cases that have been thus far reported in the literature, there appears to be a slight male predominance. The clinical presentation usually involves symptoms related to a slowly expanding mass lesion in the sella and includes visual disturbances, headaches, and signs of hypopituitarism. On imaging, the lesions are typically well-demarcated and uniformly contrast-enhancing.

Pathologically, the pituicytoma is a solid, well-circumscribed lesion. The tumor consists of elongated bipolar spindled cells arranged in interlacing fascicles and a storiform pattern (Figure 18.29). Scattered tumor cells may be marked by abundant eosinophilic cytoplasm. Nuclear atypia or pleomorphism is not a salient feature of this tumor. Mitotic figures are unusual. Oncocytic features are not present.

In terms of immunohistochemistry, pituicytomas generally stain positively with antibodies to vimentin, GFAP, and S-100 protein. Markers of neural differentiation or neuroendocrine markers including synaptophysin, chromogranin as well as cytokeratins are negative. Occasional EMA immunoreactivity may be observed. Cell proliferation indices are low, in most cases less than 2%. Currently, there is a paucity of literature regarding molecular or genetic characteristic features of these tumors. Behaviorally, these are slow growing, low grade lesions (WHO grade I). Tumors which are subtotally resected may recur.

Spindled Cell Oncocytoma

This lesion is a new addition to the World Health Organization Classification of Tumors of the Central Nervous System and comprises less than 1% of all of sellar neoplasms. Most of the tumors that have been reported thus far have occurred in adults. Clinical presentation and imaging studies are similar to nonsecreting pituitary adenomas.

Microscopically, the tumor is marked by a mixture of spindled and epithelioid cells with eosinophilic and oncocytic cytoplasm (Figure 18.30). Focal nuclear pleomorphism
may be evident. Mitotic activity is generally low. Ultrastructurally, these tumors are marked by cytoplasm filled with mitochondria. In contrast to adenomas, these lesions lack cytoplasmic secretory granules. By immunohistochemistry, these tumors usually demonstrate immunoreactivity with antibodies to vimentin, S-100 protein, and EMA. Antibodies to pituitary hormones, GFAP, cytokeratins, synaptophysin, chromogranin, CD34, and muscle-related antibodies such as smooth muscle actin and desmin are negative. Cell proliferation indices are generally low. Most tumors behave in an apparently benign fashion, although tumor recurrence with an increased MIB-1 labeling index and necrosis has been documented.125

**Metastatic Tumors**

The incidence of metastases is variable in the literature, ranging as high as 25% in some autopsy series. In surgical pathology practice, however, a metastatic lesion to the pituitary gland is seldom the target of a surgical procedure. The clinical presentation may be asymptomatic or if the neurohypophysis is involved, diabetes insipidus may develop. There is no obvious gender predilection. Lung and gastric cancers are among the more frequent tumors to metastasize to the pituitary.127-131 In addition, metastases from breast carcinoma in women and prostate carcinoma in men are also relatively common. Lymphomatous and leukemic involvement of the gland are relatively common in patients who have systemic disease.

Imaging studies are nonspecific. Destruction of adjacent bone, cavernous sinus invasion, development of cranial neuropathies, infundibular stalk invasion, and evidence of metastases elsewhere in the brain are possible clues on imaging that a lesion may be metastatic. Rare reports of metastasis to pituitary adenomas have been documented132,133 (Figure 18.31).

**Miscellaneous Neoplasms**

There are a variety of other tumors that rarely can be encountered in the pituitary gland and sellar regions. Germ cell tumors arising as primary neoplasms in the sellar region have been described134,135; the suprasellar region is the second most common site of involvement following the pineal gland. Most of these tumors present in the first two decades of life with a clear male predominance. Germ cell tumors may be associated with hypopituitarism, diabetes insipidus, and visual disturbances. Large lesions may cause symptoms related to hypertension, hydrocephalus, and altered mentation. The most common of these tumors is the germinoma, which on imaging presents as a well-demarcated lesion that has high signal intensity on a CT scan and enhances with contrast (Figure 18.32). Adipose tissue and calcifications which may be part of a teratoma can alter the imaging appearance. All types of germ cell tumors, including germinoma, teratomas, embryonal carcinoma, endodermal sinus tumor, and choriocarcinomas have been described, at one time or another, in this region. The morphologic appearance of these lesions and their immunohistochemical profile

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**Fig. 18.31.** A rare example of a metastatic colonic adenocarcinoma to a nonfunctioning pituitary adenoma (hematoxylin and eosin, original magnification 100x).

**Fig. 18.32.** Sellar germinoma marked by large germ cell and intermixed benign lymphocytes (hematoxylin and eosin, original magnification 200x).
18. Nonneoplastic and Neoplastic Pituitary Diseases

are well established and can be useful in distinguishing these tumors from each other and from other primary tumors arising in the location. Molecular genetic testing is not routinely employed in the evaluation of these neoplasms in the central nervous system. Rare cases of lymphoma, leukemia, or plasmacytoma arising as a primary lesion in the sellar area have been reported (Figures 18.33 and 18.34). Presenting symptoms are those of a mass lesion or hypopituitarism. The histologic appearance of the lesions are similar to those arising elsewhere in the body. Antibodies typically employed in the evaluation of these disorders can be similarly applied to tumors in this location. Likewise, gene rearrangement studies may also be of utility in selected instances in demonstrating monoclonality.

Cases of Langerhans cell histiocytosis or histiocytosis X have been rarely described in this location. The symptoms of hypopituitarism and diabetes insipidus are the most common presentations. On imaging studies, CT scans show an ill-defined, contrast enhancing hypodense mass with areas of edema. Lesions may be hypointense and demonstrate slight increased T1 and contrast enhancement and increased T2 signal intensity on MRI studies. Microscopically, these lesions show the typical morphology of Langerhans cells characterized by kidney shaped nuclei with abundant cytoplasm. These cells are usually admixed with acute and chronic inflammatory cells including prominent numbers of eosinophils. CD1a and S-100 immunoreactivity may be helpful to confirm the diagnosis by immunohistochemistry. Ultrastructural examination for Birbeck granules may also be employed to confirm a diagnosis.

There are a variety of vascular and mesenchymal tumors that have been documented to arise in this location. These lesions include cavernous hemangiomas, glomangioma, hemangiofibromas, chondromyxoid chondromas, chondrosarcomas, lipomas, and alveolar soft part sarcomas or sarcomas have been variously described (Figure 18.35).

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Section 5
Adrenal Diseases
Introduction

Diagnosis and treatment of patients with adrenal lesions is both challenging and fascinating owing to the wide range of biologically important compounds produced by these small glands. Tumors of the adrenal cortex may produce mineralocorticoids, glucocorticoids, sex steroids or in specific cases, combinations of these. Tumors of the adrenal medulla may produce catecholamines and other related compounds making them particularly challenging to diagnose and treat. Malignant disease of the adrenal glands may also be hormonally active.

In this chapter, we review the work-up and treatment of benign, functional masses and malignant diseases of the adrenal cortex and medulla. The appropriate application and interpretation of biochemical and imaging tests in the setting adrenal disease are explored. The role of laparoscopic and open surgery and the outcomes in benign and malignant disease are also presented. In addition, medical therapies and their role in the preoperative and postoperative setting or as primary treatment of adrenal disease are touched on.

Hypercortisolism: Work-Up

The clinical manifestations of Cushings Syndrome (CS) include centripetal obesity, rounded face, abdominal striae, and muscle weakness. More recently however, the term Sub-Clinical Cushing’s (SCC) has been used to describe patients with adrenal incidentalomas, autonomous cortisol production but more subtle clinical and laboratory abnormalities including hypertension, impaired glucose tolerance, osteoporosis, depression and renal stones. If these patients are followed, approximately 12.5% will develop overt CS by 1 year.

The differential diagnosis of endogenous CS can be divided into ACTH dependent and independent causes. Overall, 80% of the patients will have an ACTH dependent etiology. Most of these will have a pituitary adenoma, (80%), producing ACTH with the remainder having an ectopic source. Tumors associated with ectopic ACTH production include small cell lung cancer, thymic and bronchial carcinoids, medullary thyroid cancer, pheochromocytoma, and pancreatic islet cell tumors. Those patients with ACTH independent CS have a primary adrenal source of cortisol production, with the two most common being unilateral adenoma, (60%) and adrenal cortical carcinoma, (40%). Rare causes include bilateral macronodular hyperplasia, primary pigmented nodular adrenal disease, and McCune–Albright Syndrome.

The incidence of endogenous CS is 1–2 per million per year. Patients with overt signs and symptoms of CS and those with adrenal incidentalomas should undergo screening tests. In addition, screening should be strongly considered in patients with osteoporosis, and poorly controlled diabetes as these populations are known to have a disproportionately high prevalence of this syndrome.

Commonly utilized screening methods include 24-h urinary free cortisol and dexamethasone suppression tests. Urinary measurements over four times the upper limit of normal are almost always indicative of CS. Pseudo-Cushing’s states such as depression, alcoholism, and chronic illness may produce lesser elevations. Other potential methods for the diagnosis of CS include an unsuppressed AM cortisol and late-night/midnight salivary. Salivary cortisol has received increasing attention because of its noninvasive nature and ease of repeating the test. Reference values are laboratory dependent, but the test has demonstrated sensitivity and specificity in excess of 90%. This test can be repeated up to three times when the index of suspicion is high as intermittent over-production may be missed.

Low dose dexamethasone suppression tests work on the principle that the hypothalamic–pituitary–adrenal (HPA) regulation is dysfunctional in patients with CS. Following administration of 1 mg of dexamethasone at 23:00, morning values less than 50 nmol/L, are considered normal. A 2-day, 2 mg form of the low dose suppression test is also used for screening purposes in some centers. Once the diagnosis has been made, measurement of serum
ACTH with an assay capable of detecting concentrations below 2 pmol/L is the appropriate next step. Values below 1.1 pmol/L are indicative of ACTH independent CS, whereas values above 3.3 pmol/L suggest corticotrophin dependent disease.1 Equivocal values may be repeated or a corticotrophin releasing hormone, (CRH) stimulation test may be utilized.6

In the majority of patients with ACTH independent CS, CT scan will demonstrate the abnormality, most commonly a unilateral adenoma or carcinoma.5 Gadolinium enhanced, pituitary MRI should be performed in patients with ACTH dependent CS. The presence of a 6 mm or greater lesion on MRI with proven ACTH dependent CS is generally considered diagnostic. However, up to 40% of patients with Cushing’s disease will have a normal appearing MRI.9 High dose dexamethasone suppression and CRH stimulation tests are used in some centers. Patients with pituitary CS will not demonstrate suppression with the low dose test but will suppress with high dose dexamethasone. In contrast, patients with an ectopic or adrenal source of excess cortisol production will not suppress with either a high or low dose test. When MRI and biochemical testing are equivocal, bilateral inferior petrosal sinus sampling may be performed to determine the source of ACTH production. Excellent sensitivity and specificity have been demonstrated, but the procedure is technically demanding.10 In cases of suspected ectopic ACTH production, CT and/or MRI of the neck, thorax, and abdomen should be performed.4

Hypercortisolism: Treatment

In patients with adrenal adenomas, laparoscopic adrenalectomy has become the “gold-standard” treatment.11,12 A recent review identified 12 series of laparoscopic adrenalectomy’s for CS.13 Overall, conversion to open was required in 8.6%, complications occurred in 15% and length of postoperative stay was 3.9 days.14 In this review, 10 of the 12 series reported a 100% rate of biochemical cure. Postoperatively, patients require glucocorticoid replacement until recovery of the Hypothalamic Pituitary Adrenal axis can occur which may take up to 18 months.12

Bilateral causes of ACTH independent CS may be treated medically or with bilateral adrenalectomy. Ketoconazole, metyrapone, and aminogluthethimide inhibit various steps of steroid synthesis through their action on cytochrome p450 enzymes.14 Side effect may limit use and effectiveness may decrease with time. Mitotane, an adrenolytic drug inhibits steroid synthesis by causing selective destruction of the zona reticularis and fasciculate of the cortex. Unfortunately, a high percentage of patients have severe side effects limiting its use.14

Trans-sphenoidal pituitary microsurgery is the appropriate first line treatment for patients with Cushing’s disease. Most patients develop transient hypopituitarism requiring short-term glucocorticoid and L-thyroxine replacement. Failed surgical therapy may be managed with re-operation or pituitary irradiation, but success rates are only around 50%. Medical therapy or bilateral adrenalectomies are also options following failed surgery. While relief from irradiation may take up to 12 months, bilateral adrenalectomy yields immediate results. Expansion of the pituitary neoplasm (Nelson’s Syndrome) following bilateral adrenalectomy is seen in up to 30% of patients by 10 years.15

Hyperaldosteronism: Work-Up

Conn’s syndrome is a constellation of hypertension and hypokalemia associated with elevated plasma aldosterone and suppressed plasma renin activity.16 Recently, more widespread use of screening, with the plasma aldosterone concentration (PAC) to plasma renin activity (PRA) ratio has resulted in increased prevalence estimates from 0.5%17,18 to 5–13% of patients with hypertension.19,20 Common causes of primary hyperaldosteronism include aldosterone producing adenoma (APA) and idiopathic hyperaldosteronism from bilateral adrenal hyperplasia (IAH).21 Less common causes include unilateral hyperplasia and familial forms.

Hypertensive patients with hypokalemia, medication resistant hypertension, a family history of hypertension and/or early stroke and those with incidentalomas should undergo initial screening with a serum PAC/PRA ratio.22 Plasma aldosterone concentration >15 ng/dL and a ratio >20 ng/dL per ng/mL/h are generally considered positive.21 A positive screening test is usually followed by confirmation with saline loading, captopril or fludrocortisone suppression testing.23

This search for a specific cause should begin with imaging. CT and MRI have similar diagnostic performance in the detection of APA’s.24 A unilateral macroadenoma (>1 cm) with a normal contralateral gland in a young (<40 years) patient may be enough to make the diagnosis of an APA and proceed to surgery.25 In patients with bilateral adrenal nodules, adrenal venous sampling is required to localize the pathology to one side.25-27 The major drawback of adrenal venous sampling lies in its technical difficulty. Radiocholesterol scintigraphy provides functional imaging of the adrenals and has been used at some centers.

Hyperaldosteronism: Treatment

Adrenalectomy is an appropriate treatment for unilateral adrenal adenoma. In properly selected patients, normalization of BP without medications can be expected in ~33%/28,29 with 80–90% experiencing improved BP control.26,28,29 Factors associated with postop normalization of BP include young age, short duration of disease, and single agent therapy.28 Bilateral hyperplasia should be treated medically with spironolactone as the primary agent.
**Pheochromocytoma: Work-Up**

Overall, the incidence of pheochromocytoma is 2 per million and less than 0.2% in patients with hypertension. The classical symptomatic triad is headache, cardiac palpitations, and diaphoresis. However, most patients do not present in the classical manner. Hypertension may be sustained, not episodic and some patients may present with an incidentaloma. The classical “rule of tens” has been challenged in recently published literature. In particular up to 25% may be extra-adrenal in location, and 15–35% of these extra-adrenal tumors are malignant, and careful screening may reveal a germline mutation in up to 25%. Hereditary pheochromocytoma is associated with Multiple Endocrine Neoplasia (MEN) 2A and 2B, von Hippel Lindau (VHL), neurofibromatosis (NF) 1, and paraganglioma syndromes (PGL) 1 and 4. Familial paraganglioma syndromes are related to mutations in various subunits of the succinate dehydrogenase gene. Screening should be performed in all patients with multifocal, extra-adrenal or malignant tumors and all those diagnosed under the age of 50.

Biochemical testing is indicated in patients with the classical presentation, patients with adrenal incidentaloma, and those with resistant or early-onset hypertension. Screening relies on the detection of excess catecholamines and/or their metabolites in plasma or urine. Twenty-four hour urinary catecholamines and metanephrines have a sensitivity of 88–90% and a specificity of 99%. While once a commonly ordered test, an isolated elevation of vanillyl mandelic acid lacks the sensitivity to serve as a screening test for these tumors. Plasma free metanephrines have a high sensitivity but a specificity of only 85%. Values below a threshold may be used to rule out pheochromocytoma, values more than four times above the upper limit of normal are nearly 100% specific and those values in the “gray area” require further testing (Figure 19.1).

Following biochemical diagnosis, imaging studies are appropriate. Both CT and MRI tests have high sensitivity, (90–95%) but limited specificity. The use functional imaging with 123I labeled metaiodobenzylguanidine (MIBG) scintigraphy is somewhat controversial. In a recent review of patients with a unilateral lesion on CT or MRI and no family history or features suspicious of malignancy, MIBG scanning did not reveal any additional disease.

**Pheochromocytoma: Treatment**

Preoperative treatment with alpha blockade is the standard care for these patients. Common agents include phenoxybenzamine and doxazosin. As prolonged vasoconstriction results in intravascular volume depletion, liberal use of fluids is also recommended. Control of hypertension, with some degree of orthostatic hypotension following volume replacement is the goal of preoperative alpha blockade. At our center, we are prepping outpatients with escalating doses of phenoxybenzamine for at least 3 weeks. In patients resistant to phenoxybenzamine, tyrosine hydroxylase inhibitors can be used. Calcium channel blockers have been advocated with patients in a few centers. In one series, appropriate preoperative medication was credited for a reduction in mortality from 18 to 2%. Beta blockade may be used in patients with adequate alpha blockade and tachycardia but should never be used alone as they may precipitate a hypertensive crisis.

Surgery is the mainstay of treatment for pheochromocytoma with laparoscopic surgery being the treatment of choice.
Sill, these patients need to be carefully managed as mortality of 2.4% and morbidity of 24% have been seen in several series.44-46 Despite initial concerns, laparoscopic adrenalectomy has proven to be a safe approach for these patients.45,47 Indications for open surgery include peri-adrenal fibrosis, evidence of local invasion, and recurrence.31 Several series have documented comparable safety of laparoscopic adrenalectomy in large, (>6 cm) and small (<6 cm) tumors, and better intraoperative blood pressure control versus open surgery.46,47

In the immediate postoperative period, patients remain at risk for hypotension and hypoglycemia.48 We follow patients closely with plasma metanephrines every 3 months and abdominal CT scans every 6 months for recurrence.38,49 Histological features can not rule of malignancy and recurrence can occur even after normal initial biochemical testing, so long term follow-up is important.38,49 In fact recurrences, both benign and malignant have been reported as long as 17 years following “successful” surgery.50

Malignant

Adrenocortical Carcinoma: Work-Up

Primary malignancy of the adrenal gland is a rare occurrence. Adrenocortical carcinoma (ACC) has an incidence of 1:1.7 million and accounts for just 0.02% of all malignancies.51 Unfortunately, more than 20% have distant metastases at the time of diagnosis, and 20–30% have loco-regional disease.52 In a series of 253 patients with ACC, overall 5-year actuarial survival was 38%, and 50% after surgery with curative intent.52 Patients may present with a hypersecretion syndrome and/or a mass. Clinical evidence of hypersecretion, most commonly resulting in CS is seen in 60–70% of patients with ACC.52,53 Other hormones including sex steroids and occasionally aldosterone may also be secreted. This tumor may present as a non-secretory mass as well. Finally, ACC may also be discovered on imaging done for an unrelated reason. A systematic review of more than 2,000 “incidentalomas” revealed that 4.7% were ACC’s.36

Absolute criteria for malignancy are limited to metastases and local invasion. Risk of potential malignancy has also been linked to tumor size.36,54 Lesions of 4 cm in size are associated with a 10% probability of malignancy, and at 8 cm the post-test probability is 47%. Many authors recommend removing all lesions above 4 cm as the risk of malignancy is doubled at this point.54 Unfortunately, ACC in lesions smaller than 3 cm have been reported.36,55 Other features, including heterogeneous tumors with necrosis, irregular margins, and patterns of contrast washout or signal intensity may be suggestive of malignancy.36,56 Image guided biopsy has played a limited role in the work up of adrenal lesions because of concerns regarding tumor seeding and limited ability to distinguish adenoma from carcinoma.

Adrenocortical Carcinoma: Treatment

Outcome in most patients is poor with an overall 5-year survival of 40% or less.52,57 Adrenalectomy provides the only chance for cure. In general, open adrenalectomy via an anterior transperitoneal approach is preferred. Laparoscopic surgery has generally been avoided in ACC because of concerns about rupturing the capsule and incomplete resection. However, some authors have suggested that laparoscopic resection of adrenal malignancy may be feasible if the principles of oncologic resections are respected.58,59 In a recent series of 253 patients in France, operative mortality was found to be 5.5% and 5-year survival was 38%.52 Factors affecting survival included stage, curative intent, and age <35 years.52 Resection of metachronous isolated metastases may be beneficial in select cases. In a recent series, three of 15 patients with resected ACC liver metastases were alive at 5 years.60 Similarly, complete resection of locoregional recurrence may result in 5-year survival rates of almost 30%.37

Adjuvant therapy for ACC has been disappointing. Mitotane is the most effective agent however, in one series a survival advantage was found, only in patients with stage 4 disease.52 Unfortunately, mitotane is poorly tolerated with toxicity sufficiently severe to discontinue therapy is seen in 30–40% of patients.61 In general, ACC is considered radiation resistant, but tumor bed radiation following resection may decrease the risk of recurrence.62

Metastases to the Adrenal Gland

The adrenal gland is a common site of metastases. A classic series of 1,000 autopsies on patients with known primary cancers revealed adrenal metastases in 27%.63 Tumors that metastasize to the adrenal include non-small cell lung cancer, renal cell carcinoma, colorectal cancer, melanoma and others.64 Image guided fine needle aspiration biopsy may be useful in confirming the diagnosis of metastatic disease in patients with known carcinoma and an adrenal mass. Resection of both synchronous and metachronous isolated adrenal metastases has been reported with 5-year actuarial survival rates of 25–30%.65 As well, laparoscopic resection of isolated adrenal metastases appears to be feasible. Some authors have suggested that similar oncological outcomes with reduced morbidity can be achieved using laparoscopic resection.58,59

Adrenal Incidentaloma

Discovery of an “incidental” or “clinical inapparent” adrenal lesion has become a more frequent occurrence as the use of radiological modalities such as ultrasound, MRI, and CT has increased. Large autopsy series have found unexpected adrenal nodules in 6% of patients. A recent study from Italy found a 4.2% incidence of benign adrenal lesions on CT scan of patients enrolled in a lung cancer screening study.66
The differential diagnosis of these, “incidentalomas” or “adrenalomas” is broad, (Table 19.1). While the majority of these lesions are benign, nonfunctioning adenomas, a smaller but significant proportion are hormonally active and/or potentially lethal tumors. Thus, the work-up of these lesions must seek to answer two questions: (1) is this adrenal lesion hormonally active; and (2) could this lesion be malignant? As always the evaluation of any disease of the adrenal gland begins with a complete history and physical examination.

Approximately 5.4% of patients with adrenal incidentalomas are found to have SCC, making it the most common hormonal abnormality in this population.36 All patients with incidentalomas should undergo biochemical screening with serum and urine 24-h catecholamines and metanephrines. Serum AM cortisol and 24-h urinary cortisol should be obtained and dexamethasone suppression testing may be required in selected cases. Serum potassium, aldosterone and rennin will complete the work-up.36 Patients in whom metastatic disease is suspected can undergo FNA only after catecholamine secretion has been ruled out. Imaging phenotype may also help to guide treatment. Malignant tumors are typically larger with irregular margins.36,67 A size cutoff of 4 cm is approximately 90% sensitive for ACC, however 76% above 4 cm are benign. Adrenal Cortical Carcinoma is usually hyperintense on T2 weighted MRI. As well incidentalomas with density greater than 15 Housfield units on non-contrast CT scan and delayed contrast wash-out are more likely malignant.36 Overall, all hormonally active tumors should be removed. Currently, in the absence of hormonal secretion, laparoscopic adrenalectomy is recommended for lesions >3–4 cm, as those with other concerning features on imaging.

**Table 19.1. Differential diagnosis of adrenal incidentalomas.**

<table>
<thead>
<tr>
<th>Benign nonfunctioning mass</th>
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<tbody>
<tr>
<td>Adenoma</td>
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<tr>
<td>Adrenolipoma</td>
</tr>
<tr>
<td>Amyloidosis</td>
</tr>
<tr>
<td>Cyst</td>
</tr>
<tr>
<td>Ganglioneuroma</td>
</tr>
<tr>
<td>Granuloma</td>
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<tr>
<td>Hamartoma</td>
</tr>
<tr>
<td>Hematoma</td>
</tr>
<tr>
<td>Hemangioma</td>
</tr>
<tr>
<td>Infection (fungal, tuberculosis, echinococcosis, cryptococcosis, nocardiosis, paragonimiasis)</td>
</tr>
<tr>
<td>Leiomyoma</td>
</tr>
<tr>
<td>Lipoma</td>
</tr>
<tr>
<td>Myelolipoma</td>
</tr>
<tr>
<td>Neurofibroma</td>
</tr>
<tr>
<td>Pseudocyst</td>
</tr>
<tr>
<td>Teratoma</td>
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<table>
<thead>
<tr>
<th>Malignant nonfunctioning mass</th>
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</thead>
<tbody>
<tr>
<td>Angiosarcoma</td>
</tr>
<tr>
<td>Ganglioneuroblastoma</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
</tr>
<tr>
<td>Malignant schwannoma</td>
</tr>
<tr>
<td>Metastatic carcinoma</td>
</tr>
<tr>
<td>Primary malignancy (adrenocortical carcinoma)</td>
</tr>
<tr>
<td>Primary malignant melanoma</td>
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<table>
<thead>
<tr>
<th>Hyperfunctioning mass</th>
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<tbody>
<tr>
<td>Congenital adrenal hyperplasia</td>
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<tr>
<td>Masculinizing or feminizing tumor</td>
</tr>
<tr>
<td>Nodular hyperplasia</td>
</tr>
<tr>
<td>Pheochromocytoma</td>
</tr>
<tr>
<td>Subclinical Cushing syndrome</td>
</tr>
<tr>
<td>Primary aldosteronism</td>
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<tr>
<td>Primary malignancy</td>
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</tbody>
</table>

<table>
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<tr>
<th>Pseudoadrenal mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mistaken vasculature</td>
</tr>
<tr>
<td>Liver</td>
</tr>
<tr>
<td>Lymph nodes</td>
</tr>
<tr>
<td>Pancreatic mass</td>
</tr>
<tr>
<td>Renal mass</td>
</tr>
<tr>
<td>Spleen</td>
</tr>
<tr>
<td>Stomach mass</td>
</tr>
<tr>
<td>Technical artifact</td>
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</tbody>
</table>

**Conclusion**

Evaluation of patients with adrenal disease begins with a thorough history and physical examination. Advances in imaging technology have resulted in increased detection of clinically in apparent adrenal masses on tests done for unrelated indications. Some of these patients will ultimately prove to have functional or malignant tumors. In all patients with adrenal lesions, efficient and appropriate use of biochemical testing and imaging is important. In the work up of all adrenal masses, the clinician should seek to answer two questions: (1) is this mass functional; and (2) could this lesion be malignant.

**References**


Introduction

Pheochromocytomas (PCC) and paragangliomas (PGL) are tumors arising from neural crest-derived chromaffin cells. PCC are located in the adrenal medulla and produce, with few exceptions, catecholamines. Sympathetic PGL (sPGL, previously also designated extraadrenal PCC) also produce catecholamines but arise in the chromaffin tissue of the sympathetic trunk that is situated along the proximal aorta reaching down to the abdominal aorta and the urinary bladder. In the developing embryo, the organs of Zuckerkandl, which serve as catecholamine-releasing organs during development of the fetal adrenals, can be identified in the sympathetic trunk, which is an important site for sPGL to occur, especially in young patients. Together, PCC and sPGL have an incidence that varies between 2 and 8 per million.1

The parasympathetic nervous tissue gives rise to small clusters of chief cells located in the head and neck region. Tumors arising from these cells are designated parasympathetic PGL (pPGL), although a wide range of terms has been applied for these tumors in the past (e.g., chemodectoma and glomus tumor), which should no longer be used. pPGL arise in the majority of cases in the carotid body and the jugulotympanic paraganglia, followed by vagal, laryngeal, and, in some cases, aorticopulmonary paraganglia. These tumors usually do not produce catecholamines. PCC, sPGL, and pPGL share many histological and immunohistochemical characteristics, but recent work has shown that they have distinct genetic aberrations, as will be specified later in this chapter.

Clinical Presentation, Histology, and Immunohistochemistry

In clinical practice, PCC and sPGL have a heterogeneous presentation and are therefore known as “the great mimic.” The vast majority of patients present with continuously or paroxysmally increased blood pressure, with headaches, signs of flushing or palpitations, although a small number of patients will be asymptomatic. In the latter cases, the diagnosis is incidental, often in the context of diagnostic imaging procedures for other purposes; these tumors are known as “incidentalomas.” One study of Mayo clinic patients demonstrated that 10% of the adrenal incidentalomas are in fact PCC.2 pPGL, which do not produce catecholamines, usually present because of mass effect, which can cause symptomatic cranial nerve palsy.

PCC and PGL usually have a characteristic histological pattern. In the context of a typical clinical presentation, the diagnosis should be relatively straightforward. Occasionally, however, these tumors must be distinguished from a variety of other endocrine and nonendocrine tumors. The classical growth pattern shows so-called “Zellballen,” formed by rounded nests of large uniform polygonal cells (Figure 20.1). These cells have granular amphophilic or basophilic cytoplasm. The cellular nests are surrounded by S100-positive sustentacular cells, which are nonneoplastic and can usually be seen with the help of immunohistochemical stains. Sometimes, the Zellballen pattern is not so clear, with a more diffuse architecture and cells arranged in large sheets. The variable degree of cytologic atypia of PCC may be impressive, to the extent of mimicking malignancy. pPGL may have more pronounced Zellballen and more eosinophilic cytoplasm, but overlap exists between the two types of tumor.

The best immunohistochemical marker currently used for PCC and PGL is chromogranin A (CgA), a major constituent of secretory granules.3 Immunoreactivity for CgA will distinguish PCC and PGL from adrenocortical tumors and nonendocrine tumors. Staining of PCC for CgA is usually moderate to strong and diffuse. This marker is so consistent that the diagnosis of PCC should be reconsidered if CgA is negative. In contrast, the vast majority of adrenocortical tumors will show reactivity to inhibin and/or Melan A, which are rare to absent in PCC and PGL.

The issue of malignancy in PCC and PGL is one that has been extensively investigated but remains unresolved. Currently, the 2004 WHO definition states that malignancy
can only be diagnosed when metastases are present in sites where chromaffin tissue does not normally occur.4 This specific definition is used to prevent overdiagnosis of carcinoma in patients who have second primary tumors in the setting of hereditary syndromes (see below). From a clinical patient management perspective, other indicators of malignancy in the primary tumor would obviously be of great benefit. Though many studies have investigated differentiating benign from malignant tumors, most suffer from low numbers of tumors and poor adherence to the strict criteria as set out by the WHO. Three important studies have been based predominantly on histological criteria. A study by Linnoila et al in 1990 examined 120 sympathetic PGL and PCC and developed a statistical model according to which >70% of the tumors could be classified correctly with >95% probability on the basis of four criteria: extraadrenal location, coarse nodularity, confluent necrosis, and absence of hyaline globules.5 Most malignant tumors had two or three of those features, while 89% of benign tumors had one or none.

In 2002, Thompson proposed the PASS system (pheochromocytoma of the adrenal scaled score) specific to the adrenal gland. This system scores multiple microscopic findings to arrive at a total score that correlates with metastatic potential.6 All tumors that metastasized had a score >4, but 17/50 with score >4 had not metastasized in a follow-up period of ~5 years. A recent study that investigated the applicability of the PASS by a group of five endocrine pathologists showed a large inter- and intraobserver variation in a large set of PCC.7 As there are no other validation studies, the practical use of the PASS is currently limited.

Numerous immunohistochemical markers have been reported to correlate with malignancy, but their usefulness is still limited to research purposes.6–10 The marker most consistently reported to be correlated with malignancy is the proliferation marker Ki-67, which is performed on paraffin sections using monoclonal antibody MIB-1.10–12 However, in some studies, MIB-1 labeling does not correlate with malignancy. Studies of MIB-1 labeling show a striking lack of methodological consistency, and many papers do not provide sufficient methodological detail to permit replication.

A 2005 scoring system proposed by Kimura for both PCC and extraadrenal sPGL combines histological, immunohistochemical, and biochemical characteristics to arrive at a score reported to predict both the metastatic potential of tumors and the prognosis for patients with tumors that metastasize.11 However, of so-called low grade tumors, there was still a considerable proportion that metastasized, limiting the practical usefulness.

Molecular Abnormalities in Sporadic PCC and PGL

Over the past two decades, six candidate genes involved in the pathogenesis of hereditary PCC and PGL have been identified, including RET, VHL, SHDB, SDHC, SDHD, and NF1. Thus, the genetic basis for several hereditary tumor syndromes has been elucidated: MEN2 caused by RET, Von Hippel–Lindau disease caused by VHL, hereditary PCC/PGL caused by one of the SDH genes, and neurofibromatosis type 1 caused by NF1. Interestingly, in the absence of known hereditary disease, it has also been demonstrated that a substantial number of patients with apparently sporadic PCC and/or PGL carry germline mutations in one of the above genes.13 Currently, it is estimated that 25–30% of PCC and PGL patients have germline mutations, whether they are from a known family or not. Consequently, truly sporadic PCC and PGL should be a diagnosis of exclusion, after genetic testing for the above genes and in the context of a negative family history.

After the identification of these important candidate genes, several studies have been performed to investigate the occurrence of somatic mutations in these genes as well. Although low frequencies of somatic RET and VHL mutations have been found, somatic mutations in SDHB and SDHD are exceedingly rare.14,15 Many other candidate genes, including both oncogenes and tumor suppressor genes have also been investigated, including c-mos, p53, and PTEN, but no mutations have been detected.16–18 The advent of techniques that allow genome-wide analysis of DNA in large series of tumors has confirmed and expanded previously known data from LOH analysis. It now appears that the majority of PCC is characterized by the loss of chromosomal regions 1p and 3q.19–20 A more detailed analysis of the short arm of chromosome 1 has been investigated by several groups with the aim of finding candidate genes in the smallest region of overlap of the various losses, but these efforts have not been fruitful.21 Likewise, other regions of chromosomal loss or gain have not yielded candidate genes, which could be further tested by mutation analysis. Recently, work from our own...
Multiple endocrine neoplasia syndromes are currently subdivided into MEN1 and MEN2. MEN1 is due to mutations in the \textit{menin} tumor-suppressor gene (TSG) located on 11q13. The occurrence of PCC in this syndrome is very rare, with only seven patients with mutations in the \textit{menin} gene described in the literature. Thus, the discussion will be limited to MEN2, which can be divided into MEN2A and MEN2B. In the past, MEN2B was also designated MEN3, but this term is no longer applied in current literature. Recently, an MEN4-syndrome has been described with mutations in the \textit{CDKN1B} gene, with an MEN1-like phenotype, but the occurrence of PCC has only been documented only in animal models, not in humans.\textsuperscript{28,29}

The incidence of MEN2 is not known, but it is estimated to be 1.25–7.5/10,000,000 per year.\textsuperscript{1} The syndrome is due to activating mutations in the \textit{RET} proto-oncogene, coding for a tyrosine kinase receptor, located on 10q11.2. In MEN2A, the mutations are mainly found in the exons coding for the extracellular domain (exon 10 and 11 of the \textit{RET} gene), but exons 13–15 can be involved in some cases. Functional changes due to these mutations are ligand-independent dimerization of the RET receptor, with subsequent activation of the RET signaling pathway. MEN2A is clinically defined by the presence of parathyroid hyperplasia, C-cell hyperplasia/medullary thyroid carcinoma, and PCC. The disease can manifest early in life, usually with medullary thyroid carcinoma (MTC), and can also manifest with a hypertensive crisis due to a PCC. PCC occur in about 50\% of all patients with MEN2-syndrome and are frequently bilateral.\textsuperscript{30,31}

In addition, as described by Carney et al, most contralateral adrenal glands show either diffuse or nodular hyperplasia, which represents a precursor for PCC. It should be noted that the absence of a characteristic family history is not sufficient to rule out the diagnosis of MEN2A, since de novo germline mutations have been reported.\textsuperscript{32}

MEN2B is also due to activating mutations in the \textit{RET} proto-oncogene, with mutations in the kinase domain of the receptor (coded by exon 16 of the \textit{RET} gene, p.M918T in 95\% of all cases). The functional consequences of this mutation are diverse with loss of kinase inhibition, dimerization, and autophosphorylation without substrate binding to the receptor, all of which lead to aberrant RET signaling.\textsuperscript{33} Clinically, the syndrome has overlapping features with the MEN2A syndrome, with the exception of the parathyroid hyperplasia. Striking in this syndrome is the occurrence of mucosal ganglioneuromas, giving a peculiar facial appearance, and skeletal deformities as described by Carney et al.\textsuperscript{34} De novo mutations causing MEN2B syndrome occur frequently, up to 50\%.\textsuperscript{35} In addition to germline mutations, somatic \textit{RET} mutations in PCC have been described (the exon 16 p.M918T), but these mutations are not clinically relevant, since they do not increase the risk for other tumors. For all MEN-syndromes, the occurrence of malignancy of PCC is rare. No pPGL and sPGL have been described in MEN2 in the literature.

Using comparative genomic hybridization (CGH), genomic alterations in PCC from patients with MEN2 syndrome have been analyzed. In MEN2A patients, there is a consistent pattern of 1p and 3q loss, indicating that not only the involvement of the \textit{RET}-oncogene itself but also potential tumor suppressor genes located on 1p and 3q may be involved in the pathogenesis of MEN2-related PCC.

Von Hippel–Lindau (VHL) disease has an incidence ranging from 1/36,000 to 1/39,000.\textsuperscript{36,37} The disease has been subdivided into VHL type 1 and VHL type 2 (2a, 2b, and 2c). PCC do not develop in VHL type 1, but a considerable risk (16–30\%) in VHL type 2 mutation carriers has been reported. Overall, PCC develop in 10–20\% of patients with VHL and are usually bilateral.\textsuperscript{38,39} The VHL protein is involved in the regulation of HIF degradation in the presence of normal oxygen tension. The majority of mutations described in PCC are missense mutations that abrogate their ability to degrade HIF. The occurrence of sPGL and pPGL is rare but has been described in VHL patients.\textsuperscript{38}

Apart from PCC, VHL type 2a patients present with hemangioblastomas. In VHL type 2b, the same tumors occur with the addition of clear cell renal cell carcinoma. Rarer are cystic adenomas and endocrine tumors of the pancreas, papillary cystadenomas of the broad ligament and epididymis as well as endolympathic sac tumors. In VHL type 2c, there is isolated familial PCC. VHL disease is due to mutations and other genetic abnormalities

**Molecular Abnormalities in Hereditary PCC and PGL**

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Using comparative genomic hybridization (CGH), genomic alterations in PCC from patients with MEN2 syndrome have been analyzed. In MEN2A patients, there is a consistent pattern of 1p and 3q loss, indicating that not only the involvement of the \textit{RET}-oncogene itself but also potential tumor suppressor genes located on 1p and 3q may be involved in the pathogenesis of MEN2-related PCC.

Von Hippel–Lindau (VHL) disease has an incidence ranging from 1/36,000 to 1/39,000.\textsuperscript{36,37} The disease has been subdivided into VHL type 1 and VHL type 2 (2a, 2b, and 2c). PCC do not develop in VHL type 1, but a considerable risk (16–30\%) in VHL type 2 mutation carriers has been reported. Overall, PCC develop in 10–20\% of patients with VHL and are usually bilateral.\textsuperscript{38,39} The VHL protein is involved in the regulation of HIF degradation in the presence of normal oxygen tension. The majority of mutations described in PCC are missense mutations that abrogate their ability to degrade HIF. The occurrence of sPGL and pPGL is rare but has been described in VHL patients.\textsuperscript{38}

Apart from PCC, VHL type 2a patients present with hemangioblastomas. In VHL type 2b, the same tumors occur with the addition of clear cell renal cell carcinoma. Rarer are cystic adenomas and endocrine tumors of the pancreas, papillary cystadenomas of the broad ligament and epididymis as well as endolympathic sac tumors. In VHL type 2c, there is isolated familial PCC. VHL disease is due to mutations and other genetic abnormalities
in the *VHL* tumor suppressor gene, located on 3p25. De
ovo mutations occur in about 20% of clinically detected
*VHL*-patients. Therefore, not only family history but also
the clinical presentation is important in PCC. There is a
slight correlation of *VHL* mutations and malignancy, but
the majority of *VHL*-related PCC is benign. In addition to
germline mutations, somatic *VHL* mutations in PCC have
been described, although these mutations do not appear to
be clinically relevant.

PCC that occur in the context of either germline or somatic
*VHL* mutations also have consistent genomic alterations as
shown by CGH or array-CGH. These mainly consist of loss
of 3p and 11p, which matches the localization of the *VHL*
tumor suppressor gene on 3p.

In neurofibromatosis type 1 (also known as von Reck-
inghausen’s disease), there is a small proportion of patients
(up to 5%) with PCC. Compared to the previously described
PCC susceptibility syndromes, this syndrome occurs much
more frequently, affecting 1:3,000 individuals. The hall-
marks of this syndrome are the occurrence of multiple neuro-
fibromas and the presence of café-au-lait pigmentation
and Lisch-nodules of the iris. However, the spectrum is much
more diverse with the occurrence of gastrointestinal stromal
tumors (GIST), central nervous system malignancies, and an
increased risk of breast cancer in young women.39,40

NF1 syndrome is caused by mutations in the *neurofibromin*
or *nf1* tumor suppressor gene located on 17q11. Because
the gene is exceptionally large and there are no mutation
hotspots, mutation analysis is not routinely performed, as
the diagnosis can also be reliably made on clinical charac-
teristics. Although autosomally dominant, the syndrome also
arises de novo in almost half of the cases. In the literature,
a subset of apparently sporadic PCC has been investigated,
of which one patient turned out to have NF1 after clinical
examination.41 There is no relationship between the type of
*neurofibromin* mutation and the occurrence of PCC in NF1
patients.42 Thus far, no somatic mutations in PCC of NF1
patients have been described. Malignancy in NF1-related
PCC is extremely rare.

In studies on genetic alterations, only few NF1-related
PCC have been investigated. Only one was investigated in
the study by Edström et al, which displayed loss of 1p and
3q. Interestingly, an NF1 mouse model was developed, with
the same phenotype as human NF1.43 The genetic profiles of
the three cell lines derived from the mouse PCC resembled
that of human PCC, also displaying loss of the mouse homo-
logs of 1p and 3q.

The PCC–PGL syndrome is caused by mutations in three of
four genes coding for the succinate–ubiquinone oxido-
doreductase complex II of the aerobic transport chain and the
tricarboxylic acid cycle. These genes are all tumor suppres-
sor genes and are named *SDHB, SDHC*, and *SDHD*, located
on 1p36, 1q21, and 11q23, respectively. The fourth gene
involved in complex II, *SDHA*, is known to be involved in
Leigh syndrome, where both alleles are inactivated through
mutations.44 *SDHB, SDHC*, and *SDHD* are the genes in the
chromosomal regions that had been designated PGL4, PGL3,
and PGL1, respectively and that were linked to the occur-
rence of PGL. Recently, the *SDH5* gene has been discov-
ered as the candidate gene in the PGL2 chromosomal region
11q13.1, and renamed to *SDHAF2*.45 It was since shown that
the gene is mutated in a limited number of families with head
and neck PGL, but not in a large series of sporadic PCC and
PGL.46 The spectrum of the PCC–PGL syndrome is expand-
bng but seems to comprise almost all patients with any com-
bination of PCC, pPGL, and sPGL. Although a positive family
history for PCC and/or PGL is indicative of the syn-
drome, a significant subset of apparently sporadic PGL has
germline mutations in the genes involved in the PCC–PGL
syndrome.47,48 It has been hypothesized that the penetrance
of the syndrome is low and is influenced by the oxygen
tension of the patients’ habitat.49

*SDHB* germline gene mutations are frequently involved in
sPGL but have also been described in PCC and pPGL. In addi-
tion, *SHDB* mutations have been found in patients or families
with the co-occurrence of GIST and PCC or PGL, reported as
the Carney–Stratakis syndrome. Also, three patients have been
described in the literature with pPGL and renal cell carcinoma
that harbored a germline *SDHB* mutation. Mutations in *SDHC*
are infrequently found and have been reported in sPGL and
pPGL so far.50–53 Also, the co-occurrence of GIST and PGL is
found due to mutations in *SDHC*. Finally, mutations involving
*SDHD* are not only frequently found in pPGL but have also
been found in PCC and sPGL. Not only patients with a posi-
tive familial history but also a subset of “apparently” sporadic
pPGL are due to germline mutations in this gene. Detection of
genetic abnormalities in each of these three SDH genes is usu-
ally performed by direct sequence analysis of the entire coding
region, but it must be noted that multiplex ligation-dependent
probe amplification (MLPA) has shown larger gene deletions
in a number of cases.54

Malignancy in SDHx-related PCC and PGL is more fre-
quent than in the aforementioned syndromes. In SDHB-
related PCC and sPGL, the risk of malignancy, defined as
the presence of metastases, appears to be 30–50% and is
suggested to be even higher by some.55–58 Also *SDHB*
ergline mutations in pPGL give an increased risk of malignant
behavior, as described by Boedeker et al in a large series of
pPGL. There has been a single case of sPGL with malignant
behavior with an *SDHC* mutation. Malignancy in patients
with *SDHD* mutations has been described, and a possible
correlation is found with the “Dutch founder mutation” of
the *SDHD* gene D92Y. All five malignant SDHD-related
tumors described by Havekes et al were PGL, including one
sPGL and four pPGL that harbored the D92Y mutation. In
the literature the occurrence of malignancy in *SDHD*-related
PCC and PGL does not seem to be increased.59,60 Somatic
mutation of the *SDHx* genes are rare, with two cases that
have been described, one *SDHD* mutation in a PCC and one
*SDHB* mutations in an sPGL.15,60
Few tumors in the context of the PCC–PGL syndrome have been investigated by CGH. However, the series of pPGL as published by Dannenberg et al contained familial pPGL that turned out to be due to SDHD-related. In this small series, the consistent feature was the small number of genomic alterations, where the hallmark of the familial tumors was loss of 11q, matching the location of the SDHD gene. The only SDHB-related sPGL CGH-analysis described in the literature showed distinct loss of chromosome 1, in accordance with the location of the SDHB gene. However, this sPGL contained a somatic and not a germline mutation. In the small series investigated so far, it appears that PCC and sPGL in patients with germline SDHB and SDHD mutations show a distinct loss of 1p and 11q, respectively, with only few alterations throughout the genome, indicating the importance of the TSG in the tumorigenesis of these tumors.

Other Susceptibility Loci

Finally, it should be mentioned that other loci are found to be involved in familial pPGL and PCC. A study using linkage analysis in PCC has found 2 loci for familial PCC located at 2cen and 16p13. However, the genes associated with these loci are not known.

Summary

Molecular markers play a crucial role in the diagnosis of hereditary tumor syndromes in whose context PCC and PGL may occur. To detect mutations and other genetic abnormalities, genetic screening is advocated for RET, VHL, SDHB, and SDHD in all PCC and PGL patients, at least under the age of 50. The frequency of SDHC mutations is too low to warrant investigation of this gene systematically. Likewise, the complexity of NF1 precludes inclusion of this gene in routine screening. Analysis should predominantly consist of direct sequencing of the entire coding region of genes, with the exception of RET, for which selected exons can be studied. However, it is evident that larger genetic abnormalities occur in several of these genes, which need other molecular approaches.

The detection of mutations has a profound effect on follow-up of the patient, depending on the specific syndrome. In addition, the detection of SDHB mutations not only has a diagnostic significance, but also a prognostic one, as they correlate strongly with malignant tumor behavior. Apart from an effect on the patient, the detection of germline mutations impinges also on family members and relatives, depending on the fact whether such mutations are de novo or inherited. Thus, genetic counseling of PCC and PGL patients and family members is imperative before embarking on genetic analyses.

Currently, there do not exist therapeutic molecular markers for PCC and PGL, but it is expected that with the gradual elucidation of tumorigenesis, these may be developed in the next decade. In this respect, it is interesting to note that recently a hypothesis has been put forward, unifying all PCC/PGL susceptibility genes in a single biochemical pathway.

References


47. Dannenberg H, Djinjens WN, Abbou M, et al. Frequent germ-line succinate dehydrogenase subunit D gene mutations in...


Introduction

Adrenal cortical diseases are relatively rare but are important to recognize and understand because of their significant morbidity and mortality. Developments in both molecular biology and molecular genetics have significantly increased our understanding of the process of steroidogenesis and steroid action. This in turn has allowed us to broaden our understanding of the various inherited diseases that affect this pathway. The main diseases and their pathogenesis will be presented in this chapter.

The investigation of the molecular pathways involved in the pathogenesis of adrenal cortical tumors has been hampered by the rarity of adrenal cortical carcinoma (ACC) and the fact that, until recently, adrenal cortical adenoma (ACA) was only identified during life if the tumor was secreting excess hormone. However, by learning lessons from other tumor types, from familial syndromes in which these tumors occur more frequently and by examining the signaling pathways involved in steroidogenesis and adrenal cortical growth, the molecular genetics of these tumors are being unraveled. Again, the current state of knowledge will be presented. Autoimmune adrenalitis also has molecular genetic aspects, but these are not dealt with here.

Normal Adrenal Structure and Function

The adrenal cortex has a critical role in homeostasis-producing glucocorticoids, mineralocorticoids, and sex steroids. It arises from the adrenogonadal primordium that develops from the urogenital ridge. The Wilm’s tumor gene (WT-1) and the WNT4 gene play early roles in development. Other important regulators include the transcription factor, steroidogenic factor 1 (SF-1), and the nuclear receptor, dosage-sensitive sex reversal, adrenal hypoplasia critical region gene 1 (DAX-1). The adult cortex comprises three zones. The outer zona glomerulosa (ZG) produces aldosterone, the main mineralocorticoid and is made up of small angular cells, dispersed focally under the capsule. The zona fasciculata (ZF) is the major component, formed of large lipid-laden cells arranged in columns from the capsule to ZG to the inner zona reticularis (ZR). It is thought to be the major source of glucocorticoids – cortisol in the human adrenal. The ZR is made up of eosinophilic (compact) cells arranged around branching vascular sinusoids. It is the source of adrenal androgens.

Cholesterol is the precursor for all steroids and the enzymes involved are four members of the cytochrome P-450 family and a 3β-hydroxysteroid dehydrogenase. The production of aldosterone is mainly under the control of the renin–angiotensin system, although potassium, adrenocorticotrophic hormone (ACTH), catecholamines, and prostaglandins also play a role, and recent evidence suggests that endothelial cell-derived factors and adipokines are involved. The production of aldosterone specifically by the ZG is because the enzyme aldosterone synthase is expressed only in this zone. The secretion of cortisol is controlled mainly by ACTH acting through the ACTH membrane receptor, a G protein-linked receptor. The G protein comprises three polypeptide subunits, (α, β and γ). On ligand binding, the α subunit (Gs) dissociates from the other subunits and when bound to guanosine triphosphate (GTP) activates adenylate cyclase, causing the production of cyclic AMP (cAMP). Cyclic AMP binds to the regulatory subunits of the tetrameric protein protein kinase A (PKA), allowing the catalytic subunits to be released, phosphorylated, and transferred to the nucleus where they influence gene transcription. Other factors possibly involved include insulin-like growth factors (IGF-1 and IGF-2), adrenomedullin, transforming growth factor β and activin A that may have an inhibitory role. Catecholamines may also have a role.
Clinical Features of Adrenal Cortical Disease

Hypersecretion of Hormones

Diseases of the adrenal can present with signs and symptoms of excess or reduced hormone secretion or with evidence of an adrenal mass or metastases from a carcinoma. Three classical syndromes are associated with hypersecretion of steroids. These are primary hyperaldosteronism (including Conn syndrome), hypercortisolism (Cushing syndrome), and hypersecretion of sex steroids (adrenogenital syndrome).

Primary Hyperaldosteronism

This is now recognized as accounting for 5–13% of cases of hypertension, mostly sporadic. About one third are associated with an adrenal adenoma and a further 60% with bilateral idiopathic hyperplasia (IHA). Unilateral hyperplasia accounts for ~2% of cases. Carcinomas are rare. Adenomas are often less than 2 cm in diameter and the cut surface is golden-yellow. Histologically, they comprise mainly cells resembling ZF, but some contain cells similar to ZG and hybrid cells sharing features of both (Figure 21.2). Focal compact cells are also found. The paraadenomatous gland may contain micronodules. The adjacent cortex may show hyperplasia of the ZG, but it is unclear whether this is related to pathogenesis or to the effects of treatment. In IHA, the ZG usually forms a continuous band rather than being focally dispersed. However, unless nodules are present, the glands in IHA may be of normal weight. Familial hyperaldosteronism is discussed later.

Cushing Syndrome

The clinical features of this syndrome are well recognized and include centripetal obesity, moon face, hypertension, striae, osteoporosis, and psychiatric symptoms. Two-thirds of cases are the result of hypersecretion of ACTH by the anterior pituitary gland, with 80–90% of patients having a corticotroph adenoma. Most have bilateral diffuse cortical hyperplasia (Figure 21.3) and the glands usually weigh 6–12 g, with broadening of the ZF and ZR and a relative prominence of ZR. Microscopic nodules are not uncommon, especially in the outer ZF. Ten to twenty percent of patients have bilateral nodular hyperplasia, with nodules visible to the naked eye. The intervening cortex is also hyperplastic. The relationship between the two is unclear, but they may be a continuum, with nodules developing in longer-standing disease.

Ectopic ACTH syndrome underlies about 15% of cases, around half associated with small-cell lung carcinoma or bronchial carcinoid. Other tumors causing the syndrome include thymic carcinoids, endocrine tumors of pancreas, medullary carcinoma of thyroid, and pheochromocytoma.
The adrenals usually show bilateral symmetrical hyperplasia, weighing an average of 15 g each. Nodules are rare. Compact cells extend out close to the capsule and pleomorphism, and mitotic figures are not infrequent.

Fifteen to twenty percent of adults have an adrenal tumor, equally split between benign and malignant. Females are more commonly affected. In children, more than half the cases are associated with tumor, the majority carcinoma. Because of the increased negative feedback to the pituitary, ACTH secretion is suppressed and the adjacent and opposite ZF and ZR are atrophic. Because of this, the ZG may appear more prominent.

ACTH-independent macronodular adrenal hyperplasia (AIMAH) is a rare variant of the syndrome. The adrenal glands are often distorted and markedly enlarged. ACTH levels are suppressed. In many of these cases, the adrenal expresses inappropriate receptors and responds to ligands such as gastric inhibitory polypeptide (GIP), where the increase in cortisol secretion is related to food intake. Other receptors identified include those for vasopressin, luteinizing hormone, and serotonin.

**Adrenogenital Syndrome**

Excess production of sex steroids causes virilization, feminization, or precocious puberty, depending on the age and sex of the patient and the nature of the steroids secreted. Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive diseases associated with inherited defects in steroidogenesis. Most present early in life. The lack of negative feedback by cortisol to the pituitary causes an increase in ACTH secretion and marked adrenal cortical hyperplasia. The clinical presentation depends on the enzyme involved and is related to the range of steroids produced. The details are discussed below. Adrenal cortical tumors can also produce sex steroids, usually androgens.

**General Aspects of Adrenal Cortical Tumors**

Adrenal cortical nodules can be found in up to 53% of adrenal glands at autopsy. It is unclear exactly what proportion is neoplastic and what hyperplastic, but by using architectural and histological features, these studies suggested that adrenal cortical adenomas (ACA) were present in ~5%. This is similar to the 4% prevalence of adrenal lesions identified by computed tomography scan. They are more common with increasing age, seen in 7% of those over 70 years. Adenomas are usually single and unilateral, although bilateral lesions have been reported. They are intraadrenal, may be unencapsulated or show a true capsule or pseudocapsule. They show an expansile pattern of growth. On slicing, they usually have a yellow color with brown foci. The majority comprise mainly lipid-laden cells resembling ZF in an alveolar pattern. Groups of compact cells may be scattered throughout and may predominate in tumors producing androgens.

Adrenal cortical carcinoma (ACC) is a rare tumor, with an estimated prevalence of between 4 and 12 per million and accounts for 0.05–2% of all malignancies. Women are more commonly affected. There is a peak in early childhood and a second in the fifth to seventh decades. The tumor is aggressive with 67–94% mortality, and median or mean survival is between 4 and 30 months. Many are locally invasive at time of presentation and up to two-thirds have metastasized. They commonly metastasize to liver, lung, retroperitoneum, and lymph nodes. Between 26 and 76% of tumors are nonfunctional. The commonest functional syndrome is Cushing syndrome, often also with androgen excess. Virilization may occur alone, but estrogen secretion is rare. There may also be nonhormonal symptoms such as abdominal fullness or pain, loin pain, fever, weight loss, or other malignancy-related features. Most respond poorly to treatment and surgery is the mainstay. Resistance to chemotherapy may be related to the expression of glutathione-S-transferases and P-glycoprotein, which have roles in conferring drug resistance.

The majority of carcinomas weigh over 100 g, but small tumors may also be malignant. They may be encapsulated, but many infiltrate adjacent tissues and there may be obvious vascular invasion. The cut surface is usually yellow to brown and is often lobulated with fibrous bands separating the lobules. Necrosis and hemorrhage are common. Resistance to chemotherapy may be related to the expression of glutathione-S-transferases and P-glycoprotein, which have roles in conferring drug resistance. Some combine...
Clinical and histologic features. However, the most commonly used is still that of Weiss where a series of nine histologic features are assessed (Table 21.1), the presence of three or more indicating malignant potential. This has been validated in a more recent study, but the authors found poorer correlation over the assessment of nuclear pleomorphism and vascular invasion than of the other features and proposed that these should be omitted from the assessment and the others incorporated into a weighted numerical score (Table 21.2). This approach has been compared with the van Slooten and Weiss scores and has been shown to correlate with diagnosis and to survival in metastasizing carcinoma.

Occasional tumors with a Weiss score of 2 or less may behave in a malignant fashion.

There is no formal staging system for adrenal cortical carcinoma from either the International Union against Cancer (UICC) or the American Joint Committee on Cancer (AJCC), but most use that of Macfarlane, modified by Sullivan (Table 21.3). Seventy percent of patients present with stage 3 or 4 disease.

### Immunohistochemistry

Adrenal tumors rarely stain strongly positively for cytokeratins, particularly in the absence of antigen retrieval and are negative for epithelial membrane antigen (EMA). Antibody D11 has been reported to give positive staining.
and the combination of inhibin-\(\alpha\)^{54,55} and melan-A clone A103^{56} is useful, while others have recommended the addition of calretinin.^{57,58} Immunopositivity for proteins important in steroidogenesis, such as SF-1, DAX-1, and steroidogenic enzymes, has been reported^{59-61} but have not found a role in diagnostic practice, probably because of the lack of good commercial antibodies. Adrenal cortical hyperplasias and tumors show positivity for a range of general neuroendocrine markers including synaptophysin^{62-64} and, therefore, chromogranin A is the only marker that will positively discriminate pheochromocytoma from a cortical tumor.

Genetic Aspects of Adrenal Cortical Tumors

Familial Syndromes Associated with Adrenal Cortical Lesions (Table 21.4)

Li–Fraumeni Syndrome

Families with this syndrome have a predisposition to a number of tumors including breast carcinoma and soft tissue sarcomas. ACC occurs in 3–4% of family members.\(^{65}\) The TP53 tumor suppressor gene located on 17q13.1 encodes a protein with pivotal roles in cell proliferation and apoptosis.\(^{66}\) Seventy percent of families with Li–Fraumeni syndrome have a germline mutation in the gene, usually in exons 5–8.\(^{67}\) Others have a heterozygous mutation in the checkpoint kinase 2 (hCHK2) gene and ACC has not been reported in these kindreds.\(^{68}\) A locus on 1q23 is involved in other families.\(^{69}\)

Beckwith–Wiedemann Syndrome

This syndrome is associated with macrosomia, macroglossia, abdominal wall defects, and a predisposition to childhood tumors including ACC.\(^{70}\) It is linked to the 11p15 locus with evidence of involvement of a number of genes including IGF2, H19, and cyclin-dependent kinase inhibitor 1C (CDKN1C or p57\(^{kip2}\)). These genes are imprinted, IGF2 showing maternal and CDKN1C and H19 showing paternal imprinting.\(^{71}\) Most individuals with the syndrome have loss of the maternal allele and paternal disomy, leading to overexpression of IGF2 and reduced expression of H19 and p57\(^{kip2}\).

Carney Complex

This is a rare autosomal dominant condition characterized by pigmented lesions of skin and mucosa, cardiac and neural myxomas, and endocrine overactivity.\(^{72,73}\) Some present with Cushing syndrome and have primary pigmented nodular adrenocortical hyperplasia (PPNAD). Both adrenal glands consist of multiple nodules, usually 1–3 mm in diameter. The combined weights range from 4 to 21 g. The nodules appear dark brown to black and the intervening cortex appears suppressed. Many of these cases have been shown to be linked to the 17q22–q24 region. Heterozygous inactivating mutations of the protein kinase A regulatory subunit 1A (PRKAR1A) gene in this region were reported initially in 45–65% of index cases and may be present in about 80% of the families presenting mainly with Cushing syndrome.\(^{74}\) The protein product is important in the cAMP pathway. Linkage to chromosome 2 has also been reported.\(^{75}\)

Multiple Endocrine Neoplasia, Type 1

This is another autosomal dominant syndrome associated with mutations in the multiple endocrine neoplasia, type 1

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**Table 21.4. Familial syndromes associated with adrenal cortical tumors.**

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene</th>
<th>Chromosomal location</th>
<th>Adrenal lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li–Fraumeni</td>
<td>TP53</td>
<td>17p13</td>
<td>ACC in 1–4%</td>
</tr>
<tr>
<td>Beckwith–Wiedemann</td>
<td>? IGF2</td>
<td>11p15.5</td>
<td>ACC in 5%</td>
</tr>
<tr>
<td></td>
<td>H19</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CDKN1C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carney complex</td>
<td>PRKAR1A</td>
<td>17q22–24</td>
<td>PPNAD in 90–100%</td>
</tr>
<tr>
<td>Multiple endocrine neoplasia type 1</td>
<td>MEN1</td>
<td>11q13</td>
<td>ACA in 5% and rare ACC</td>
</tr>
<tr>
<td>Congenital adrenal hyperplasia</td>
<td>CYP21B (rarely CYP11B, CYP17A or HSD3B2)</td>
<td>6p21.3</td>
<td>BAH in 100%, ACA in 82% and rare ACC</td>
</tr>
</tbody>
</table>

ACAs adrenal cortical adenoma; ACC adrenal cortical carcinoma; BAH bilateral adrenal cortical hyperplasia; PPNAD primary pigmented nodular adrenocortical disease.
General Aspects of the Genetics of Sporadic Adrenal Cortical Tumors (Table 21.5)

Researchers have approached the investigation of adrenal cortical tumors in a number of ways. Chromosomal gains and losses have been examined by comparative genomic hybridization (CGH) and loss of heterozygosity (LOH) studies. Oncogenes and tumor suppressor genes important in other tumor types have been targeted. There is little evidence to support a role for many of the classic oncogenes. Genes involved in the genetic syndromes associated with adrenal cortical tumors have been investigated. A more recent approach has been to investigate components of the signaling pathways important in steroidogenesis and adrenal growth. Microarray technology has been applied both to identify novel genes and to attempt to find panels that will differentiate between benign and malignant tumors and provide prognostic information in carcinoma.

Tumor Clonality

Clonality studies based on X chromosome inactivation are often used to examine tumor development, the premise being that monoclonal lesions are neoplastic, the result of expansion of a clone from a single cell with genetic alterations, while polyclonal populations are still responding to external stimuli. However, this may not fully apply, at least in the endocrine system.82 Three studies on adrenal cortical tumors have shown that all 28 ACCs were monoclonal, 64 of 78 ACAs were monoclonal, and 53 of 67 nodular hyperplasias were polyclonal.83,85 This may reflect different tumorigenic pathways in benign tumors, or the change from a polyclonal to a monoclonal population as a step in tumor progression. Interestingly, studies on AIMAH have shown both polyclonal and monoclonal lesions in the same patient85 suggesting that neoplasms may arise in hyperplasia. This is also supported by the development of tumors in patients with congenital adrenal hyperplasia.86

Chromosomal Changes

The data from chromosomal studies have given inconsistent data, probably due to differences in methodology and the small numbers in individual series. In situ hybridization studies have shown that adrenal tumors commonly have chromosomal gains and less common losses.87-88 A number of CGH studies have been performed. The first report demonstrated losses on chromosomes 2, 11q, and 17p, and gains on 4 and 5 and increasing numbers of changes with size and malignancy.89 Further studies have shown changes in all ACCs and up to 61% of adenomas, although there are some discrepancies in changes detected.90,91 ACC gains were seen on chromosomes 5, 12, 9, and 4 and losses were most frequently seen at 1p, 17p, 22, 2q, and 11q. The most common change in adenomas was gain of 4q.90 Numerous loci of high-level amplification were seen in the other study.90

Loss of Heterozygosity

LOH has been shown in sporadic tumors at loci associated with familial syndromes (Table 21.4). LOH at 17p13,92-94 11q13,95-97 and 11p1598,99 are more common in ACC than in ACA. In contrast, LOH at 17q22–2499 has been identified only in ACA. LOH has also been demonstrated at 18p11,100 the locus of the gene encoding the ACTH receptor.

Specific Gene Involvement

IGF2 Signaling Pathway

IGF2 is a fetal growth factor. The gene lies within the 11p15 imprinted region as discussed above. IGF-2 is highly overexpressed in ~90% of ACC.98,101,102 There is paternal disomy even in sporadic cases. Reduced expression of both H19 and p57kip2 may also play a role.98,103,104 The type 1 IGF-receptor, through which IGF2 acts, is also overexpressed.102 The abnormal expression of this pathway is the most common event reported in ACC.

ACTH Signaling Pathway

This pathway, involving a G protein-linked receptor, cAMP, and protein kinase A has been discussed in detail in the section on steroidogenesis. Increased stimulation of the adrenal by ACTH is associated with hyperplasia and there is an increased incidence of ACA in congenital adrenal hyperplasia.86 The pathway is involved in the pathogenesis of tumors in other endocrine organs; for example, the Gsα mutations in a subpopulation of somatotroph tumors in the pituitary.105,106 The same mutations are present in McCune–Albright syndrome107 where patients develop AIMAH.

Table 21.5. Genetic changes in sporadic adrenal cortical tumors.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosomal locus</th>
<th>LOH</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>17p13</td>
<td>Up to 30% of ACA and 87.5% of ACC</td>
<td>0–6% of ACA and 20–27% of ACC</td>
</tr>
<tr>
<td>IGF2</td>
<td>11p15</td>
<td>Up to 34% of ACA and 83% of ACC</td>
<td>10% of ACA; not found in ACC</td>
</tr>
<tr>
<td>PRKAR1A</td>
<td>17q23–q24</td>
<td>23% of ACA and 53% of ACC</td>
<td>7% of ACA and ACC</td>
</tr>
<tr>
<td>MEN1</td>
<td>11q13</td>
<td>25% of ACA and 100% of ACC</td>
<td></td>
</tr>
<tr>
<td>GNAS</td>
<td>20q13.2</td>
<td>LOH loss of heterozygosity; ACA adrenal cortical adenoma; ACC adrenal cortical carcinoma.</td>
<td></td>
</tr>
</tbody>
</table>
As discussed above, Carney complex (CNC) is associated with PRKARIA mutation. There is little evidence to support a significant role for the ACTH receptor in tumorigenesis as no activating mutations have been found in benign or malignant tumors. However, since LOH has been found in 1 of 16 ACA and 2 of 4 ACC, it has been suggested that loss of the gene may result in dedifferentiation and tumor progression. Mutations in G proteins are exceedingly rare, 3 in all detected in four studies. Mutations in PRKARIA have been identified in only a small subset of ACA and not in ACC. A recent study has demonstrated a subgroup of adrenal adenomas characterized by underexpression (4% of normal) of another of the regulatory subunits of PKA, R2B, although levels of messenger RNA (mRNA) were normal suggesting a posttranscriptional mechanism. These tumors were all small cortisol-secreting adenomas. A further interesting observation has been the presence of inactivating mutations in the PDE9A gene on 2q31–35 in patients with PPNAD and other forms of bilateral hyperplasia. This encodes a dual-specificity phosphodiesterase that can hydrolyze both cAMP and cGMP, again implicating the cAMP pathway. There has been a recent proposal that most benign adrenal hyperplasias and adenomas are linked to abnormalities in this pathway and that other changes, such as TP53 mutations, overexpression of IGF2 and other growth factors, are associated with carcinoma.

Wnt Signaling Pathway

The Wnt proteins are a family of growth factors important both in development and adult life. Wnt signaling results in cytoplasmic accumulation of β-catenin and translocation to the nucleus and regulates gene transcription. Abnormal Wnt signaling is involved in the development of a number of cancers. In one study of adrenal tumors, accumulation of β-catenin was demonstrated in 13% of ACA and 77% of ACC, and activating mutations of β-catenin were found in 27% of ACA and 31% of ACC. The changes were more commonly seen in nonfunctional tumors. The presence of such mutations has been confirmed in sporadic adenomas and in PPNAD.

TP53

Acquired mutations in TP53 gene are common in most human cancers. Mutations have been found in only up to 27% of ACC and 6% of ACA. In one of these studies, ACC with mutations showed a trend towards poorer survival whereas a previous study showed no correlation with either survival or disease-free survival. In contrast to the mutations in exons 5–8 seen in these studies, mutations were found in exon 4 in 60% of ACAs in a series from Taiwan. This mutation was not demonstrated in a series of white patients, raising the possibility of ethnic differences. A number of germline TP53 mutations have been identified in 50–80% of childhood ACC, in the absence of Li–Fraumeni syndrome, suggesting the possibility of low penetrance mutations. However, given the low rate of mutations compared to LOH, it is possible that this locus contains other TSGs.

The incidence of ACC is ten times higher in Southern Brazil than in other geographic areas. A germline mutation (R337H) has been demonstrated in these cases and also in adult cases in the area. There is loss of the wild-type allele and accumulation of the mutant protein. It is estimated that one in ten carriers of this mutation develop ACC.

MEN1

LOH at 11q13 has been reported in <20% of ACA but in >90% of ACC. However, mutation of the MEN1 gene has been demonstrated in only one ACA and in one ACC. The level of gene expression was similar in normal adrenals, ACA, and ACC. These suggest that the gene is not important in the pathogenesis of sporadic adrenal cortical tumors.

Microarray Studies

More recently, there have been a number of studies based on microarray expression profiling. Most have been based on a wide array of genes and have confirmed the importance of IGF2 and other growth related genes in carcinoma, and have been able to make a differentiation between benign and malignant tumors. They have identified a range of novel genes that require validation, but the genes identified have varied between the studies, probably relating to the small numbers of tumors studied and the variation in methodology. One study took a different approach and compared the expression of a panel of 230 selected genes linked either to steroidogenesis and the cAMP pathway, including steroidogenic enzymes and StAR protein or to the IGF2 pathway in a series of 33 ACA and 24 ACC. They showed that the steroidogenesis group were more highly expressed in 93% of the benign tumors and the IGF2 group in 75% of the malignant group. Using a combination of eight genes from the IGF2 cluster and 14 from the adrenal cluster, it was possible to predict malignancy but only at the same level as the Weiss histologic score. However, using 14 genes, it was possible to separate recurring from nonrecurring tumors in a group of 13 carcinomas. These approaches need further validation in larger series and refinement.

Prognostic Features in Adrenal Cortical Carcinoma

Our tools for the prediction of outcome in carcinoma are still limited. The most important feature at present is tumor stage. In one series, 5-year survival was 60% for stage 1, 58% for stage 2, 24% for stage 3, and 0% for stage 4. Mitotic rate of >20 per 50 high power fields has been shown to correlate with poorer outcome and MIB-1 (Ki-67) index of >3% to indicate poorer disease-free survival. More recent molecular analysis has suggested that overexpression of IGF2 and
LOH at 17p13 predict recurrence in carcinoma. As indicated above, gene expression analysis may improve our ability to predict outcome.

**Primary Hereditary Disease of the Adrenal Cortex (Table 21.6)**

**Congenital Adrenal Hyperplasia**

These are a group of autosomal recessive diseases in which there are deficiencies in the various enzymes involved in steroidogenesis (Figure 21.1). In most forms, the lack of cortisol feedback leads to increased ACTH levels, adrenal cortical hyperplasia, and the diversion of precursor steroids into androgen synthesis. The most common enzyme involved is 21-hydroxylase, accounting for more than 90% of cases. The classical form presents at birth with varying degrees of masculinization of external genitalia in female infants. Boys may have normal genitalia at birth but may develop precocious puberty. In 65–75% of cases, there is also a defect in aldosterone synthesis that results in hyponatremia, hyperkalemia, hypovolemia, and shock. The overall incidence is one in 15,000 live births, but this varies with geography and ethnic origin. In the Yupic Eskimos of Alaska, it is one in 280 and in the inhabitants of La Réunion, one in 2,100. The nonclassical form is the most common autosomal recessive condition recognized, with a prevalence of one in 1,000 in the white population and higher in other ethnic groups. This variant may be asymptomatic or present with features of androgen excess.

The gene encoding 21-hydroxylase (CYP21A2, CYP21B) is located within the HLA histocompatibility complex at 6p21.3. It lies close to an inactive pseudogene with 98% homology (CYP21A1P, CYP21P). About 90% of the mutations are the result of recombinations of the two genes with transfer of sequences from the pseudogene to the active gene, leading to an inability to encode a normal enzyme. Most patients are compound heterozygotes, with different mutations on the two alleles. The severity of disease is usually related to the allele that retains most activity. The 11β-hydroxylase enzyme is involved in 5–8% of cases with a range of mutations. The signs of androgen excess are accompanied by hypertension because of the accumulation of precursors with mineralocorticoid activity. A variety of mutations are recognized. The 17α-hydroxylase enzyme accounts for about 1% of patients. Deficiency of 3β-hydroxysteroid dehydrogenase 3β-HSD) is caused by mutations in the type 2 3β-HSD gene. Affected males are incompletely masculinized because of lack of testicular androgens. In all of these variants, the adrenals show bilateral cortical hyperplasia and have a characteristic cerebriform appearance. The cortex is lipid-depleted because cholesterol is being used for steroidogenesis and is not stored. Adrenal tumors, mainly adenomas, have been reported frequently in patients with CAH but carcinomas are rare.

An unusual variant of CAH is congenital lipid hyperplasia in which there is hyperplasia with cholesterol accumulation in the cortex. A minority of cases is caused by mutations in the side-chain cleavage enzyme that converts cholesterol to pregnenolone, but the majority have mutations in the gene encoding the StAR protein that is integral to the transport of cholesterol to the mitochondrion to start steroidogenesis. Since all steroidogenesis is affected, the condition is often lethal.

**Other Diseases**

Primary congenital adrenal hypoplasia is a rare X-linked disease usually resulting from mutations and deletions of the DAX-1 gene on Xp21. There is weight loss, dehydration, and salt loss. The condition may be fatal and the adrenals are small and difficult to find at autopsy. Occasional cases may be due to mutations in the ACTH receptor gene (MC2R). Adrenoleukodystrophy (Addison–Schilder disease) is also X-linked. It is a peroxisomal disorder in which there are mutations in a transmembrane transporter gene on Xq28 leading to the accumulation of very long chain fatty acids in the adrenal cortex and the white matter of the brain. More than 100 mutations have been identified, about 50% missense, but also deletions and insertions. The adrenals undergo gradual atrophy and vary in size at autopsy. The ZG may be normal, but the ZF contains nests of degenerated balloon cells. In the later stages, these may disappear and the gland may resemble autoimmune Addison disease without a lymphoid infiltrate. There is evidence to suggest that it may be the cause of clinical Addison’s disease in a significant proportion of young men without adrenal antibodies.

Familial glucocorticoid deficiency is a rare condition that affects both sexes and is characterized by very low cortisol levels, relatively normal mineralocorticoids, and very high ACTH levels. It is caused by mutations in the ACTH receptor (MC2R) on 18p11.2 in 25–40% of cases. However, other genes including melanocortin 2 receptor accessory protein (MRAP) may also be responsible.

Two rare forms of familial hyperaldosteronism (FH), types I and Type I, also known as glucocorticoid remediable aldosteronism, is an autosomal dominant disease in which there is unequal crossover between the ACTH responsive part of the 11β-hydroxylase gene (CYP11B1) and the functional part.

**Table 21.6. Nonneoplastic familial diseases of the adrenal cortex.**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene</th>
<th>Chromosomal location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital adrenal hyperplasia</td>
<td>CYP21B (rarely CYP11B)</td>
<td>6p21.3</td>
</tr>
<tr>
<td></td>
<td>CYP17A1 or HSD3B2</td>
<td>8q21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10q24.3, 1p13.1</td>
</tr>
<tr>
<td>Congenital lipid hyperplasia</td>
<td>STAR (rarely P450SCC)</td>
<td>8p11.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15q23–q24</td>
</tr>
<tr>
<td>Adrenoleukodystrophy</td>
<td>ALD</td>
<td>Xq28</td>
</tr>
<tr>
<td>Familial glucocorticoid insufficiency</td>
<td>MCR2</td>
<td>18p11.2</td>
</tr>
<tr>
<td></td>
<td>MRAP</td>
<td>21q22.1</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td></td>
</tr>
</tbody>
</table>
of the aldosterone synase gene (CYP11B2), thus bringing aldosterone synthesis largely under ACTH control. Type II is associated with familial occurrence of aldosterone-producing adenosmas or bilateral hyperplasia or both and is linked to 7p22.

**Conclusion**

The further developments in our understanding of the inherited adrenal cortical diseases will no doubt come from the identification of additional specific mutations and their effects on the expression and activity of the encoded proteins.

The major area of future development, however, will be in the area of tumor diagnosis, biology, and prognosis. By drawing on the single gene and signaling pathway studies, it should be possible to design more specific and refined microarrays to diagnose malignancy. This approach should eventually allow a more targeted approach to both diagnosis and therapy in adrenal carcinoma, a tumor that still has a dismal prognosis for most patients.

**References**


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Section 6
Pancreatic Neuroendocrine Tumors
Introduction

Pancreatic endocrine neoplasms (PEN) typically generate a great deal of clinician interest because of the rarity with which they occur and their associated hormonal syndromes. While their rarity has limited research efforts into the pathophysiology of these tumors to some extent, recent advances in molecular biology techniques have allowed investigators to provide new insights into the pathogenesis of these lesions allowing the development of novel techniques for the diagnosis, localization, and treatment of PENs.

The mainstay of therapy for these tumors has consisted of surgical resection for locoregional disease in the setting of malignancy, as well as for the relief of hormonal hypersecretion syndromes due to secretion of various hormones such as vasoactive intestinal peptide (VIP), gastrin, glucagon, somatostatin, and insulin. In the setting of metastatic disease, curative surgical resection is not usually feasible. However, the generally indolent biology of these tumors, the fact that these patients frequently have a high performance status and preserved organ function, and the presence of symptomatic hormonal hypersecretion favor aggressive approaches to treatment even in the presence of widespread metastases. Traditional cytotoxic chemotherapeutic regimens typically are not successful in effecting significant responses in these tumors. Consequently, there has been a great deal of interest in the development of novel molecular methods with which to target these tumors. In this chapter, we review recent advances in molecular techniques being utilized to diagnose, localize, and treat PEN.

Diagnosis

PENs can be divided into two general categories, those that cause syndromes of hormone overproduction and those that do not. The clinical presentation of these tumors reflects the nature of these categories, with the symptoms of hormonal hypersecretion prompting evaluation and subsequent discovery of the pancreatic neoplasm in cases of functional lesions, while nonfunctioning lesions present because of mass effect or are discovered incidentally during imaging studies obtained for unrelated reasons.

While biochemical studies are utilized to establish the diagnosis of functional PENs, tumor markers are also important in establishing their diagnosis, particularly in the setting of nonfunctioning neoplasms. Chromogranin-A, a 439 amino acid secretory protein produced by dense core vesicles of neuroendocrine cells, has been found to be the most sensitive marker for the presence of PENs. However, several other conditions can result in elevations of this peptide, such as renal failure, heart failure, and atrophic gastritis, limiting its specificity. Sensitivity has been reported as ranging between 80% and 100% for functional PENs and 50% and 70% for nonfunctional PENs. In addition to diagnosis, this marker is useful in the postoperative surveillance of patients with these tumors and has been shown to correlate with prognosis as well.

While chromogranin-A is the primary tumor marker utilized in the diagnosis and surveillance of pancreatic neuroendocrine tumors, others have been investigated. Many PENs secrete pancreatic polypeptide (PP), a 36 amino acid peptide secreted by islet cells primarily in the head of the pancreas. The physiologic role of this peptide has yet to be fully elucidated, but it has also been considered a potential marker for PENs. Several studies published in the early 1980’s found that the sensitivity of PP was not sufficient to be clinically useful. However, a more recent study published in 2004 evaluated the utility of PP in conjunction with chromogranin A as markers for neuroendocrine tumors. They found that in the 68 patients studied, the addition of PP increased the sensitivity of detection of all PENs from 74% to 94% and nonfunctioning PENs from 68% to 93% when compared with chromogranin A alone (p < 0.05). These results suggest a role for PP as a tumor marker in patients with nonfunctional PENs.

Other tumor markers, such as neuron-specific enolase and the alpha subunit of glycoprotein, have been evaluated for their utility in the diagnosis and follow-up of PENs, but have been found to be lacking in clinical utility because of...
low sensitivity and specificity. Neuroendocrine secretory peptide-55 (NESP-55) is a 241 amino acid peptide secreted by neuroendocrine cells and a member of the chromogranin family. This peptide has been shown to be secreted by PENs and pheochromocytomas, but not carcinoid tumors, suggesting that this marker could play a role in the diagnosis and localization of gastroenteropancreatic endocrine tumors. Other published reports have shown evidence that chromogranin-A is processed differently by various neuroendocrine tumors and that by analyzing the breakdown products of this marker, or chromogranin-related peptides, one can gain insight into the type of tumor present. However, these potential applications have not been borne out by additional studies and have not gained widespread clinical acceptance, with chromogranin-A remaining the standard of care in the diagnosis and follow-up of neuroendocrine tumors (Table 22.1).

Localization

Localization of neuroendocrine tumors was initially limited to anatomic imaging studies such as computed tomography (CT), ultrasound (US), or magnetic resonance imaging (MRI). More recently, functional imaging of these tumors has been developed and become an essential step in the management of many of these tumors. This development was based on the fact that many neuroendocrine tumors express somatostatin receptors on their cell surfaces. Somatostatin is a cyclic neuropeptide containing 14 (SST-14) or 18 (SST-18) amino acids. This peptide has an inhibitory effect on several organ systems by decreasing neurotransmission, the secretion of growth hormone and thyrotropin-stimulating hormone, gastric acid production, pancreatic exocrine secretion, and secretion of insulin and glucagon. These effects are mediated by interaction of SST with its receptors on various target cells. There are five SST receptors (SST-R1-5) that have been described.

Somatostatin Receptor Sintigraphy

The fact that neuroendocrine tumors express relatively more SST-R than normal tissues has been the basis of the development of these functional imaging techniques, referred to as somatostatin receptor sintigraphy (SRS). Native SST-14 and SST-18 are not stable in the bloodstream, so various SST analogues were developed that were much more resistant to degradation and consequently had significantly longer half-lives, including octreotide, lanreotide, and vapreotide (Table 22.2). The high affinity of these peptides for SST-R (SST-R2 > SST-R5 > SST-R3) and the internalization of the peptide–receptor complex facilitate the retention of the radiopeptide in receptor-expressing tumor cells. Additionally, their small size (Octreotide is eight amino acids in length) allows for rapid clearance from the bloodstream. Subsequently, these analogues were conjugated with various radioisotopes and bound to a chelator such as diethylenetriaminepentaacetic acid (DTPA) to ensure stability. For example, conjugating octreotide with 111In and DTPA forms DTPA-D-Phe-Octreotide (Octreoscan). Use of this radiopharmaceutical has become the mainstay of molecular imaging studies for the localization of neuroendocrine tumors, including those of the pancreas, with sensitivities ranging from 60% to 90% depending on the tumor type.

Other radiopharmaceuticals have continued to be developed in an effort to improve upon the sensitivity of detecting neuroendocrine tumors. Somatostatin analogues have also been conjugated with 1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetraacetic acid (DOTA) and 111In to form In-DOTA-DPhel-Tyr3-octreotide, In-DOTA-lanreotide, and In-DOTA-octreotate. Of these, In-DOTA-octreotate has been shown to have the highest affinity for SST-R2.

Vasoactive Intestinal Peptide Scintigraphy

In addition to various SST analogues, other peptides have been evaluated for their utility in the localization of neuroendocrine tumors. Included in these is vasoactive intestinal peptide (VIP) comprised of 28 amino acids. VIP receptors are found in a wide variety of tissues, including pancreas, GI mucosa, lung, thyroid, and lymphoid tissues. VIP has been conjugated with radiolabelled iodine (123I) and technetium (99Tc), and used...
clinically in the imaging of PENs. 20,21 This radiopharmaceutical has shown some benefit in patients with neuroendocrine tumors receiving somatostatin therapy as this decreases the sensitivity of SRS. Additionally, some studies have shown that this radiopptide is more sensitive for the localization of insulinomas, suggesting potential clinical applicability, however, currently this has not become routinely used in practice.20

Cholecystokinin-B/Gastrin Receptor Scintigraphy

One other target that has been explored as a candidate for molecular imaging of PENs is the cholecystokinin (CCK) receptor. Two primary receptor subtypes have been identified for CCK, called CCKAR and CCKBR. Of these, CCKAR is expressed in the pancreas, in addition to areas of GI tract smooth muscle, the gastric mucosa, and regions of the central nervous system. CCKBR, which is also the receptor for gastrin, is expressed in the nervous system and various aspects of the gastric mucosa.22 Both of these CCK receptors have been found to be overexpressed in gastroenteropancreatic PEN, which has led to the development of several CCK and gastrin derivatives to be used as potential in vivo receptor targeting for localization or therapy. One group showed that radioiodinated gastrin could be used to specifically target CCKBR in a xenograft model of medullary thyroid cancer (MTC) and in a patient with metastatic MTC.23 DTPA-coupled derivatives of this peptide have also been characterized, with one such radiopptide being evaluated in a clinical trial of MTC.24,25 These studies have validated the potential utility of CCK/gastrin analogues for targeted imaging or therapy of patients with neuroendocrine tumors, particularly in the setting of MTC. Further studies are needed to determine whether or not this will be applicable to PENs.

Positron Emission Tomography

Positron emission tomography (PET) scanning has gained widespread oncologic applicability in the treatment of various tumors. PET scanning using [11C]fluoro-2-deoxy-d-glucose (FDG) has been increasingly utilized for diagnosis, staging, restaging, and evaluation of response to treatment of many tumor types. FDG–PET scanning relies on the principle that tumor cells are more metabolically active than normal cells and therefore utilize glucose at a higher rate. FDG can be taken up by cells by the same mechanism as glucose, but FDG cannot continue along the glycolytic pathway leading to accumulation of this tracer in the tumor cells, which can then be imaged. While this radiopharmaceutical has shown to be useful for the imaging of many tumor types, it has not been successful at localizing neuroendocrine tumors. This is due to the fact that most neuroendocrine tumors are well-differentiated, slow-growing tumors with largely normal glucose metabolism. To combat this problem, 11C-5-hydroxy-l-tryptophan (11C-HTP) and 18F-l-dihydroxyphenylalanine (18F-DOPA) were developed for PET imaging of these tumors. The principle of the use of these radiopharmaceuticals is based on the ability of neuroendocrine cells to take up these amine precursors, transform them by decarboxylation into biogenic amines, and retain them in storage vesicles. 11C-HTP was found to have greater uptake by PENs in a study comparing it and 18F-DOPA. Subsequent studies showed that 11C-HTP-PET had higher sensitivity than CT and SRS for visualizing neuroendocrine tumors and was able to detect many small, previously overlooked lesions by these other imaging modalities.26–28

Another recent study compared 11C-HTP-PET with 18F-DOPA-PET in the localization of 24 carcinoid tumors and 23 PENs. The authors found that the sensitivity for detection of carcinoid tumors was greatest with 18F-DOPA-PET/CT (98%), while the greatest sensitivity for detecting islet cell tumors was 11C-HTP-PET/CT (96%). Both of these modalities were superior to SRS and SRS/CT for the detection of PENs.29 It has become clear that PET scanning is a valuable tool in the diagnosis, staging, and follow up of patients with PENs. Table 22.3 summarizes the molecular markers used in the localization of PENs and their targets.

### Therapy

PENs are a heterogeneous group of tumors with varying malignant potentials. While these tumors often display indolent biologic behavior, they frequently have metastasized at the time of initial presentation. Historically, the mainstay of therapy for systemic disease was SST analogues. While these were fairly successful in controlling symptoms from hormonal hypersecretion, with various studies reporting improvement in symptoms in 50–60% of patients, rarely did they result in tumor regression (3–5% of patients). Additionally, traditional chemotherapeutic agents have not been effective at generating significant responses in these tumors. Consequently, there has been a great deal of research performed in an effort to develop more effective therapeutic options for patients with metastatic PENs.

#### Targeted Peptide Receptor Radiotherapy

During the past decade, that research effort has led to the development of peptide receptor radionuclide therapy. Similar to the imaging modalities discussed previously, this therapy

<table>
<thead>
<tr>
<th>Imaging Modality</th>
<th>Marker</th>
<th>Target Receptor</th>
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<tbody>
<tr>
<td>Somatostatin analogues</td>
<td>SST-R1-5</td>
<td></td>
</tr>
<tr>
<td>VIP analogues</td>
<td>VIP-R</td>
<td></td>
</tr>
<tr>
<td>Cholecystokinin/Gastrin</td>
<td>CCKAR/CCKBR</td>
<td></td>
</tr>
<tr>
<td>5-Hydroxy-l-tryptophan</td>
<td>NET secretory vesicles</td>
<td></td>
</tr>
<tr>
<td>l-Dihydroxyphenylalanine</td>
<td>NET secretory vesicles</td>
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</table>

NET neuroendocrine tumor, VIP vasoactive intestinal peptide, CCK/BR cholecystokinin A&B receptors, SST-R somatostatin receptor.
exploits the fact that many PENs express SST receptors on their cell surfaces. While SST itself does not lead to significant tumor regression, researchers hoped to utilize the affinity of SST to neuroendocrine tumor cells to create a delivery mechanism for cytotoxic agents resulting in tumor destruction. Several radioisotopes have been conjugated with SST analogues to serve as the cytotoxic agent. In the following section, we review the results of studies examining this therapeutic modality.

**111In-DTPA-Octreotide**

As this was the most widely used radiopeptide for the imaging of PENs, 111In-DTPA-octreotide also became the first to be examined for its therapeutic efficacy against advanced tumors. Initial studies showed limited responses, but no therapeutic effect was observed in the setting of larger tumors or widespread metastases. The limited effect was likely due to the fact that this radioisotope emits mainly $\gamma$-radiation which is a relatively low-energy emitter with limited particle range and therefore tissue penetration.

**90Y-DOTA-Octreotide**

The next generation of SST analogues, such as DOTA-octreotide, were also examined for their potential therapeutic utility. The use of DOTA as a chelator allowed stable radiolabeling with $\beta$-emitting radionuclides such as yttrium-90 ($^{90Y}$) which is advantageous in that these higher-energy particles have sufficient energy to cause cell damage without penetrating significantly into surrounding tissues. Additionally, DOTA has higher affinity for SST-R2 than DTPA. Figure 22.1 shows the structure of these ligands. Several phase I and II trials using $^{90Y}$-DOTA analogues have been performed with objective response rates ranging from 9 to 33%.

$^{177}$Lu-DOTA-Tyr$^3$-Octreotate

Lutetium-177-DOTA-Tyr$^3$-octreotate ($^{177}$Lu-DOTATOC) is a third generation SST analogue which has even higher affinity for SST-R2, showing increased binding to SST-R2 positive tissue in vitro and in vivo. An additional advantage in this third generation SST analogue is the ability to deliver higher adsorbed radiation doses to tumors without increasing the radiation dose to dose-limiting organs, such as the kidneys and bone marrow. In 2003, Kwekkeboom and colleagues published the results of a study looking at the effects of $^{177}$Lu-DOTATOC on 34 patients with neuroendocrine tumors of varying sizes. They reported 1 complete remission (3%), 12 partial remissions (35%), 14 patients with stable disease (41%), and progressive disease in seven patients (21%) after 3 months of therapy. They also found a significant improvement in quality of life for these patients, even those with progressive disease.

A more recent study looked at the results of therapy in 131 patients treated with this radiopharmaceutical, with three patients with complete remission (2%), 32 with partial remission (26%), 24 with a minor response defined as a decrease in tumor diameter of 25–50% (19%), and 44 patients with stable disease (35%). Twenty-two patients developed progressive disease despite therapy (18%). Median time to progression for responders was 36 months. In both of these studies, clinical response correlated with uptake on their diagnostic pretreatment $^{111}$In-octreotide scan.

The availability of several SST-analogues with varying characteristics offers the potential of therapy tailored to a particular tumor type. For example, the emitting $\beta$-particle range of both $^{177}$Lu and $^{90}$Y exceeds the target cell diameter, allowing irradiation of neighboring tumor cells. This is particularly important in tumors with heterogeneous SST-R expression, such that even tumor cells without SST-R can be treated, and offers an advantage over $^{111}$In analogues. $^{177}$Lu has lower tissue penetration than $^{90}$Y, making it a better choice for smaller tumors, while $^{90}$Y may be a better alternative for larger tumors. Another clinical advantage of $^{177}$Lu is that, unlike $^{90}$Y, it emits low-energy $\gamma$-rays, which allows for dosimetry and imaging immediately following DOTATOC therapy. Serious side-effects related to therapy with radiolabeled SST analogues are rare, but include myelodysplastic syndromes, renal toxicity, and hepatic toxicity. See Table 22.4 for a summary of commonly used radionuclides in the localization and treatment of neuroendocrine tumors.

**Somatostatin Receptor Upregulation**

**Radiation-Induced Upregulation**

The data from these and other studies examining the efficacy of targeted peptide receptor radiotherapy for gastroenteric and pancreatic neuroendocrine tumors have confirmed the potential of this therapeutic modality for unresectable or widespread tumors. Ongoing studies continue to search for
been developed which inhibit vascular endothelial growth factor receptors (VEGF-R), a tyrosine kinase receptor. As PENs are known to be vascular tumors and have been shown to over-express VEGF-R, some of these drugs have been examined for their therapeutic potential in treating advanced neuroendocrine tumors. A phase-II trial evaluating the use of Sunitinib, a tyrosine kinase inhibitor with activity against several VEGF-R’s and other tyrosine kinases. One hundred seven patients, 41 with PENs, received treatment with Sunitinib, with objective responses by RECIST criteria seen in 11 patients (17%), and stabilization of disease in 45 patients (68%). Monoclonal antibodies against VEGF, such as bevacizumab, have also been studied in the treatment of advanced neuroendocrine tumors in phase II trials, but thus far clinical trials have been limited to patients with carcinoid tumors.45

**mTOR-Inhibitors**

The mammalian target of rapamycin (mTOR) is an intracellular protein critical to control of cell growth, protein synthesis, and autophagy. Downstream targets include the tyrosine kinases VEGF and insulin-like growth factor (IGF), and abnormalities in this pathway have been implicated in the pathogenesis of several tumor types, including neuroendocrine tumors. A phase-II trial evaluating the use of the mTOR inhibitor everolimus in patients with neuroendocrine tumors, including carcinoids and PENs, showed an overall response rate of 13%, with 73% of patients with stable disease and progression-free survival at 24 weeks of 64%. Based on these results, additional clinical trials are currently in accrual. Table 22.5 summarizes the status of clinical trials for VEGF and mTOR inhibitors in the treatment of neuroendocrine tumors.
Conclusion

The treatment of advanced PENs has traditionally been restricted to medical therapy for symptomatic relief of hormone-related symptoms. Recent advances in the development of new somatostatin analogues, radiodeptides, and chelators have significantly improved our ability to use these radiodeptides in the localization and treatment of these tumors. Additionally, advances in our understanding of the molecular pathogenesis of PENs have led to the development of exciting new molecular treatment strategies utilizing inhibitors of critical signaling pathways in neuroendocrine tumor cells resulting in arrest of tumor progression or even significant reduction in tumor volume in some of these patients. Ongoing studies attempting to improve upon these promising therapeutic modalities continue offering new hope to patients with advanced PENs.

References


Pancreatic Endocrine Neoplasms

Ahmed S. Bedeir and Alyssa M. Krasinskas

Introduction

Pancreatic endocrine neoplasms (PENs) are foregut endocrine tumors that arise in the pancreas and appear morphologically similar to other neuroendocrine (carcinoid) tumors throughout the body. However, the biology of endocrine/neuroendocrine tumors tends to depend upon the site of origin. PENs can produce various hormones, though most are nonfunctional. They are generally low grade, well-differentiated tumors. PENs can be classified according to grade, size, or functional status. The nomenclature of these neoplasms can be confusing, partly because of the variable terminology, including different names for the same neoplasm (including “islet cell tumors,” “pancreatic neuroendocrine tumor,” and “pancreatic endocrine tumors”) and partly because well-differentiated malignant tumors can either be called “malignant pancreatic endocrine neoplasms” or “pancreatic endocrine carcinomas.” The authors of the AFIP fascicle prefer to consider all well-differentiated PENs as potentially malignant (with the exception of microadenomas less than 0.5 cm in size). The term “carcinoma” is reserved for poorly differentiated lesions. Similarly, the term PEN will be used in this chapter to refer to well-differentiated tumors.

Sporadic Pancreatic Endocrine Neoplasms

PENs are uncommon neoplasms; the incidence in the United States is about 2.2 per 1,000,000.2 There is a slight male predominance and the mean age is 58.5 years.2 The vast majority of PENs are sporadic and up to 90.8% are nonfunctional.2 A PEN not associated with a syndrome of hormone overproduction is, by default, a nonfunctional tumor. Nonfunctional tumors are discovered incidentally or may be associated with abdominal pain. PENs associated with a syndrome of hormone overproduction are classified as functional PENs and include insulinomas, glucagonomas, somatostatinomas, gastrinomas, VIPomas, serotonin secreting tumors, as well as other rare and mixed hormone-producing entities. The clinical presentation of functional tumors depends on the type of hormone that is secreted.

About 60% of PENs arise in the pancreatic tail,3 but they can occur throughout the pancreas. Grossly, PENs are usually solid, well-circumscribed, sometimes encapsulated lesions. The cut surfaces can be uniform or heterogeneous with solid or spongy, yellow to red parenchyma. PENs have many various histological patterns, including the traditional organoid or trabecular growth pattern seen in well-differentiated neuroendocrine tumors at other sites. The cytologic features can also be typical of other neuroendocrine tumors. The neoplastic cells tend to be small and polygonal with abundant amphophilic cytoplasm and uniform round to oval nuclei with the coarsely stippled “salt and pepper chromatin.” However, variable cellular size, nuclear size, nuclear shape, and chromatin patterns are common. The variation in growth patterns and cytologic features makes PENs a unique and interesting group of endocrine tumors.1

Useful immunohistochemical markers include antibodies to most neuroendocrine cells, such as synaptophysin, protein gene product (PGP) 9.5, CD 57 (Leu7) and CD 56 (neural cell adhesion molecule); chromogranin is inconsistently expressed in PENs and its staining can be patchy and dependent on the antibody that is used.4 When confronted with a metastatic (neuro)endocrine tumor, antibodies to CDX-2 and TTF-1 can be very helpful. PENs tend to lack staining for both of these markers, while TTF-1 is expressed in lung primaries and CDX-2 in gastrointestinal primaries.5 Immunohistochemical stains can also be used to detect specific peptides such as insulin, glucagon, somatostatin, and pancreatic polypeptide, but the protein expression does not always correlate with the functional status of the PEN.

In an attempt to better predict the prognosis of PENs, several immunohistochemical markers have been studied, but none have replaced classic clinical and morphologic parameters. Ki-67 (MIB-1) proliferation index has been
shown to have prognostic value in PENs, and is used by the WHO to distinguish PENs with “benign” behavior from those with “uncertain” behavior based on a cutoff of 2%. Another proposed grading scheme uses mitoses and ki-67 labeling index to separate low and intermediate grade PENs from high grade pancreatic endocrine carcinomas. However, one group has proposed simplifying the WHO classification system by using clinical parameters of tumor size and metastases with grading information based on necrosis and mitotic rate. Another promising prognostic marker, CK-19, was found to be expressed in more aggressive PENs and its expression was correlated with a shorter disease free survival and was independent of the WHO criteria.

### Genome Wide Study of Sporadic Pancreatic Endocrine Neoplasms

#### Comparative Genomic Hybridization
Using comparative genomic hybridization, numerous studies have identified many chromosomal alterations in PENs with chromosomal losses being slightly more common than gains. Commonly described alterations include losses at 1p, 3p, 6q, 9p, 10p, 11p, 18q, 22q, Y and X and gains at 4p, 5q, 7p, 7q, 9q, 12q, 14q, 17p, 17q and 20q. One of the most consistent chromosomal alterations is loss of 11q, which harbors the MEN1 gene. Some chromosomal alterations have been associated with tumor grade, stage, and prognosis. The overall number of genetic changes per tumor, especially gains, has been associated with both tumor size and disease stage. Losses of 3, 6, and 21q and gains of 4, 7, 14q, 17, 20q, and Xq were found to be associated with malignant behavior and/or metastatic disease. Prognostic significance was also linked to changes in the sex chromosome. In males, Speel et al noted that all of the four male patients with malignant insulinomas studied had loss of chromosome Y in addition to gain of Xp. This finding was further supported by Missiaglia et al who showed that PENs from female patients show frequent loss of chromosome X and PENs from male patients show relatively frequent loss of chromosome Y, and that loss of a sex chromosome was associated with the presence of metastases, local invasion, and with poor survival.

#### Loss of Heterozygosity (LOH)
A number of studies have used LOH to identify chromosomal loci that may harbor potential tumor suppressor genes involved in the pathogenesis of PEN. LOH can detect smaller deletions than CGH. In addition to those losses noted by CGH, additional deletions of interest have been detected by LOH at 3p14.2-3p21, 3p23, 6q22, 7q31-32, 9p, 11q13, 13q14, 17q21, 18q21, and 22q12. LOH at loci 3p25.3-p23, 6q, 17p13, and 22q have been associated with aggressive behavior and metastatic disease. Importantly, these assays have been reported in fine needle aspirations, as well as in histologic material. In addition to their importance as useful markers for clinical behavior, these chromosomal changes direct attention to particular chromosomal regions as candidates for a more detailed analysis with respect to genes involved in PEN development.

### Gene Expression Profiling of Sporadic PENs
Several DNA microarray studies using different approaches have reported novel genes that may be important in the pathogenesis and prognosis of PENs. Up-regulated genes potentially involved in pathogenesis of PENs include oncogenes (MLLT10/AF10), growth-factor-related genes (IGFBP3), cell adhesion and migration molecules (fibronectin), endothelial elements (MCAM/MUC18, PECAM1/CD31, ANGPT2/Ang2), potential biomarkers (SERPINA10, BIN1) and therapeutic targets (LCK/SRC-like kinase, BST2); down-regulated genes include cell cycle check point genes (CDKN1A/p21), cell surface glycoproteins (CD99/MIC2), putative metastasis suppressor genes (NM23, transcription factors (JUND) and apoptosis-related genes (IER3, PHLDA2, IAPP, and SST). One DNA microarray study compared nonmetastatic with metastatic PENs and found differential expression of genes related to growth-factor-related molecules (IGFBP1, IGFBP3), cytoskeleton-related molecules (b1-tubulin), cell cycle regulation (CHEK1), developmental regulation (TBX3), intracellular signaling (UPK1B), and DNA damage repair (MGMT), while another study found no significant differentially expressed genes between primary tumors and their metastases. Due to poor concordance between these published studies, further analysis of these genes may reveal important insight into the pathogenesis and prognosis of PENs.

### Individual Genes in Sporadic Pancreatic Endocrine Neoplasms
Most of the molecular studies of PEN have failed to demonstrate a strong role of the common oncogenes and tumor suppressor genes in the molecular pathogenesis of most PENs. Because of its involvement in MEN1 patients, the MEN1 gene has been examined for mutations in sporadic PENs. Although loss of MEN1 locus at 11p13 occurs in 43–68% of sporadic PENs, somatic mutations have been found in only 15–26% suggesting involvement of another tumor suppressor gene at this location. Similarly, in patients with sporadic PENs, LOH on chromosome 3p was identified in 33% of cases, but no mutations in VHL were detected. For other tumor suppressor genes
and oncogenes, there appears to be no significant role of TP53, CDKN2A (p16), PTEN, SMAD4/DPC4, ERBB2/HER2, BCL2, KRAS, or BRAF in the pathogenesis of sporadic PENs.18,22,28,39–43

Epigenetic Changes in Sporadic Pancreatic Endocrine Neoplasms

In addition to genetic mutation and chromosomal loss, aberrant epigenetic changes including abnormalities in methylation have been demonstrated in PEN. Chan et al demonstrated that the methylation profile of gastrointestinal neuroendocrine (carcinoid) tumors differs from PENs, reflecting different molecular pathogenesis.54 Among the most commonly methylated genes in PEN are RASSF/RASSF1A, CDKN2A/p16, MGMT, and MLH1.45–47 The hypermethylation of RASSF1 promoter is frequent in many human cancers, and there is an inverse correlation between RASSF1 silencing by methylation and KRAS activation.38,49 As mentioned earlier, it has been shown that well-differentiated PENs lack KRAS or BRAF mutations.32,50 It is therefore interesting that the RASSF1 gene is the most frequently methylated gene in PEN.45–47 This finding suggests that the RAS pathway may still be involved in well-differentiated neuroendocrine tumors mostly by gene silencing of RASSF1 gene through methylation. The frequency of CDKN2A/p16 methylation ranges from 19% to >50% in the literature.45–47 The MGMT gene is an important tumor suppressor gene responsible for removing alkyltransferase of DNA at the O6 position of guanine and playing a major role in DNA repair. Methylation of CpG islands in the promoter region of MGMT can cause gene silencing.33 Another tumor suppressor gene important in DNA repair is MLH1. The association between loss of MLH1 function and microsatellite instability in colorectal cancer is well documented. Like microsatellite unstable colorectal cancer, MLH1 methylation leads to MSI in PENs and this was shown to be associated with favorable prognosis.46,53 Similarly, a few studies have suggested that the methylation status of specific tumor suppressor genes is predictive of PEN behavior. In a study of gastrinomas, Serrano et al reported that CDKN2A/p16 gene methylation correlated with malignant tumors associated with lymph node but not liver metastases.28 In a larger study of all types of PEN, House et al found that methylation at the CDKN2A/p16 gene locus was associated with decreased 5-year survival and tumor recurrence within 24 months; two molecular markers that can predict patient outcome after surgical resection. Methylation at 3 or more genes or at the CDKN2A/p16 gene locus is associated with decreased 5-year survival and tumor recurrence within 24 months; 37% of CDKN2A/p16 methylated PEN recurred, compared with 26% of tumors without CDKN2A/p16 methylation.46 They also showed that the methylation of multiple (≥3) tumor suppressor genes may be associated with more aggressive tumors.46

Other Techniques

Telomerase in Sporadic PENs

Chromosomal telomeres (terminal chromosome regions) are made up of several thousand copies of repeating nucleotide sequences. Their main functions are believed to be the stabilization and protection of chromosomal ends.34 In a normal somatic cell, there is approximately 50–100 basepair loss of telomeric DNA from the end of every chromosome during each cell cycle. With progressive erosion of telomeres, these unprotected ends may participate in end-to-end chromosomal fusions, which are lethal to cells.34 Telomerase is an enzyme believed to be involved in the de novo synthesis of telomeric DNA onto chromosomal ends.35–37 Telomerase is not detected in most somatic cells. Conversely, it is activated in most human cancers suggesting an important role in cancer cell survival.37 Telomerase is a ribonucleoprotein complex including a telomerase catalytic subunit (telomerase reverse transcriptase protein subunit, TERT; an internal RNA strand (TR), and an RNA-binding protein (TEP1)). TERT gene expression seems to be the rate-limiting determinant of telomerase activity. A few published studies have shown that telomerase activity is closely related to the malignant potential of human pancreatic endocrine tumors.38,39 In the study by Lam et al, three of ten pancreatic endocrine tumors had telomerase activity. Two of these cases were frankly malignant tumors with liver metastases and the third was pancreatic endocrine tumor occurring in the setting of MEN type 1 and histologically showed an infiltrative border, vascular and perineural tumor infiltration.38 This finding suggested that telomerase activity might be useful in distinguishing between benign and malignant pancreatic endocrine tumors. A more recent study employed real-time quantitative RT-PCR in the quantification of TERT mRNA 23 cases of PEN. Telomerase was negative in 12 out of 12 benign pancreatic tumors (100%) and positive in 6 out of 8 malignant (metastatic or not) pancreatic tumors (75%), resulting in a positive predictive value of 100% and a negative predictive value of 85.7%. In addition, telomerase activity was helpful in identifying metastatic tumors. Sixteen of eighteen nonmetastatic pancreatic tumors were telomerase negative (88.99%), while 5 out of 5 metastatic pancreatic tumors were telomerase positive (80% and 100% positive and negative predictive values, respectively).40

MicroRNA

MicroRNAs are small (about 22 nucleotides in length), single-stranded forms of noncoding RNA that are involved in the normal functioning of our cells. The dysregulation of microRNA has been linked to human disease, including cancer. One study has examined the role of microRNAs in PEN. Comparing nontumor pancreas to pancreatic tumors (insulinomas, nonfunctioning PEN and acinar cell carcinomas), the expression of miR-103 and MIRN107/miR-107 in conjunction
with a lack of expression of MiR155/miR-155 distinguished tumors from normal. A set of 10 microRNAs were upregulated in PEN and MiR204/miR-204 was found to be primarily expressed in insulinomas. Interestingly, the overexpression of MiR21/miR-21 was strongly associated with both a high Ki67 proliferation index and presence of liver metastasis.61

Hereditary Pancreatic Endocrine Tumors

In a small subset of patients, PENs are inherited as part of a genetic syndrome. Recognition of these patients is important for both treatment and evaluation of family members. The study of these inherited neoplasms also plays an important role in our understanding of the pathogenesis of PEN. The most common hereditary syndromes associated with the development of PEN are multiple endocrine neoplasia type 1 (MEN1) and von Hippel–Lindau syndrome (VHL).

MEN1 is a rare autosomal dominant disorder with a prevalence that ranges from 1 in 20,000 to 1 in 40,000.62 It is characterized by the combination of parathyroid tumors, pancreatic endocrine cell neoplasms, and pituitary hyperplasia/tumors. Tumors can also arise in the upper gastrointestinal tract, lung, thyroid, thymus, and adrenal gland. Parathyroid tumors occur in nearly all patients by age 50, and pancreatic tumors arise in approximately 80% of patients.63,64 The pancreatic tumors are often multiple, including microadenomas and PENs, and the PENs may undergo malignant transformation. While the majority of PENs are nonfunctional, most patients will have at least one functional tumor. The most common functional tumors are gastrinomas, followed by insulinomas, glucagonomas, and others.

Using genetic linkage analysis, a region on the long arm of chromosome 11 (11q13) was implicated as the potential site that harbored the key gene that is dysfunctional in MEN1 syndromes (Table 23.1).65 The candidate gene was identified and designated the MEN1 gene by Chandrasekharappa et al in 1997.66 Multiple subsequent studies confirmed that MEN1 gene mutations are detected in most MEN1 patients.67,68 This tumor suppressor gene contains 10-exon that encodes for a 610-amino-acid nuclear protein, Menin, that interacts with a variety of transcription factors, DNA repair proteins, DNA repair proteins, and cytoskeletal proteins.69,70 At least 400 different MEN1 mutations have been described.71 It is important to note that mutations in the MEN1 gene locus are also reported in 27–39% of sporadic PENs.23,30,72 The mutations in the MEN1 gene in sporadic PENs appear to be distributed throughout the nine coding exons.23 Mutations are present in the MEN1 gene in both benign and malignant sporadic PENs suggesting that they are an early event in the molecular pathogenesis.23,72

The second syndrome that is associated with the tendency to develop PEN is VHL syndrome. The prevalence ranges from 1 in 30,000 to 1 in 50,000.73 The disease penetrance is over 90% by age 65.74 This autosomal dominant disorder is associated with many tumors, most notably hemangioblastomas, clear cell renal cell carcinomas and pheochromocytomas.74–76

PENs are reported to be present in 17% of patients with VHL and they behave in a malignant fashion in up to 8.3%.77 The majority of PEN are nonfunctional75 and many (60%) have a foamy clear cell appearance.78

Using genetic linkage analysis, a region on the short arm of chromosome 3 (3p25-p26) was found to harbor the VHL gene and the gene was subsequently cloned (Table 23.1).73,79 More than 300 germline mutations have been identified in familial VHL.79 Functioning as a tumor suppressor gene, its product, VHL protein, targets several proteins with which it forms stable complexes that binds to ubiquitin that then get degraded by proteasomes.80,81 Hypoxia-inducible factor-1 (HIF-1) is one of the major proteins regulated by VHL. This protein is involved in erythropoiesis through its ability to induce transcription of mRNA coding for erythropoietin. In patients with VHL disease, loss of functioning VHL results in HIF-1alpha not binding to ubiquitin and thus not degraded by proteasomes.80,81 In addition to erythropoietin, other factors known to be regulated through the HIF-1alpha system include vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF)-beta, and transforming growth factor (TGF)-alpha.80,81 VHL also affects several other factors potentially involved in tumorigenesis that are not regulated through the HIF-1alpha system. These targets include matrix metalloproteinases (MMP) such as MMP1, MMP inhibitors, and atypical protein kinase C.80,81 Although the mechanism of tumorigenesis remains unproven, the combined effect of various angiogenic factors and other growth factors may be to create an autocrine loop that provides an uncontrolled growth stimulus.82 Although loss of 3p is well documented in sporadic PEN,24 mutation of this gene is usually not targeted in sporadic PEN.25 Interestingly, 78% of patients with metastatic

| Table 23.1. Hereditary syndromes associated with pancreatic endocrine neoplasms. |
|------------------------------------------|----------|------------------------------------------------|
| **Multiple endocrine neoplasia type-1**  | **Gene** | **Protein product**                             |
|                                          |         | Interacts with transcription factors            |
|                                          |         | – JunD, NF-kB, Smad3, Pem                       |
|                                          |         | DNA repair proteins                             |
|                                          |         | – RPA2                                          |
|                                          |         | DNA processing factors                          |
|                                          |         | – nm23                                          |
|                                          |         | Cytoskeletal proteins                           |
|                                          |         | – GFAP, vimentin                                |

<table>
<thead>
<tr>
<th><strong>von Hippel Lindau disease</strong></th>
<th><strong>Gene</strong></th>
<th><strong>Protein product</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VHL</td>
<td>Targets several proteins that bind to ubiquitin</td>
</tr>
<tr>
<td></td>
<td>VHL</td>
<td>to be degraded by proteasomes:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Hypoxia-inducible factor-1 (HIF-1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Vascular endothelial growth factor (VEGF)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Platelet derived growth factor (PDGF)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Tumor growth factor alpha (TGFa)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Matrix metalloproteinases (MMP)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– MMP-inhibitors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Atypical protein kinase C (APK-C)</td>
</tr>
</tbody>
</table>
disease had exon 3 mutations in the VHL gene, compared with 46% of patients without metastases, suggesting that detection of exon 3 mutations will be helpful in assessing aggressiveness of PEN and guiding surgical management in these patients. 77

**Summary**

Even though two well-known hereditary conditions are associated with PENs, MEN1 and VHL, the molecular pathogenesis of these two diseases has provided only limited insight into the much more common sporadic form of the pancreatic neoplasms. Sporadic PENs harbor MEN1 gene mutations in only a minority of cases and they tend not to have VHL mutations, but rather show loss of the locus where the VHL gene resides. Abundant research has been performed on sporadic PENs that has provided insight into the molecular pathogenesis of these tumors. Rather than resulting from dysfunction of a single gene, several pathways appear to be involved with the formation of PENs, including losses and gains of genomic material, methylation of some key regulatory genes and expression of particular microRNAs.

While PENs are typically easy to diagnose, their behavior is difficult, if not impossible, to predict. Immunohistochemistry has limited value in assessing prognosis, but new markers are always being developed. Molecular studies have provided some targets that may be helpful in assessing the aggressiveness and metastatic potential of these tumors, and some have even been reported to predict survival (Table 23.2). Some prior studies need to be validated, but a panel of markers utilizing various techniques may be needed to accurately predict the behavior of these tumors.

### References


Section 7
Other Neuroendocrine Lesions
Introduction

Gastrointestinal neuroendocrine tumors (GI-NETs) arise from diffuse neuroendocrine system cells, which express neuroendocrine markers, produce certain peptide or amine hormones, and contain dense core secretory granules. NETs still retain these features after the transformation of normal neuroendocrine cells.

The neuroendocrine features of these tumors are summarized in Table 24.1. Histologically, individual tumor cells have monomorphic nuclei with a “salt and pepper” chromatin pattern. The cytoplasm may be clear or granular. Four morphologic patterns of growth have been recognized: insular or nested, trabecular, tubular or acinar, and poorly differentiated. Histochemical stains can be used to identify endocrine cells. Argentaffin is a silver stain, which selectively demonstrates serotonin-producing EC cells. Argyrophil stain is less specific to secretory type and identifies all GI endocrine cells.

Some immunohistochemical markers are routinely used to identify or confirm NETs. Chromogranin A and B are specific secretory granular markers. Synaptophysin is a synaptic vesicle protein widely expressed in neuroendocrine cells although it can also be found in normal adrenal cortex and its tumors. Cytoplasmic markers such as neuron-specific enolase and protein gene product 9.5 may highlight cells containing very few secretory granules. However, its presence in some non-endocrine cells has limited its utilization.

General Overview

Histopathologic Classification

The classification of GI-NETs has been tedious, controversial, and sometimes confusing. Various schemes (Table 24.2) will be adapted in this text. It can be simply classified by the embryologic sites into foregut, midgut, and hindgut tumors. NETs arising from each location share similar clinical, biochemical, and secretory features. For instance, foregut NETs are argentaffin-negative and have low serotonin levels. These tumors can metastasize to bone and associate with atypical carcinoid syndrome. Midgut NETs are argentaffin positive and may produce high level of serotonin associated with classical carcinoid syndrome. They uniformly express intestinal transcription factor CDX2. Hindgut NETs are argentaffin negative and rarely secrete serotonin or other vasoactive peptides. Bone metastasis is uncommon.

GI-NETs can also be characterized by the secretory products of endocrine cells. There are at least 12 types of peptide- or amine-secreting endocrine cells in the gut. GI-NETs mainly arise from 5 major types of cells: (1) gastrin-producing G-cells; (2) somatostatin-producing D-cells; (3) entero-glucagon-producing L-cells; (4) serotonin-producing EC-cells; and (5) histamine-producing ECL cells. Tumors arising from cells containing other hormones such as secretin, GIP, CCK, motilin, or neurotensin are either very rare or non-existent.

Pure neuroendocrine tumors from various sites used to be called carcinoid based on their similar morphology and seemingly benign course. It has become apparent that this term is insufficient to cover the full range of malignant potentials. The WHO 2000 classification categorizes pure neuroendocrine tumors into 4 groups (1) well-differentiated neuroendocrine tumor, (2) well-differentiated neuroendocrine carcinoma, (3) poorly differentiated neuroendocrine carcinoma, and (4) mixed exocrine/endocrine carcinoma. The distinction of well-differentiated tumor and carcinoma is based on the tumor size, mitotic rates, and angio-lymphatic invasion. The diagnosis of poorly differentiated neuroendocrine carcinoma is the same as that of small cell carcinoma. When a non-endocrine element, such as adenocarcinoma, is also present, it should be designated as mixed endocrine-exocrine tumor.

WHO 2000 classification also established the morphological criteria for assessing the prognosis of GI-NETs based on tumor size, histological differentiation, proliferation...
activity (Ki67 index), hormonal activity, angioinvasion, and the presence of metastasis to adjacent organs. In the original publication, these prognostic criteria were further subdivided into localizations including the stomach, duodenum (and proximal jejunum), ileum (and distal jejunum), appendix, and colon-rectum. To eliminate redundancy, these criteria can be summarized into a simple table (Table 24.3) and easily applied to all anatomic sites in the GI tract.

There was no accepted TNM staging system although the European Neuroendocrine Tumor Society (ENETS) has recently proposed a grading system based on the tumor size. This proposal requires future validation by clinicopathological correlation.

**Organ Distribution and Clinical Features**

The true incidence rates and survival rates from each anatomic site were not well-documented. The Surveillance, Epidemiology, and End Results (SEER) is the largest nationwide cancer registry for GI-NETs. The raw-data between the periods of 1973–1991 and 1992–1999 are summarized in Table 24.4 for comparison.

Within the gut, small bowel, rectum, and colon are the three most frequent sites for GI-NETs. The incidence of gastric NETs increased during the two periods of comparison probably due to increasing gastroscopic surveillance for *Helicobacter pylori*. The 5-year survival throughout all anatomic sites improved with time and is best for rectum (88%) and appendix (71%), followed by stomach (63%), colon (62%), and small bowel (61%).

GI-NETs are usually benign, slow-growing, and asymptomatic. They are frequently found incidentally during an abdominal procedure or surgery unrelated to the tumor. The typical carcinoid syndrome including flushing, diarrhea, and asthma are present only in less than 10% of patients. These symptoms were caused by circulating hormones and substance such as serotonin, histamine, and substance P. Since these substances are quickly metabolized during their first pass through the liver, carcinoid syndrome is usually seen in patients with liver metastasis.

**Molecular Genetics**

The molecular genetics of GI-NETs is heterogeneous and poorly understood. Many genes commonly involved in other epithelial tumors, such as RAS, MYC, and FOS do not appear to be involved in GI-NETs. Instead, only a small subset of genes (Table 24.5) is altered in GI-NETs. These genes are discussed in this section according to their preferential

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### Table 24.2. Nomenclature used in various classification of GI-NETs.

<table>
<thead>
<tr>
<th>Embryologic/anatomical classification</th>
<th>Foregut (stomach, duodenum, proximal jejunum)</th>
<th>Midgut (distal jejunum, ileum, appendix, cecum)</th>
<th>Hindgut (colon and rectum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major cell types of NETs and their hormonal production</td>
<td>G-cell tumor, producing gastrin</td>
<td>D-cell tumor, producing somatostatin</td>
<td>L-cell tumor, producing enteroglucagon</td>
</tr>
<tr>
<td></td>
<td>EC (enterochromaffin)-cell tumor, producing serotonin</td>
<td>ECL (enterochromaffin-like)-cell tumor, producing histamine</td>
<td></td>
</tr>
<tr>
<td>Histopathologic grading</td>
<td>Pure neuroendocrine tumors</td>
<td>Well-differentiated neuroendocrine tumor (a.k.a carcinoid)</td>
<td>Well-differentiated neuroendocrine carcinoma (a.k.a malignant carcinoid)</td>
</tr>
<tr>
<td></td>
<td>Poorly differentiated neuroendocrine carcinoma (a.k.a small cell carcinoma)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed endocrine-exocrine tumors</td>
<td>Biological behavior</td>
<td>Benign</td>
<td>Benign or low-grade malignant</td>
</tr>
<tr>
<td></td>
<td>Low-grade malignant</td>
<td>High-grade malignant</td>
<td></td>
</tr>
</tbody>
</table>

### Table 24.3. Criteria for assessing the prognosis of GI-NETs.

<table>
<thead>
<tr>
<th>Biologic behavior</th>
<th>Histological differentiation</th>
<th>Tumor size</th>
<th>Ki-67 index</th>
<th>Invasion of m. propria</th>
<th>Angio-invasion</th>
<th>Metastasis</th>
<th>Hormonal syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>Well-differentiated</td>
<td>≤1 cm</td>
<td>&lt;2%</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Benign or low grade malignant</td>
<td>Well-differentiated</td>
<td>≤2 cm</td>
<td>&lt;2%</td>
<td>–</td>
<td>–</td>
<td>📈</td>
<td>–</td>
</tr>
<tr>
<td>Low grade malignant</td>
<td>Well-differentiated</td>
<td>&gt;2 cm</td>
<td>&gt;2%</td>
<td>📈</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>High grade</td>
<td>Poorly differentiated</td>
<td>Any</td>
<td>&gt;30%</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

1Exception: duodenal gastrinomas are usually smaller than 1 cm and confined to the submucosa.

2Exception: benign NETs of the appendix usually invade muscularis propria.

involvements in the tumors of foregut, midgut, and hindgut. Each gene will then be discussed in appropriate organ sections.

The MEN1 gene is located on chromosome 11q13. Germ-line mutations in this gene are responsible for multiple endocrine neoplasia syndrome, type 1 (MEN1),21 which is often associated with endocrine tumors of the stomach, duodenum, pancreas, as well as pituitary and parathyroid glands.

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Somatic mutation of MEN1 gene can be found in about one third of foregut NETs, including those from the stomach22 and duodenum.23

The NF1 gene is located at chromosomal locus 17q11. Germ-line mutations in this lead to neurofibromatosis, which has increased risk of periacillary tumors, especially duodenal somatostatinoma24 and gangliocytic paraganglioma.25

Loss of heterozygosity on chromosome 18q is frequently seen in midgut and hindgut NETs.26 The alterations in these loci probably involve Smad2, Smad4, or DCC genes. LOH in X-chromosome27 or 17q13 are seen only in malignant NETs but not in benign tumors. The mutation of Reg
cene (Reg) is associated with ECL cell tumors in patients with hypergastrinemia.28

In the following sections, GI-NETs will first be classified by their organ sites, then by the cell types or histopathologic entities. Any specific features pertinent to the organ site will be discussed in that section.

Esophageal Neuroendocrine Tumors
Esophageal NETs are extremely rare, accounting for only 0.05% of all GI-NETs and 0.02% of all esophageal cancers.29 The tumors are usually located in the distal esophagus and may be associated with achalasia or adenocarcinoma in the setting of Barrett’s esophagus.30 Three histopathologic entities have been reported in the esophagus: (1) classical NETs, (2) high-grade NETs, and (3) combined adenocarcinoma/ neuroendocrine tumors. Patients having these tumors usually have a poor prognosis.31

Gastric Neuroendocrine Tumors (G-NETs)
The normal stomach has two major types of endocrine cells, the gastrin-producing G cells32 (Figure 24.1a) and histamine-producing ECL cells33 (Figure 24.1b). G cells are located at the neck region of the antrum whereas ECL cells are found at

![Fig. 24.1. Endocrine cells in the normal stomach and their regulation of gastric acid secretion. (a) Normal G-cells are located at the neck region of the antrum (gastrin immunostain). (b) Normal ECL-cells (arrows) are found at the base of fundic glands (synaptophysin immunostain). (c) Schematic diagram of the regulation of gastric hydrochloric acid by G- and ECL cells.](image-url)
the base of fundic glands. In normal stomach, G cells secrete gastrin in response to negative feedback by hydrochloric acid (Figure 24.1c). Gastrin, in turn, drives the ECL cells to produce histamine, which is the main activator of acid secretion by parietal cell. Most G-NETs arise from ECL cells and rarely from G cells.

The incidence of G-NET has increased in past five decades. Since 1950, the proportion of G-NET among all GI-NET has increased from 2.6% to 8.7%. This increase is due to increased endoscopic surveillance and improved pathological evaluation. The recent propensity of using strong acid-suppressing medications such as proton pump inhibitors has been suspected a risk factor of G-NET secondary to the resulting hypergastrinemia. However, this theory has not been confirmed. Three distinct types of G-NETs will be described as follows.

**Type 1 G-NETs Associated with Type A (autoimmune) Chronic Atrophic Gastritis**

Among various types of G-NETs (Table 24.6), type 1 is most common and it often has a benign course. Histologically, the tumor is usually small (<2 cm), multiple (Figure 24.2b), and confined to the fundic mucosa, which exhibits various degree of chronic inflammation and glandular atrophy. The antral mucosa is characterized by G-cell hyperplasia (Figure 24.2a). Lymph node metastasis is very uncommon. Given its favorable outcome, endoscopic mucosal resection or antrectomy are considered as an appropriate treatment. Tumor regression has been reported following antrectomy, which removes the entire population of G cells.

Type A chronic atrophic gastritis is caused by autoantibodies to parietal cells or intrinsic factor. It may or may not be associated with pernicious anemia. The disease involves the oxyntic mucosa of the fundus, leading to glandular atrophy and achlorhydria secondary to the loss of parietal cells, which produces hydrochloric acid (Figure 24.2c). Lacking the negative feedback of hydrochloric acid, the antral G-cells undergo hyperplasia and hypersecretion of gastrin. Hypergastrinemia, in turn, causes ECL cell hyperplasia (Figure 24.2b) and its subsequent transformation into G-NET.

The pathogenesis of type 1 G-NET is mostly unclear. Hypergastrinemia is required for the hyperplasia of ECL cells but insufficient for their transformation into G-NET. Only 3% of patient with A-CAG develops G-NETs. Therefore, some other genetic factors are still needed in the development of tumors. LOH analysis showed allelic loss of the 11q13 regions (MEN-1 gene locus) in 48% cases of type 1 G-NETs.

**Table 24.6. Features of gastric neuroendocrine tumors.**

<table>
<thead>
<tr>
<th>Tumor features</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of all gastric NETs</td>
<td>68–85%</td>
<td>8%</td>
<td>23%</td>
</tr>
<tr>
<td>Size</td>
<td>&lt;2 cm</td>
<td>&lt;2 cm</td>
<td>&gt;3 cm</td>
</tr>
<tr>
<td>Number</td>
<td>Single/multiple</td>
<td>Multiple</td>
<td>Single</td>
</tr>
<tr>
<td>Location</td>
<td>Fundus</td>
<td>Fundus</td>
<td>Antrum or fundus</td>
</tr>
<tr>
<td>Clinical features</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma gastrin levels</td>
<td>Increased</td>
<td>Increased</td>
<td>Normal</td>
</tr>
<tr>
<td>Gastric acid</td>
<td>Low</td>
<td>High</td>
<td>Normal</td>
</tr>
<tr>
<td>Associated conditions</td>
<td>A-CAG, ZES/MEN I</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Pathology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antral G cells</td>
<td>Hyperplasia</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Fundic ECL cells</td>
<td>Hyperplasia/dysplasia</td>
<td>Hyperplasia/dysplasia</td>
<td>Normal</td>
</tr>
<tr>
<td>Fundic mucosa</td>
<td>Atrophic</td>
<td>Hypertrophic</td>
<td>Normal</td>
</tr>
<tr>
<td>Prognosis</td>
<td>5-year survival</td>
<td>&gt;70%</td>
<td>&gt;70%</td>
</tr>
<tr>
<td>Metastases</td>
<td>Rare</td>
<td>Up to 30%</td>
<td>50–100%</td>
</tr>
<tr>
<td>Treatment</td>
<td>Endoscopic removal</td>
<td>Endoscopic removal</td>
<td>Surgery</td>
</tr>
<tr>
<td>Genetic factors</td>
<td>LOH of 11q13 (MEN1 locus)</td>
<td>48%</td>
<td>75%</td>
</tr>
</tbody>
</table>
24. Gastrointestinal Neuroendocrine Tumors

Type 2 G-NETs Associated with Zollinger-Ellison Syndrome (ZES) with or Without Type 1 Multiple Endocrine Neoplasia (MEN-1)

ZES is characterized by multiple intractable peptic ulcers secondary to gastrin-producing tumors in the pancreas or duodenum. ZES may be sporadic (rare) or commonly associated with MEN-1, which contains pancreatic gastrinoma as a component.42

These lesions are usually multiple and less than 2 cm in size. Background pathology includes hypertrophic, hypersecretory gastropathy (Figure 24.3a) secondary to the trophic effects of gastrin.43,44 Unlike type 1 G-NET, hypergastrinemia in this setting is arising from gastrinoma (Figure 24.3b) rather than antral G cells, which appear normal in type 2. In addition to hyperplasia, dysplasia of ECL cells is more commonly seen than that in type 1 G-NETs. The pathogenetic pathway is summarized in Figure 24.3c. The treatment should aim at the localization and surgical excision of gastrinoma. Spontaneous regression of G-NET has been reported following the removal of gastrinoma.45 Palliation by endoscopic mucosal excision or somatostatin analog may be considered in nonsurgical candidates.

Type 3 Sporadic G-NETs

This type of lesions is gastrin-independent and not associated with hypergastrinemia.36 It accounts for about a quarter of all G-NETs and has a worse prognosis than other 2 types of G-NET. Patients usually have a large, solitary tumor presented in the antrum or fundus. The adjacent antral or fundic mucosa appears histologically unremarkable (Figure 24.4). Metastatic disease is more common than type 1 and 2 G-NET. In light of the malignant potential of this tumor, aggressive therapy should be considered. In addition to surgery, combined chemotherapy and radiation may be required.46

Unlike type 1 and 2, endocrine cell hyperplasia plays no role in these lesions. Genetic abnormalities are considered the major pathogenic mechanisms. It is interesting to note that LOH of 11q13 has been reported in 25–62% of cases, suggesting a common genetic basis may be shared by all 3 types of G-NET.

Hyperplasia-Dysplasia-Neoplasia Sequence in G-NET

Nonneoplastic endocrine cell hyperplasia has been proposed as the precursor lesion of GI-NETs in several anatomic sites. The hyperplasia-dysplasia-neoplasia sequence was best studied in the stomach and its histopathologic classification in 1988 by Socia et al47–50 (Table 24.7) (Figure 24.5). This process can be seen in both type 1 and 2 gastric NETs. By definition, ECL hyperplasia is a lesion that lacks inherent evolutive potential and less than 150 μm in size. ECL dysplasias are pre-carcinoid lesions that measure between 150 and 500 μm and display progressive capacity. ECL neoplasias are those
lesions larger than 500μm with invasion into the mucosa or beyond. Table 24.7 lists the histological criteria of all subcategories of three major entities.47

**Table 24.7. Hyperplasia-dysplasia-neoplasia sequence of gastric neuroendocrine tumors.**

<table>
<thead>
<tr>
<th>Classification</th>
<th>Lesion size</th>
<th>Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hyperplasia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simple (or diffuse)</td>
<td></td>
<td>Cell number &gt;2 standard deviation normal</td>
</tr>
<tr>
<td>Linear (or chain-forming)</td>
<td>≤5 cells; 2 chains/mm mucosa</td>
<td></td>
</tr>
<tr>
<td>Micronodular</td>
<td>≥5 cells; 30–150μm</td>
<td></td>
</tr>
<tr>
<td>Adenomatoid</td>
<td>≥5 micronode</td>
<td></td>
</tr>
<tr>
<td><strong>Dysplasia (Pre-neoplasia)</strong></td>
<td>&lt;500μm</td>
<td>Cluster of cells &lt;150μm</td>
</tr>
<tr>
<td>Enlarge micronodular</td>
<td>Loss of basement membrane in between</td>
<td></td>
</tr>
<tr>
<td>Fusing micronodular</td>
<td>Infiltrating the lamina propria</td>
<td></td>
</tr>
<tr>
<td>Microinvasive lesion</td>
<td>Microlobular or trabecular structure</td>
<td></td>
</tr>
<tr>
<td>Nodule with newly formed stroma</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Neoplasia (carcinoid)</strong></td>
<td>≥500μm</td>
<td>Confined to the mucosa</td>
</tr>
<tr>
<td>Intramucosal carcinoid</td>
<td>Microscopic lesion not seen grossly</td>
<td></td>
</tr>
<tr>
<td>Microcarcinoid</td>
<td>Multiple microcarcinoids</td>
<td></td>
</tr>
<tr>
<td>Microcarcinoidosis</td>
<td>Penetrating muscularis mucosae</td>
<td></td>
</tr>
<tr>
<td>Invasive carcinoid</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Small Bowel Neuroendocrine Tumors**

**Duodenal Neuroendocrine Tumors**

Duodenal NETs (Table 24.8) are rare and can be classified into five major types: (1) G-cell tumors, (2) D-cell tumors, (3) other well-differentiated NETs, (4) gangliocytic paraganglioma, and (5) poorly differentiated endocrine carcinomas.51 G-cell tumors are either sporadic or hereditary associated with MEN-1.52 Most G-cell tumors from the latter group are small, multiple, and functioning.53 Gastrin-producing G-cell tumor can generate Zollinger-Ellison syndrome. In a recent report of 18 cases of G-cell tumors arising in the duodenal bulb, there is a high association with long-term use of proton pump inhibitors and with *H. pylori* gastritis.54 Histologically, G-cell tumors form trabecular or acinar architecture surrounded by a delicate fibrovascular stroma (Figure 24.6a).

Almost all D-cell tumors occur in the region of ampulla of Vater.55 Although containing somatostatin, these tumors are usually nonfunctioning and do not produce symptoms related to somatostatin hypersecretion such as diarrhea and diabetes. There is a high association (50%) between D-cell tumors and type 1 neurofibromatosis56,57 and von Hippel Lindau disease58 with unclear molecular mechanism. Histologically, D-cell tumors contain glands lined by columnar cells and filled with psammoma bodies in

---

**Fig. 24.5. Hyperplasia-dysplasia-neoplasia sequence of gastric ECL cell tumors (a) Linear and micronodular hyperplasia. (b) Enlarged and fusing micronodular dysplasia. (c) Intramucosal carcinoids. (d) Invasive carcinoids in the submucosal stroma. (synaptophysin immunostains).**
the lumens (Figure 24.6b). In patients with NF1 associated with D-cell tumors, gangliocytic paraganglioma is another common lesion found in the duodenum.

### Jejunal and Ileal Neuroendocrine Tumors

Jejuno-ileal region is the second most common site for GI-NETs and accounts for up to 30% cases of the latter. EC cell tumor is the most common type of jejuno-ileal NETs. The etiology is unknown although it is interesting to note its association with Crohn’s disease or other non-carcinoid neoplasms of the GI-tract in some patients. Lymph node metastasis is commonly found during the surgery, but it does not seem to affect patients’ survival. Histological features are characterized by multiple solid or insular nests of packed cells, which frequently invade the bowel wall (Figure 24.7a, b).

### Appendiceal Neuroendocrine Tumors

NETs are found in (0.3–0.9 %) of appendectomy specimens, but they account for up to 75% of all appendiceal neoplasms. Its incidence among all GI-NETs appears decreasing during the period of 1973–1997. A classification based on WHO is shown in Table 24.9.

---

**Table 24.8. Neuroendocrine tumors of the small intestine.**

<table>
<thead>
<tr>
<th></th>
<th>Duodenal NETs</th>
<th>Jejuno-ileal NETs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primitive gut</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of total GI-NET</td>
<td>22%</td>
<td>23–28%</td>
</tr>
<tr>
<td><strong>Histologic types</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-cell tumor (gastrinoma)</td>
<td></td>
<td>EC cell tumors</td>
</tr>
<tr>
<td>D-cell tumor</td>
<td>(somatostatinoma)</td>
<td>L cell tumor</td>
</tr>
<tr>
<td>Other hormone-producing tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gangliocytic paraganglioma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuroendocrine carcinoma</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Associated conditions**

| Hereditary          | MEN-1               | –                |
|                     | Neurofibromatosis   |                  |
| Nonhereditary       | Zollinger-Ellison syndrome | –             |
|                     | Proton-pump inhibitor |                |

---

**Fig. 24.6.** Duodenal neuroendocrine tumors (H&E stains) (a) G-cell tumor (gastrinoma). (b) D-cell tumor (somatostatinoma) with psammoma bodies.

**Fig. 24.7.** Neuroendocrine tumors of the jejunum (a) and ileum (b) (H&E stains).
Serotonin-producing EC cell carcinoids are the most common type of appendiceal NETs. Most of them are located at the tip of the appendix (Figure 24.8a, b) and arise from the subepithelial neuroendocrine cells, which are most abundant in the tip. The majority of tumors is less than 1 cm and associated with better prognosis. L cell carcinoids are uncommon. They are small and localized at the tip of the appendix.

Mixed endocrine-exocrine neoplasms are rare but interesting variants because of their differentiation into multiple cell lineages. Three morphological types were recognized in a large series of 64 cases by Armed Institute of Pathology: (1) tubular carcinoids, (2) goblet cell carcinoids, and (3) mixed carcinoid-adenocarcinomas.61

Tubular carcinoids are typically less than 5 mm and poorly defined in the stroma or smooth muscle. Most of them were diagnosed incidentally by microscopic examination without gross evidence of lesions. Histology shows tubular structures of cuboidal cells with occasional goblet cells. Patients have excellent prognosis without recurrence or metastasis.

Goblet cell carcinoid is also known as adenocarcinoid, mucin-producing carcinoid, and crypt cell carcinoma. Histology is characterized by small nests of goblet cells and neuroendocrine cells (Figure 24.9a) with scattered glandular formation. Patients have a prognosis intermediate between typical carcinoids and adenocarcinoma. Of those tumors confined to the appendix and excised with negative surgical margins, a cure without recurrence or metastasis is the norm. A third of patients may have extensive disease outside the appendix and eventually die of the disease.

Despite its rarity, goblet cell carcinoid has drawn the attention of investigators regarding its histogenesis. Stancu et al found loss of heterozygosity of chromosomes 11q, 16q, and 18q occurring 25%, 38%, and 56% respectively in 16 cases of goblet cell carcinoid.63 The profile was similar to that of ileal carcinoid, but different from non-ileal carcinoids. They did not find any mutations of \( K-ras \), \( \beta\)-catenin, or DPC-genes, which supports that this tumor follows the usual pathogenetic pathway of endocrine tumors rather than exocrine adenocarcinoma. Another study by Kuroda

<table>
<thead>
<tr>
<th>Table 24.9. Classification of appendiceal neuroendocrine tumors.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Typical carcinoids</strong></td>
</tr>
<tr>
<td>EC cell carcinoid</td>
</tr>
<tr>
<td>L cell carcinoid</td>
</tr>
<tr>
<td><strong>Mixed endocrine-exocrine neoplasms</strong></td>
</tr>
<tr>
<td>Tubular carcinoid</td>
</tr>
<tr>
<td>Goblet cell carcinoid</td>
</tr>
<tr>
<td>Mixed carcinoid-adenocarcinoma</td>
</tr>
</tbody>
</table>

![Fig. 24.8. Typical EC cell carcinoid of the appendix. (a) Cross section of the appendix shows a 5 mm yellowish, well-circumscribed carcinoid tumor (arrow). (b) Microgram of the carcinoid (H&E stain).](image)

![Fig. 24.9. Mixed endocrine-exocrine neoplasms of the appendix. (a) Goblet cell carcinoid. (b) Mixed carcinoid-adenocarcinoma (H&E stain).](image)
et al indicated that reduced expression of E-cadherin in this tumor is more similar to typical carcinoid rather than to adenocarcinoma.\textsuperscript{64}

Mixed carcinoid-adenocarcinoma\textsuperscript{61,65} is associated with the worst prognosis among 3 variants of amphicrine tumors. Most patients died of metastatic carcinoma within 2 years. In addition to clusters of endocrine cells and goblet cell carcinoid, this variant also shows typical morphology of adenocarcinoma (Figure 24.9b), such as glandular formation or signet-ring cell features, in more than 50% of the tumor mass.\textsuperscript{61}

Colonic and Rectal Neuroendocrine Tumors

Large intestine NETs are most common in the rectum (54%), followed by the cecum (20%), sigmoid colon (7.5%), and ascending colon (5%).\textsuperscript{29} Some cases were reported in patients with inflammatory bowel disease.\textsuperscript{66} However, a direct association of NETs and inflammatory bowel disease has not been substantiated.

Excluding the rectum, about half of the colonic NETs are located at the cecum. The remaining half has a roughly equal distribution among the ascending, transverse, descending, and sigmoid colon. As their counterpart of adenocarcinoma, NETs of the right colon tend to be larger and present at more advanced stage compared to NETs of the left colon. The diagnosis is commonly established by colonoscopic biopsies and histological examination. Surgical treatment follows the same principle of colonic adenocarcinoma, i.e., complete resection of the tumor and lymph node spread. The surgeon should expect higher rate of local invasion and liver metastases than adenocarcinoma.

Rectal NETs are frequently small (<2 cm), asymptomatic, and have an indolent course. Carcinoid syndrome is uncommon (<10% of cases) because of the secretion of hormone products into the portal circulation and detoxication in the liver.

Histologically, large intestinal NETs can be classified into (1) typical carcinoids, including EC- and L-cell tumor, (2) large cell neuroendocrine carcinoma, and (3) small cell carcinoma (Table 24.10). Typical carcinoids are those well differentiated NETs, which exhibit trabecular pattern (Figure 24.10a). Large cell neuroendocrine carcinoma is a malignant tumor composed of large cells with organoid, nesting, and palisading patterns (Figure 24.10b). Small cell carcinoma is rare and morphologically similar to that of the lung.

Treatment

The treatment of GI-NETs varies depending on the disease stages and symptoms related to hormonal secretion. The major options can be summarized as follows:

1. Surgery: Surgical resection, either curative or palliative, is considered the first choice for patients with localized disease. Appendectomy may be adequate for appendiceal NET unless the tumor is larger than 2 cm or have angiolympathic invasion or positive margin. Then, right hemicolectomy should be performed. Endoscopic removal is considered appropriate for rectal carcinoid less than 1 cm without invasion into the muscularis propria.\textsuperscript{67} However, small bowel NETs require wider resection according to the stage due to the high risk of metastasis.

2. Biotherapy: Low dose interferon-\(\alpha\) or somatostatin analogues (e.g., octreotide) has been used to manage the clinical symptoms with more than 50% response rate.\textsuperscript{68} However, tumor reduction is only seen in less than 15% of patients.

<table>
<thead>
<tr>
<th>Table 24.10. Classification of colorectal neuroendocrine tumor.</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC cell tumor</td>
</tr>
<tr>
<td>L-cell tumor</td>
</tr>
<tr>
<td>Small cell carcinoma</td>
</tr>
<tr>
<td>Large cell neuroendocrine carcinoma</td>
</tr>
</tbody>
</table>

Fig. 24.10. Colorectal neuroendocrine tumors (H&E stains) (a) Colonic carcinoid tumor. (b) Rectal large cell neuroendocrine carcinoma.
3. Chemotherapy: Clinical response to single agent, such as 5-fluorouracil, streptozocin, doxorubicin, actinomycin D, and cyclophosphamide, has been around 10%. Combination chemotherapy may increase response rate to 30%, but complete response is rare.

References


Introduction

The head and neck region contains a number of disparate organs ranging from skin to brain, and many of the neuroendocrine tumors of these organs have been discussed elsewhere in this book (skin, thyroid, and pituitary gland). Extra-adrenal paragangliomas, although also discussed elsewhere, will be mentioned and discussed briefly as they pertain to the ear and upper aerodigestive tract. This chapter will concentrate on neuroendocrine tumors of the ear, upper aerodigestive tract, and salivary glands. Focusing on these entities leaves only a few head- and neck-specific tumors to discuss, including middle ear adenoma, neuroendocrine carcinomas of the upper aerodigestive tract and salivary glands, uncommon and unique neuroectodermal tumors of the area, and, as mentioned, paraganglioma. Even if taken together, these tumors are exceedingly rare and little is known regarding the genetics of the tumors. While this limits our discussion, it does leave open some exciting avenues of possible research.

Upper Aerodigestive Tract

Neuroendocrine Carcinomas

Neuroendocrine carcinomas of all grades (well, moderately and poorly differentiated neuroendocrine carcinomas, also called carcinoid tumors, atypical carcinoid tumors, and small cell carcinomas) occur throughout the upper aerodigestive tract, most often within the larynx or sinonasal tract. \(^1\)–\(^9\) Distinguishing these tumors histologically is important, as they have very different prognoses.

Well-differentiated neuroendocrine carcinomas or carcinoid tumors are by far the rarest of these malignancies. \(^1\)–\(^3\), \(^8\) They develop most frequently in the supraglottic larynx of older men. Up to a third of the patients can eventually develop distant metastases, but only 10% of patients die from their disease.

These tumors resemble carcinoid tumors from other sites and have an organoid, nested or trabecular architecture, and are composed of uniform epithelioid cells. \(^1\)–\(^3\), \(^8\) The cells may have a variable amount of cytoplasm that sometimes appears granular or oncocytic. Nuclei are usually also uniform in size and are round to ovoid with granular chromatin. By definition, mitotic figures are usually not seen and necrosis is absent. Background amyloid or sclerosis is sometimes seen.

Moderately differentiated neuroendocrine carcinomas or atypical carcinoid tumors are much more common. \(^1\)–\(^3\), \(^8\) They also develop in the submucosa of the supraglottic larynx of older men but have a worse behavior than well-differentiated neuroendocrine carcinomas. Nearly half will present with regional lymph node metastases and less than one half of patients survive 5 years.

These tumors have much the same histologic appearance as typical carcinoid tumors; however, they show more variable architecture and can focally display sheet-like growth (Figure 25.1). \(^1\)–\(^3\) Characteristically, they have a moderate amount of nuclear and cellular pleomorphism and occasional mitotic figures. Furthermore, necrosis is often seen. Oncocytic and mucinous changes have been noted and amyloid can also be found.

Small cell carcinomas also can occur anywhere throughout the upper aerodigestive tract; however, they also most often involve the supraglottic larynx. \(^1\)–\(^3\), \(^7\)–\(^8\), \(^10\), \(^11\) These occur more frequently in men, usually in the sixth to seventh decades of life and have a dismal prognosis; less than 5% of patients diagnosed with these lesions are alive 5 years after their diagnoses.

Histologically, small cell carcinomas of the upper aerodigestive tract are indistinguishable from small cell carcinomas seen elsewhere. \(^1\)–\(^4\), \(^7\) They are composed of variably sized sheets, cords, nests, and ribbons of small cells with little identifiable cytoplasm (Figure 25.2). Large areas of confluent necrosis are often seen. Nuclear molding and focal crush artifact are often also seen with abundant mitotic figures and single-cell apoptotic figures. Tumor cell nuclei are hyperchromatic and have granular chromatin without prominent
nucleoli. The Azzopardi effect is often noted as is true vascular invasion. The tumors may contain areas diagnostic of non-small cell carcinoma, and varying differentiation has been noted throughout the upper aerodigestive tract.

Immunohistochemically, neuroendocrine carcinomas of the upper aerodigestive tract react with antibodies to keratins and neuroendocrine antigens, such as CD56, NSE chromogranin, and synaptophysin. Small cell carcinomas often exhibit “dot-like” staining with antikeratin antibodies similar to immunoreactivity described with these tumors at other sites (Figure 25.3). Small cell carcinomas also are more likely to lack immunostaining with antibodies to chromogranin and synaptophysin. Moderately differentiated neuroendocrine carcinomas of the larynx have been noted to react with antibodies to calcitonin in approximately 75% of cases. As the tumors sometimes are also associated with amyloid, some have noted their resemblance to medullary thyroid carcinomas.

Scant information is available regarding genetic changes seen with well and moderately differentiated neuroendocrine carcinomas and small cell carcinomas of the upper aerodigestive tract. Overexpression of p53 protein has been seen immunohistochemically in 50% of the laryngeal moderately differentiated neuroendocrine carcinomas tested in two separate studies.

Finally, some large cell undifferentiated carcinomas of the upper aerodigestive tract will show a prominent neuroendocrine phenotype, both histologically and immunohistochemically, resembling large cell neuroendocrine carcinomas of the lung. While these tumors can occur throughout the tract, in our experience, they occur most frequently in the sinonasal area; they are considered by some to be within the spectrum of sinonasal undifferentiated carcinomas (SNUCs). These tumors can occur in men and women of any age, although they most often occur in older patients. They are very aggressive and have a poor prognosis, even with multimodality therapy.

Large cell carcinomas with neuroendocrine differentiation have larger cells than typical small cell carcinomas, frequently with more vesicular chromatin and prominent nucleoli (Figure 25.4). They are very high grade, and necrosis, mitotic figures, vascular, and perineural invasion are usually seen. Immunohistochemically, undifferentiated carcinomas typically express keratins. P63, a marker of squamous differentiation, is variably expressed depending on tumor site and features; most SNUCs will not express the protein. By definition, those undifferentiated carcinomas with a neuroendocrine phenotype should react with antibodies to one or more neuroendocrine antigens.

Conventional cytogenetics has only been performed with a few SNUCs. One case was found to have two translocations, t(1;6)(p22;p23) and t(12;17)(q13;p13); another had a very
complex karyotype with numerous derivative chromosomes, deletions, and additions. SNUCs have been noted to frequently overexpress p16 and p53 proteins by immunohistochemistry (Figure 25.5). Mismatch repair proteins, MLH-1 and MSH-2, have both found to be intact by immunohistochemistry. C-kit has also been noted to be overexpressed, although activating kit mutations have not been identified. EGFR has also been overexpressed by immunohistochemistry whereas Her2/neu has not been shown to be overexpressed.

Paragangliomas

Paragangliomas of the upper aerodigestive tract are almost always sporadic and benign, although both familial and malignant cases have been reported. The lesions occur most frequently in the larynx and are more common in women. Within the larynx, tumors are most frequently supraglottic and this has suggested to some that the superior paired paraganglia of the larynx more frequently develop tumors than the inferior paraganglia. In Lack’s description of 69 head and neck paragangliomas, only one arose in the larynx. Histologically, immunohistochemically, and genetically, upper aerodigestive tract paragangliomas are identical to those of the ear and other extra-adrenal sites (see below).

Neuroectodermal Tumors

Melanotic neuroectodermal tumor of infancy (MNTI) is, as the name implies, a tumor of early childhood that is more common in boys. The tumors most frequently involve the maxilla, mandible, or skull. Although the tumors often recur, they rarely metastasize.

Grossly, the lesions appear lobulated and circumscribed, sometimes darkly pigmented, and are usually less than 5 cm in size. Histologically, the tumors are composed of larger, more epithelioid cells and small round cells (Figure 25.6). The larger cells typically have abundant eosinophilic cytoplasm with melanin pigment and are arranged in tubular or alveolar structures, while the smaller cells are often nested. Necrosis is generally not seen (unless related to treatment) and mitotic figures are uncommon. Larger cells are immunoreactive with antibodies to keratins and EMA while both cell types are immunoreactive with antibodies to NSE, synaptophysin, and HMB45. Little is known regarding the cytogenetic and molecular changes of these tumors. A cell line established for an MNTI has revealed a complex karyotype.

Olfactory neuroblastomas (ONBs) are rare tumors that develop in the upper nasal cavity in the area of the olfactory epithelium. The tumors occur in patients of either sex at over a wide age range. Patients present with nonspecific symptoms, sometimes with visual changes. With current multimodality treatments, patients with ONB do quite well and some have reported survival rates at 10 years to be greater than 80%.

Microscopically, tumor cells grow either diffusely or in discrete, circumscribed nests that are separated by fibrous or edematous stroma (Figure 25.7). Homer Wright or more equivocal type rosettes are seen and an eosinophilic fibrillary background is usually present, sometimes with calcifications. Rarely, ganglion cells or even glandular differentiation are seen. The neoplastic cells are small and usually have minimal eosinophilic cytoplasm with round monomorphic nuclei. Although most cases have few mitotic figures, occasional cases can have marked mitotic activity. ONBs have been graded according to the Hyams grading system, which takes into account tumor architecture, nuclear atypia, mitotic activity, necrosis, and the presence or absence of background fibrillary material. Most ONBs will show immunoreactivity with antibodies to synaptophysin or chromogranin, as...
well as with antibodies to less specific markers of neural or neuroendocrine differentiation such as NSE and CD56.33,38 Focal immunoreactivity with antibodies to cytokeratins is sometimes seen. S100 immunoreactivity is usually limited to supporting sustentacular cells.

Little is known regarding the molecular abnormalities of ONBs. The tumor is not believed to be a member of the Ewing sarcoma group of tumors and the typical t(11;22) seen in those tumors is not seen with ONBs, despite an earlier paper claiming the contrary.10,39,40 Array comparative genomic hybridization has identified numerous gains and losses.41 The most frequent changes included gains at 7q11.22–q21.11, 9p13.3, 13q, 20p/q, and Xp/q, and losses at 2q31.1, 2q33.3, 2q37.1, 6q16.3, 6q21.33, 6q22.1, 22q11.23, 22q12.1, and Xp/q. Frequent changes seen with higher stage tumors were gains at 13q14.2–q14.3, 13q31.1, and 20q11.21–q11.23, and loss of Xp21.1. Gains at 5q35, 13q, and 20q, and losses at 2q31.1, 2q33.3, and 6q16–q22, were present in 50% of cases in one study. Immunohistochemistry has identified a number of possible molecular abnormalities. Cyclin-D1 has been noted to be overexpressed in 63% of cases.42 Sixty percent of cases have been shown to express bcl-2, and HIF-1alpha expression has been noted in most cases.43

Salivary Glands

Neuroendocrine Carcinomas

Neuroendocrine carcinomas of the salivary glands are very uncommon and the best described and overwhelmingly most common type is small cell carcinoma.44–48 Only rarely have other neuroendocrine carcinomas been described, though, interestingly, a family with apparently hereditary low-grade neuroendocrine carcinomas or carcinoid tumors has been described.49,50 Also of note, other poorly differentiated salivary gland malignancies can sometimes show neuroendocrine differentiation by immunohistochemistry.51 Small cell carcinomas occur most often in the major salivary glands, although some have been described to involve minor salivary glands (many of these may have been basaloid squamous cell carcinomas or SNUCs). The tumors occur in older patients of either sex (the rare small cell carcinomas reported in the very young may have represented other small blue cell tumors). Patients typically present with symptoms secondary to their mass lesions.

Histologically, small cell carcinomas of nonpulmonary sites are generally defined as having the histologic features of pulmonary small cell carcinomas.44,47 There are sheets, ribbons, and nests of small, oval to round cells that have minimal cytoplasm and hyperchromatic nuclei. Nuclear chromatin is often fine, and prominent nuclei are usually absent. Mitotic figures and necrosis (both coagulative and single cell) are abundant, and perineural and vascular invasion are usually found. Nearly half of cases will have foci showing either squamous or glandular differentiation. We have seen a case associated with a pleomorphic adenoma.

Immunohistochemically, neoplastic cells should react with antikeratin (AE1/AE3 or CAM5.2) antibodies (frequently with perinuclear dot-like staining) and with antibodies to neuroendocrine antigens such as NSE, CD57, synaptophysin, and chromogranin.48,52 Immunoreactivity with antibody to CK20 and TTF1 have also been noted and some have suggested that the two stains can help distinguish pulmonary from Merkel cell types, the latter having improved survival.48 The prognosis is bad in either case, although patients may do better than those with small cell carcinomas of other nonpulmonary sites. Aside from the fact that about half of cases overexpress p53 protein by immunohistochemistry, there is no other specific information known regarding molecular changes in these tumors.48
The Ear

Middle Ear Adenoma

The most common epithelial neuroendocrine tumor of the ear per se seen at the University of Virginia is Merkel cell carcinoma. These develop in the sun-exposed auricle and have been discussed with skin tumors. Those that develop on the ear do not appear to be unique in any way.

Adenomas of the middle ear (i.e., middle ear adenoma), on the other hand, do appear to be unique to the site. These tumors have been given a variety of names throughout the years including middle ear adenomatous tumor, carcinoid tumor of the middle ear, and neuroendocrine adenoma of the middle ear. The designation used here is currently advocated by the World Health Organization. These tumors are very rare and represent approximately 18% of middle ear neoplasms and just 9% of neoplasms of the middle ear and temporal bone when considered together. The tumors present with equal frequency in men and women and occur almost exclusively in adults over a wide age range (mean age of 45 years). Patients present with nonspecific symptoms, especially hearing loss. A familial association has not been noted with these tumors.

Middle ear adenomas usually present as nondestructive, unilateral middle ear mass lesions, although they can be invasive into bone. The tumors are typically small, and the mean aggregate size has been reported to be less than 1 cm. Histologically, the tumors show a great deal of variety, similar to well-differentiated neuroendocrine carcinomas or carcinoid tumors seen throughout the rest of the body. They may be glandular, trabecular, solid, infiltrating, organoid, or grow as single cells (Figure 25.8). Frequently, more than one growth pattern is noted in a single tumor. Necrosis and ulceration are very rare. The neoplastic cells are typically epithelioid, although occasional cases can have more spindled or plasmacytoid cells. They have indistinct cells borders with eosinophilic, often granular cytoplasm. The nuclei are round to oval, centrally or eccentrically placed, and typically have finely granular chromatin. Nucleoli and mitotic figures are usually not seen. Occasionally, two apparent cell types can be seen, especially with tumors having a glandular growth pattern. Here, the luminal cells appear more flattened as the basal cells appear more cuboidal.

Neoplastic cells are immunoreactive with antibodies to cytokeratins (cocktail, CK7, and CAM5.2) and with antibodies to neuroendocrine antigens (chromogranin, NSE, human pancreatic polypeptide and synaptophysin) (Figure 25.9). Some cases will be immunoreactive with antibodies to S100 protein and vimentin. Interestingly, cases displaying more than a single cell type will show differential staining with luminal cells reacting with antibodies to CK7 while the more basal cells react with antibodies to neuroendocrine antigens.

Middle ear adenomas are almost always benign. Recurrences have been noted in up to 20% of cases and are usually associated with less aggressive initial therapy. Rare cases have been noted to metastasize. Specific histologic features or prognostic markers have not been identified that are associated with behavior for these tumors. Finally, there have been no studies regarding the molecular changes seen with these tumors.

Jugulotympanic Paraganglioma

Jugulotympanic paragangliomas represent approximately one-third of all middle ear or surrounding neoplasms. As with middle ear adenomas, jugulotympanic paragangliomas have been given a number of appellations such as jugulotympanic chemodectoma and jugular or tympanic...
glomus tumor. The tumors are more common in women and occur over a wide age range; however, most occur in middle age and the average age at presentation is 50 years. Most arise at the site of the jugular bulb and thus involve the petrous bone. Approximately, 12% primarily involve the middle ear space (typanic paraganglioma) and a very small percentage involves the external ear canal only. The tumors usually present with conductive hearing loss; however, a number of other nonspecific symptoms have been noted.

Jugulotympanic paragangliomas tend to be very small and most are less than 5 mm in greatest dimension. They have a typical nested or “zellballen” architecture described in other portions of this text, although our experience suggests that this is less pronounced with these tumors than with those seen at other sites (Figure 25.10). The immunophenotype is also identical with typical immunoreactivity for antibodies to endocrine antigens such as chromogranin and synaptophysin and not with antibodies to cytokeratins. Supporting cells or “sustentacular cells” are typically immunoreactive with antibodies to S-100 protein.

Few cases of jugulotympanic paragangliomas behave aggressively and metastasize. This is generally impossible to predict based on histology. The tumors are mostly sporadic; however, in Alford and Guilford’s seminal study, approximately 6% were associated with paragangliomas elsewhere. Others have noted a higher familial incidence, especially given that the inheritance is autosomal dominant with genomic imprinting (thus, maternal transmission is more likely to metastasize). Both sporadic and familial paragangliomas frequently exhibit mutations of the genes encoding subunits of succinate dehydrogenase (SDHB, SDHC, and SDHD). Rarely, they are associated with mutations of the VHL and RET genes. These are discussed in more detail in the chapters discussing pheochromocytomas and extra-adrenal paragangliomas. Interestingly, paragangliomas associated with germline SDHB mutations are more likely to metastasize.

Fig. 25.10. This jugulotympanic paraganglioma is obviously nested and composed of round, epithelioid cells.

References


25. Head and Neck Neuroendocrine Tumors


Merkel Cell Carcinoma

Steven D. Billings and Melissa P. Piliang

Introduction

Merkel cells are neural crest-derived cells found in the basal layer of the epidermis and tactile hair discs of Pinkus that serve as mechanoreceptors. Ultrastructurally, they demonstrate neuroendocrine differentiation with membrane-bound dense core neurosecretory granules. Primary neuroendocrine carcinomas of the skin are thought to derive from Merkel cells and have thus been designated Merkel cell carcinoma.

Clinical Features

Merkel cell carcinoma is a rare sporadic cutaneous malignancy that occurs primarily on actinically damaged skin. With an incidence of 0.44/100,000 people, Merkel cell carcinoma is 20 times more common in whites than blacks. The average age of onset is 60–80 years old. The tumor classically presents as a painless red-purple nodule on the head and neck and can occur less commonly on the upper extremities and back. Merkel cell carcinoma has rarely been reported on the trunk, genitalia, and oral mucosa. Risk factors for Merkel cell carcinoma include being a fair-skinned male with extensive sun-exposure. Like other sun-induced skin cancers, immunosuppression is associated with increased risk of developing Merkel cell carcinoma.

Merkel cell carcinoma is an aggressive tumor with a high rate of regional and lymph node metastasis and is the second most common cause of nonmelanoma skin cancer death. The overall recurrence rate is 55–70% with local and regional lymph nodes being the most common sites. The majority of recurrences occur 6–12 months after diagnosis. Prognosis depends on the stage of disease at the time of diagnosis. Small tumors (<2 cm) without lymph node involvement have a 90% 5-year survival rate. Approximately, half of patients with aggressive advanced disease will succumb to disease within 9 months of diagnosis. Pathologic staging is closely associated with disease survival probabilities. Patients with negative sentinel lymph node biopsies have a better survival and a risk of nodal recurrence of only 11%.

Histology

Merkel cell carcinoma can display many growth patterns. Most commonly, Merkel cell carcinoma is a dermal-based tumor usually with a diffuse to nodular (Figure 26.1) or, less commonly, trabecular growth pattern (Figure 26.2). A nested, clustered, or rosette pattern may also be seen. Often, tumors display multiple growth patterns. Epidermal involvement may be seen in up to 10% of cases, with a pagetoid growth pattern rarely reported (Figure 26.3).

Merkel cell carcinoma is composed of small, round to oval monomorphic blue cells with scant cytoplasm and prominent, stippled chromatin imparting a “salt and pepper” appearance to the nuclei (Figure 26.4). Due to the small amount of cytoplasm, the nuclei are closely spaced and molded. The cells are mitotically active with frequent atypical mitotic figures and apoptotic cells.

Immunohistochemistry

Immunohistochemistry is critical in the confirmation of suspected Merkel cell carcinoma. Merkel cell carcinoma displays positive staining with cytokeratin 20 (CK20), epithelial membrane antigen (EMA), neurofilament protein, and neuroendocrine markers synaptophysin, chromogranin, and neuron-specific enolase, indicating both neuroendocrine and epithelial differentiation. CK20 staining classically is in a perinuclear dot-like pattern (Figure 26.5), although diffuse staining is often seen. Immunohistochemistry with “pan-keratin” and neurofilament protein stains also will often display the perinuclear dot pattern similar to CK20. Cytokeratin 7 (CK7) expression is generally absent, but a case series of CK20−/CK7+ cutaneous neuroendocrine carcinoma has been reported. Importantly, Merkel cell carcinoma is negative for S-100 and TTF-1, helping to differentiate it from melanoma and metastatic small cell lung carcinoma, respectively (see Table 26.1).
Electron Microscopy

Historically, due to the difficulty of diagnosing Merkel cell carcinoma, electron microscopy has been used to confirm the diagnosis. Ultrastructurally, the most characteristic finding is the aggregation of intermediate filaments in a perinuclear dot location and dense core neurosecretory granules, usually concentrated in the periphery or in dendrite-like processes. The ultrastructural findings parallel those of normal Merkel cells.

**Table 26.1. Immunohistochemistry in Merkel cell carcinoma.**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>CK20</th>
<th>CK7</th>
<th>NSE</th>
<th>TTF-1</th>
<th>CD45</th>
<th>S-100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merkel cell carcinoma</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Small cell lung carcinoma</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Small cell melanoma</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**Fig. 26.1.** Most Merkel cell carcinomas display a nodular to solid growth pattern.

**Fig. 26.4.** High power showing tumor cells with scant cytoplasm, nuclear molding, prominent, stippled chromatin pattern, atypical mitotic figures, and apoptotic cells.

**Fig. 26.2.** Merkel cell carcinoma displaying a trabecular growth pattern.

**Fig. 26.5.** Cytokeratin 20 showing diffuse staining with accentuation in a perinuclear dot pattern.

**Fig. 26.3.** A small percentage of Merkel cell carcinomas may have epidermal involvement that sometimes has a pagetoid pattern.
With the advent of immunohistochemistry, electron microscopy now plays little role in the diagnosis.

Pathogenesis

Merkel cell carcinoma occurs sporadically and is believed to arise from Merkel cells residing in the epidermis. Little is known about the etiologic mechanism in Merkel cell carcinoma. It has been linked with chronic sun-exposure and is therefore seen most commonly in individuals with advanced age (>60 years), fair-skin, and excessive lifetime sun-exposure. Impaired immune function appears to play a role in the development of Merkel cell carcinoma in some cases; patients with HIV/AIDS, lymphoma, solid-organ transplants and iatrogenic immunosuppression are at increased risk for the development of Merkel cell carcinoma.

Increased incidence in elderly and immunosuppressed individuals suggests a potential role of an infectious agent in the pathogenesis. Recently, Feng et al identified a previously unknown polyomavirus, Merkel cell polyomavirus (MCV), in 80% (8 of 10) Merkel cell carcinoma’s studied, but only 16% (4 of 25) of controls. In some cases, but not all, MCV was found to be integrated in the tumor genome in a clonal pattern. The authors hypothesize that MCV could be involved in tumorigenesis via MCV T-antigen expression and/or insertional mutagenesis of tumor suppressor genes.

Molecular Aspects

Despite numerous studies of oncogenic pathways, little is known about the molecular basis of Merkel cell carcinoma. Ras, p53, B-Raf, MAP kinase, Wnt, c-Kit, PTEN, and bcl-2 have all been evaluated in Merkel cell carcinoma. Kennedy et al and Plattenberg et al found bcl-2 expression in 15 of 20 Merkel cell carcinoma tumors in 2 separate studies. Decreasing bcl-2 expression in vivo in an SCID mouse/human tumor xenograft model resulted in tumor shrinkage. An important protein in inhibiting apoptosis, bcl-2 expression is found in many cancers and suggests a mechanism to avoid cell death. Expression of bcl-2 alone does not provide clues to the proto-oncogenes involved in tumor development.

Loss of heterozygosity tumor suppressor gene PTEN was found in 43% of the tumors examined (9/21), but only one tumor was found to have a concurrent PTEN mutation.

The Wnt-signaling pathway, which regulates various cell cycle processes including cell proliferation, cell fate, and morphogenesis, also seems an unlikely candidate to play an important role in oncogenesis in Merkel cell carcinoma. Twelve cases were studied for alterations in the Wnt-pathway by looking for alteration of expression of β-catenin and for mutations in the genes coding for β-catenin, APC, and AXIN. Only one case of Merkel cell carcinoma was found to have nuclear accumulation of β-catenin and mutations in the Wnt-pathway genes.

Although the MAP kinase pathway has been found to play a role in the development of melanoma and squamous cell carcinoma, it does not seem to be important in Merkel cell carcinoma. Houben et al evaluated 44 Merkel cell carcinoma’s for activating BRAF mutations, Raf kinase inhibitor protein (RKIP), extracellular signal-regulated kinase (ERK), and phosphorylation status of ERK. All tumors were negative for the BRAF mutation. A high expression of Raf kinase inhibitor protein was found in primary and metastatic Merkel cell carcinoma. Forty-two of forty-four had no phosphorylation of ERK indicating that the MAP kinase pathway was silent. The RAS gene, which is also involved in the RAS–RAF–MEK pathway, was examined by Popp et al. In their study of six Merkel cell carcinomas, no activating mutations in NRAS, HRAS, or KRAS genes were found. Similar findings were reported in another series of 21 Merkel cell carcinomas. In sum, these findings suggest that the MAP kinase pathway does not play a role in carcinogenesis in Merkel cell carcinoma. A follow-up study found that activation of MAP kinase pathway induced apoptosis of a single cell line of Merkel cell carcinoma, suggesting a potential therapeutic target.

As in other ultraviolet light-induced neoplasms, such as melanoma and squamous cell carcinoma, mutations in the tumor suppressor gene p53 may be found in Merkel cell carcinoma, though in a minority of cases. In a study of 15 Merkel cell carcinomas, 3 of 15 tumors harbored p53 mutations. Of the p53 mutations found, most were the type that have been associated with UV exposure. In the same study, only one case demonstrated mutations in p73, a gene that encodes for a protein with structural and functional similarities to p53. The relative rarity of p53 and p73 mutations argues against a strong role for these genes in the majority of Merkel cell carcinomas.

Epigenetic studies on cancer have revealed altered gene methylation in many cancers. A study of methylation of p14ARF promoter DNA and p16INK4a promoter DNA and mutations in common oncogenes in 21 Merkel cell carcinomas demonstrated no mutations in Ras, c-Kit, or p14ARF. However, methylation of p14ARF promoter DNA was found in 8 of 19 tumors (42%), suggesting that p14ARF silencing may represent a mechanism for tumorigenesis in some Merkel cell carcinomas. This pathway may provide a potential epigenetic therapeutic target for treatment with demethylating agents.

Merkel cell carcinoma has been shown to express the KIT transmembrane tyrosine kinase receptor by immunohistochemistry. Unlike gastrointestinal stromal tumors, where activating mutations of the c-Kit proto-oncogene are important in tumorigenesis, activating mutations have not been found in Merkel cell carcinoma. These findings suggest that therapeutic interventions that inhibit KIT protein kinase likely will not be effective in treating Merkel cell carcinomas.
Summary

Merkel cell carcinoma is a rare but aggressive cutaneous malignancy with an increasing incidence. Despite extensive study of common cancer-associated pathways and genes, very little is known about the molecular pathogenesis of Merkel cell carcinoma. To date, only bcl-2 and p14ARF methylation have been identified as potentially important factors in tumorigenesis. The identification of the novel Merkel cell polyoma virus suggests a possible infectious etiology and provides a potential focus for future studies.

References

Neuroendocrine Lung Tumors

Sanja Dacic

Introduction

Morphologic and clinical characteristics of neuroendocrine tumors (NE) of the lung represent a wide spectrum of entities. At one end of the spectrum is the clinically and biologically indolent typical carcinoid (TC). At the other end of the spectrum, large cell neuroendocrine carcinoma (LCNEC) and small cell lung carcinoma (SCLC) show aggressive behavior and shorter survival. The clinical behavior of atypical carcinoid (ATC) lies somewhere between these two extremes. NE tumors of the lung may occur in pure or mixed forms with other types of non-small cell lung carcinomas (NSCLC). In contrast to NE tumors occurring in other organ sites, histologic diagnostic criteria for lung neuroendocrine tumors are well defined and correlate with clinical behavior and outcome. Diagnosis of TC, ATC, and SCLC is usually made on morphology alone, while diagnosis of LCNEC requires immunohistochemical confirmation of neuroendocrine differentiation. The majority of NE tumors of the lung occur as sporadic isolated tumors, although rare cases are components of complex familial endocrine, such as multiple endocrine neoplasia type 1 (MEN1). The most important goal of molecular profiling studies of NE tumors of the lung to date has been to identify diagnostic, prognostic, and therapeutic targets for these tumors. This chapter will briefly review the current literature regarding pathways involved in tumorigenesis of these tumors. Recent advances in understanding of potential prognostic and diagnostic markers discovered by RNA- and DNA-based analysis will be discussed.

Sporadic (Nonhereditary) Lung NE Tumors

Background

Clinical

TC and ATC are more frequent in nonsmokers (20–40% of patients). In contrast, almost all patients with SCLC and LCNEC are cigarette smokers. Most of these tumors occur sporadically. TC and ATC may occur in patients with MEN 1, where they tend to be multicentric. TC may present as a central or peripheral mass, whereas AC and LCNEC are more commonly located in the peripheral regions. SCLC usually presents as hilar or perihilar masses and is often accompanied by mediastinal lymphadenopathy; liver or bone marrow metastases are common at the time of primary diagnosis. Paraneoplastic syndromes are also common in patients with SCLC but are rare in patients with LCNEC. LCNEC are similar in their clinical presentation to other types of NSCLC. Carcinoid syndrome, which includes skin flushing, diarrhea, and shortness of breath, is rare in all of these pulmonary NE tumors and usually only occurs with widespread metastatic disease. The 5-year survival is 90–98% for TC, 61–73% for ATC, and 13–57% for LCNEC. Survival rates for patients with SCLC remain dismal, despite aggressive therapy.

Histology

All NE tumors of the lung show variations on the typical features of neuroendocrine morphology, including organoid, nested, or trabecular growth patterns, cellular palisading, and rosette-like structures. The main distinguishing features are the mitotic count and the presence or absence of necrosis. TC is defined as a NE tumor with no more than 2 mitoses per 10 HPF and absence of necrosis. Mitotic count between 2 and 10 per 10 HPF and/or necrosis are needed for a diagnosis of ATC. Mitoses are readily identified in LCNEC and SCLC with 11 or more mitoses per 10 HPF. These tumors also have more extensive necrosis than ATC. LCNEC shows larger cell size, abundant cytoplasm, prominent nucleoli, vesicular or coarse chromatin, and less prominent nuclear molding. These features, which are much more similar to other NSCLCs, are in contrast to the cytologic features SCLC.

Immunohistochemistry

Markers of neuroendocrine differentiation such as synaptophysin, chromogranin, and CD56 (N-CAM) are often positive in NE tumors of the lung, and are particularly prominent in TC.
for these markers in ATC may be focal. More than 90% of SCLC are also positive for all neuroendocrine markers. Expression of these markers is requisite for diagnosis of LCNEC but not for the diagnosis of the carcinoid tumors or SCLC. Expression of TTF-1 depends on the tumor type. Up to 90% of SCLC is positive for TTF-1.6,10 Conflicting results have been published, however, for the staining of TTF-1 in TC and ATC.6,8,11,12 Several reports have shown TTF-1 to be absent in central carcinoids, as compared to peripheral carcinoids that are frequently positive. About 50% of LCNEC will express TTF-1.12,13 Cytokeratins are variably expressed, with more than 80% of carcinoids being positive; SCLC will usually show very focal cytokeratin positivity.

**Important Pathways**

Neuroendocrine carcinomas share genetic alterations in three common pathways with other types of NSCLC. These include the p53, retinoblastoma, and Fas pathways.

Genetic alterations of the p53 gene are the most common genetic abnormality identified in high-grade NE lung tumors. These alterations are extremely rare in TC.15,16 Chromosome 17p13, which includes the p53 locus, shows hemizygous deletion in 90% of SCLC (loss of heterozygosity). Mutational inactivation of the remaining allele occurs in 75% of SCLC, which is in contrast to ATC, which shows only a 10% mutation rate.17 p53 mutations in lung tumors are considered to be a direct result of mutational damage from cigarette smoking. Smoking-related p53 mutations consist mostly of G:T transversions, which are the most frequently identified mutations in SCLC. The much less common p53 mutations in ATC are usually nonsense mutations and not the G:T mutations of smoking. It has been suggested that p53 alterations correlate with poor survival.18

Downstream of the p53 pathway, upregulation of Bcl-2 (antiapoptotic), and downregulation of Bax (proapoptotic) are also very common events in SCLC and LCNEC. Carcinoids have a low level of Bcl-2 and a high level of Bax. These results are expected, since most of the higher grade tumors will also harbor alterations in p53.15

The second most common genetic alteration in lung NE tumors is inactivation of the pathway controlling the retinoblastoma gene (RB). RB, a tumor suppressor gene located on chromosome 13q11, encodes for the RB protein, which functions as a gatekeeper for the G1 to S transition of cell cycle. The pathway includes another tumor suppressor gene, CDKN2a/p16 (located on 9p21), and CCND1, which encodes cyclin D1. Alterations in the pathway can be seen in any of these three targets, with loss of heterozygosity or promoter methylation of CDKN2a or overexpression of cyclin D1. There is inverse correlation between loss of Rb protein, p16 inactivation, and cyclin D1 overexpression.

At the DNA level, inactivation of both RB alleles is common in high-grade NE tumors (68% LCNEC and 78% SCLC), and protein abnormalities are reported in more 90% of tumors. Defects in the RB pathway are less common in the lower grade tumors, however, with TC retaining Rb expression and only 20% of ATC showing loss. Despite the fact that the RB pathway is affected in both SCLC and NSCLC, mechanisms of alteration of this pathway are different between the two tumor categories. Loss of Rb protein expression can be detected in 80–100% of high-grade NE tumors while retaining normal p16 and cyclin D1 expression.19,20 A mutual exclusion between Rb loss and p16/cyclin D1 alterations is observed in low-grade NE tumors.

Fas (CD95) is a p53 transcriptional target gene, and downregulation of Fas represents the third most common abnormality in NE lung tumors. Low FAS expression is seen in 80% of carcinoids, while FasL is expressed in 40% of the cases. SCLC and LCNEC show a strong downregulation of Fas with a very low to negative Fas expression.21 Caspase-8 located on chromosome 2q33, which is a downstream effector of the Fas/FasL pathway, is frequently lost and occasionally methylated in SCLC. About 18% of carcinoids show caspase-8 methylation.

**Genetics**

**Oncogenes**

The MYC family of genes, including C-MYC, N-MYC, and L-MYC, is frequently altered in SCLC. Only one of these genes is generally activated in any individual tumor. Activation of MYC gene may occur through gene amplification or through transcriptional dysregulation.22 Both of these mechanisms result in protein overexpression. Activation of C-MYC proto-oncogene is seen in both SCLC and NSCLC at fairly high rates,23 but amplification of N-MYCN and L-MYC also occur in SCLC.24–26 Amplification of C-MYC appears to have some correlation with tumor grade and survival. It is absent in TC but is seen in up to 17% of ATC, 23% of LCNEC, and up to 70% of SCLC.27

**Tumor Suppressor Genes**

LOH at chromosome 3p is the most common change in NE of the lung. It occurs in 40% TC, 73% ATC, and in over 80% of LCNEC and SCLC (Table 27.1).18 Putative tumor suppressor genes have been identified at four widely separated regions including 3p12–13 (ROBO1/DUTT1), 3p14,2 (FHIT), 3p21,3 (multiple genes including RASSF1A, FUS1, HYAL2, BAP1, Sema3B, Sema 3F, and beta catenin), and 3p24–6 (VHL and RAR-beta). The FHIT gene has been studied in detail and LOH was observed in 23%TC, 35–50% of ATC, 90% LCNEC, and 67% of SCLC.18,28 However, no somatic point mutations of FHIT gene have been discovered in these tumors.

Another relatively common finding in NE carcinomas is LOH at multiple areas on 5q (46% of LCNEC and 86% of SCLC).29–32 Again, this genetic alteration is also thought to be associated with a poor survival. LOH at 5q21 is not seen in TC and is only present in approximately 25% of ATC.18,33
Epigenetics

DNA methylation appears to be a relatively infrequent event in NE tumors of the lung, particularly when compared to other types of NSCLC. The most frequently methylated gene is RASSF1A (Ras-associated domain family 1A), which is located in a small 120-kb region of minimal homozygous deletion in 3p21.3. Methylation of RASSF1A correlates with loss of gene expression. The gene is methylated in 80% of SCLC, 71% of ATC, and 45% of TC (Table 27.2). Methylation of RAR-beta gene promoter located (chromosome 3p24 region) is also relatively common in NE lung tumors (72% of SCLC). Methylation of this gene is one mechanism of silencing RAR-beta2 and RAR-beta4 isoforms expression. It is known that retinoids, which play an important role in lung development, have chemopreventive effects and therefore aberrant methylation in SCLC may be of potential future diagnostic and therapeutic significance.

Gene Expression Profiling

A limited number of gene expression studies of lung NE tumors have been published. Most of the studies have been able to reproduce histologic classification of NE lung tumors at the gene expression level. Furthermore, two potential novel biomarkers of lung NE tumors with prognostic and diagnostic significance have been discovered through this molecular approach: carboxypeptidase E (CPE) and gamma-glutamyl hydrolase (GGH). CPE, located on chromosome 4p33, is a secreted neuropeptide-processing enzyme, which is present in membrane-bound and soluble forms in normal tissues. GGH, located on chromosome 8q12.1, is a lysosomal enzyme that catalyzes the hydrolysis of polyglutamyl-gamma-glutamates and antifolypoly-gamma-glutamates by the removal of gamma-linked polyglutamates and glutamate. By immunohistochemistry, positive CPE and negative GGH were most frequently observed in TC and ATC, and are associated with a good prognosis. In contrast, the negative CPE and positive GGH are a poor prognostic immunophenotype, and are observed in LCNEC and SCLC. Interestingly, GGH has been implicated in methotrexate resistance in sarcomas and leukemias, indicating a potential use of this marker for prediction of therapeutic response in NE lung tumors.

Proteomics

Only one proteomics study comparing different histologic groups of NE lung tumors has been published. This analysis showed that protein expression profiles of TC are similar to normal lung tissue. There was no clear difference between AC and LCNEC, but SCLC formed a distinct group. MALDI analysis detected 9 surrogate endpoint biomarkers with cytokeratin 18 and p63 being most useful as a potential diagnostic and prognostic markers.

Other Techniques

Comparative Genomic Hybridization

CGH studies have been mostly restricted to high-grade NE lung carcinomas but have demonstrated multiple chromosomal alterations shared between SCLC and LCNC. Some potential distinguishing alterations have been suggested, including gains at 2q, 3q, 5p, 5q, 8q, and deletions at 1p, 3p, 4q, 4p, 9p, 19p, 13q, and 20q. As expected, the most common alterations in SCLC are deletions of 3p chromosome, with VHL (3p25) and FHIT (3p14.2) genes as the most likely candidates. Losses at 22q and gains in 17q24–25, which have also been suggested to be a potential marker for brain metastases in SCLC, were also reported.
CGH studies have also identified that allelic deletions of different loci at 11q chromosome are common chromosomal alteration in TC and AC. Almost half of the TC and AC cases studied showed underrepresentation of 11q13. Further work on these tumors has determined that deletions of 11q13, which is the location of the MEN1 gene locus, are confirmed by the presence of LOH in TC and AC. These deletions of MEN1 gene are almost entirely absent in a high grade NE carcinomas.

Summary

A morphologic spectrum of NE tumors of the lung is well defined. These tumors that can exist in pure or mixed forms with other types of lung carcinomas. The diagnosis of these tumors is largely based on morphology alone, with a small role for immunohistochemistry in cases of LCNEC. Molecular studies have demonstrated many shared abnormalities between the NE tumors and other NSCLC. These shared abnormalities include smoking-related genetic changes, such as p53 alterations. Extensive studies of 3p, 5q 11q, and 17p have shown the importance of multiple tumor suppressor gene pathways and have even documented potential prognostic significance. However, none of these markers have current clinical applications. Currently, many of the high-grade tumors in the spectrum of NE lung tumors are diagnosed at an advanced stage and have a very poor prognosis. In the future, the hope is that a better molecular characterization of these tumors will reveal new therapeutic targets and eventually lead to improved survival.

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