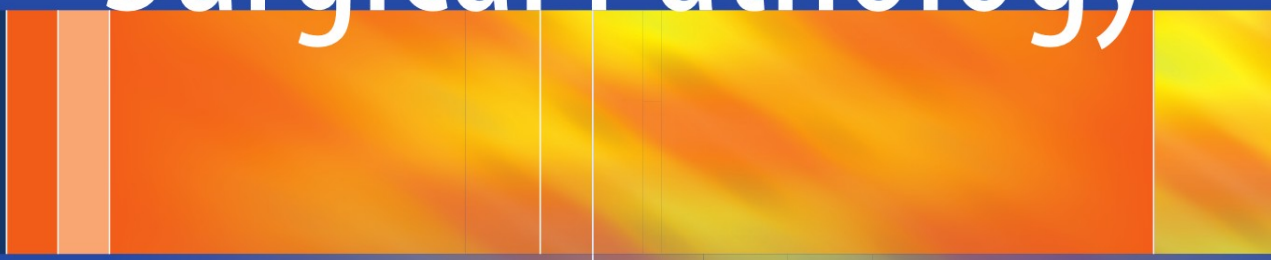
Liang Cheng
John N. Eble *Editors*

Molecular Surgical Pathology



Springer

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ISBN 978-1-4614-4899-0 ISBN 978-1-4614-4900-3 (eBook)
DOI 10.1007/978-1-4614-4900-3
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2012951054

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface to Molecular Surgical Pathology, First Edition (Springer, NY)

Molecular pathology is a driving force towards personalized medicine and is already an integral part of current surgical pathology practice. High expectations of excellence in surgical pathology exist in this era of genomic medicine as specific genetic alterations become amenable to tailored personalized therapy. The role of the surgical pathologist involves more than just providing an accurate diagnosis, but also involves developing strategies for effective genomic characterization and integrating various ancillary test results necessary for disease prognosis and clinical management. Fundamental knowledge and understanding of molecular pathogenesis and pathways of human diseases, as well as molecular diagnostic tools, are essential for the daily practice of surgical pathology.

The goal of this new textbook is to provide a concise review of recent advances in molecular pathology for each organ system. This book is not intended to replace comprehensive sources of existing pathology textbooks that have heavily emphasized morphologic criteria. This is intended to be a "first knowledge base" in a rapidly evolving field, organized in a user friendly outline format.

The specialty of surgical pathology has grown enormously as a result of recent breakthroughs in molecular pathology such as testing for predictive cancer biomarkers, decoding of cancer molecular profiles, and next generation sequencing for entire genomes. In this volume, we will discuss how the advent of genomic medicine, disruptive technological innovations, and targeted cancer therapies have impacted our understanding of the disease process, created paradigm shifts in cancer treatment, and revolutionized our practice of surgical pathology in an unprecedented manner. The text is authored by leading international experts. Each chapter is organ-based and covers important aspects of molecular pathology with emphasis on their impact to daily surgical pathology practice. The topics presented herein constitute fundamental and core basic knowledge required for surgical pathologists.

The book consists of 17 chapters covering the molecular pathology of tumors from major organ systems, including colorectal cancer, pancreatic cancer, liver tumors, gallbladder cancer, lung cancer, breast cancer, ovarian cancer, kidney tumors, prostate cancer, urinary bladder cancer, testicular cancer, cutaneous melanoma and nonmelanoma skin cancer, head and neck cancer, soft tissue and bone tumors, brain tumors, and endocrine cancer.

A detailed table of contents is provided at the beginning of each chapter for quick reference. This book focuses on the practical utility of molecular techniques and on molecular biomarkers for the practicing surgical pathologist. The emphasis is on the impact of molecular pathology for tumor classification, diagnosis and differential diagnosis, as well as its implications for patient management and personalized care. Numerous tables, diagrams, and color illustrations are included. Given space limitations, basic molecular biology techniques and principles of molecular genetics are not covered in this text. Readers are encouraged to consult other textbooks focusing on basic concepts and principles of molecular biology. Selected references are provided in the Suggested Reading section for each chapter.

Molecular Surgical Pathology is designed for use by pathologists in training and by surgical pathologists in their daily practice. There is increasing emphasis on knowledge of molecular pathology by the American Board of Pathology. This book will serve as a practical, readily accessible source of information for pathologists who are preparing for Board and in-service examinations. It is our hope that this book will also be a unique and invaluable resource for medical oncologists, treating physicians, other medical professionals, and basic research scientists who have an interest in the molecular pathology of human cancer.

We are greatly indebted to all individuals who have been involved in the preparation of this book. Our profound gratitude goes to the contributing authors who have shared their knowledge and experience with our readers and with us. We express our appreciation to Ms. Tracey Bender for her outstanding and ebullient editorial assistance. We also thank the dedicated and talented staff at Springer, especially Richard Hruska, Maureen Alexander, Michael Doblados, Joseph Quatela, and supportive colleagues who have given invaluable support throughout the development and production of this book.

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Molecular Pathology of Colorectal Cancer

1

Fred T. Bosman

Contents

Introduction

- Colorectal cancer is one of the most intensively studied cancer types, partly because of its high prevalence but also because of the existence of precursor lesions, known as adenomas, which can be detected endoscopically and removed. Theoretically, removal of these adenomas would prevent most cases of colorectal cancer from developing
- Characteristic morphological steps in the evolution of these precursor lesions have been elucidated at a molecular level and the adenoma–carcinoma sequence, as this has become known, is one of the classical examples of stepwise progression of cancer. Gaining this knowledge has been facilitated by the occurrence of a variety of forms of familial intestinal cancer, the molecular genetic background of which has been largely clarified
- Apart from early detection of familial forms of colorectal cancer and its use in genetic counseling, until recently this knowledge has had little impact on the clinical management of colorectal cancer. Classical clinicopathological parameters remain the essential parameters determining how a colorectal patient will be treated. This has dramatically changed in the last five years. With drugs specifically targeting the EGF receptor having been shown effective in colorectal cancer, mechanisms responsible for resistance have been explored. The finding that *KRAS*-mutated cancers do not respond to anti-EGFR treatment has had a profound impact on clinical management and on molecular diagnostics of colorectal cancer
- With new targeted drugs in the pipeline it is highly likely that companion diagnostics will

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L. Cheng and J.N. Eble (eds.), *Molecular Surgical Pathology*,
DOI 10.1007/978-1-4614-4900-3_1,

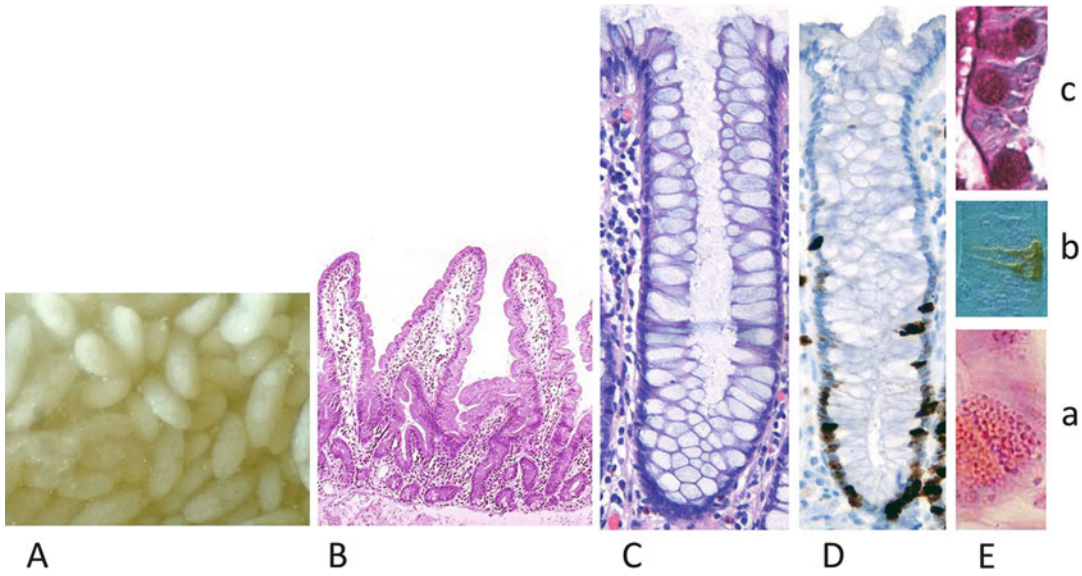

Fig. 1.1 Architecture and cell types of intestinal mucosa. (**A**) Atereomicroscopical image of the normal intestinal mucosa. Note the villous surface; (**B**) microscopical image of the small intestinal mucosa. The villus and crypt compartments are clearly discernible; (**C**) mucosal crypt of the colon, (**D**) stained for proliferating cells (Ki67). The proliferating compartment is in the bottom of the crypts. (**E**) cell types occurring in the normal intestine: in the bottom of the crypt Paneth cells with striking eosinophil granules (*a*), mostly in the lower half of the crypt neuroendocrine cells (*b*). Stained with an antichromogranin antibody and towards the upper half of the crypt goblet cells and enterocytes (*c*). Stained with PAS stain which stains the mucin vacuole in the goblet cells and the brush border of the enterocytes *red*

go through a period of explosive development. In this chapter we will discuss the biology of intestinal mucosa and its disturbance in cancer

- We will review cancer family syndromes and the impact of understanding these on a molecular level has had on our understanding of colorectal cancer. Finally we will review how molecular diagnostics now contribute to the clinical management of colorectal cancer and what can be expected in this field in the near future

The Biology of Intestinal Mucosa

Overview of Renewal and Differentiation of Intestinal Mucosa

- Intestinal mucosa is a highly dynamic tissue, of which the entire epithelial lining is renewed every 3–4 days. The basic architecture of the mucosal epithelium consists of crypts, tube-like pits lined by epithelial cells. The surface of the large bowel is flat, that of the small bowel beset with villi, which increases the resorptive surface (Fig. 1.1)
- Crypt epithelium is in principle a monoclonal cell population derived from a single crypt base stem cell. The stem cell division is asymmetrical: one of the daughter cells remains a stem cell; the other develops into a proliferative cell type that expands the epithelial cell population in a proliferation zone just above the bottom of the crypt. The crypt stem cell gives rise to distinctly different mature epithelial cells:
  - Enterocytes
    - Have a predominantly resorptive function
    - Are characterized by the presence of a brush border at the luminal surface of the cell which increases its resorptive surface
    - Specifically express villin, a brush-border-associated molecule
  - Goblet cells
    - Are responsible for the production of mucins, water binding proteoglycans resulting in a viscous layer of mucus on

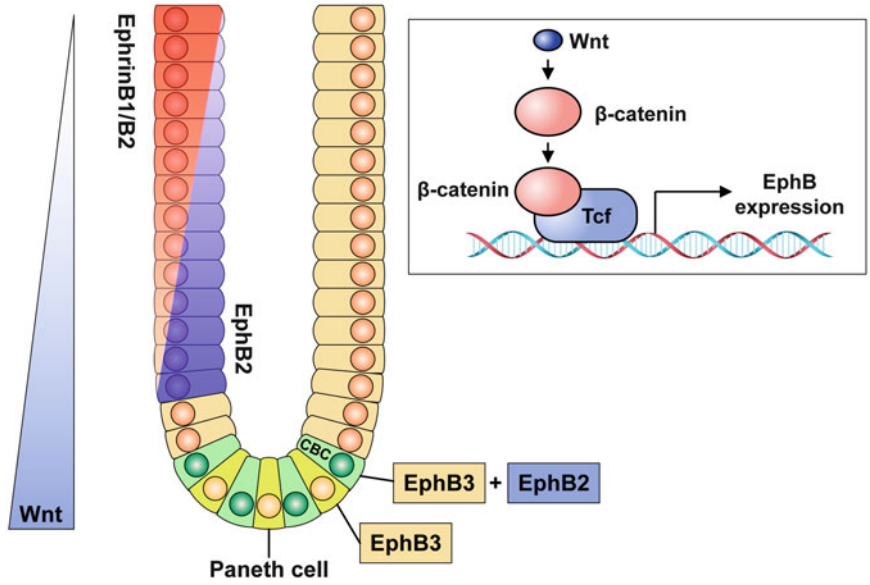

Fig. 1.2 Role of the Wnt pathway in the maintenance of proliferation, differentiation, and migration of intestinal mucosa. Interaction between Wnt-induced Ephrin B receptors and EphB ligands direct cellular localization and migratory behavior within the crypt. A gradient of Ephrin B1/B2 ligands exists within the crypt with cells at the crypt–villus junction expressing high levels of these molecules. An opposing gradient of EphB2 expression exists beginning at the crypt base. Thus the level of Ephrin B ligand and Wnt-induced EphB expression determines cell location. Paneth cells express EphB3 and no Ephrin B ligands, which restricts the location of these cells to the crypt base. (adapted from Scoville Current view: intestinal stem cells and signaling. Gastroenterology 2008;134:849–864)

the mucosal surface. Characteristic for intestinal goblet cells are MUC1, MUC2, and MUC6

- Endocrine cells
  - Secrete peptide hormones and bioactive amines (such as serotonine)
  - Express endocrine specific markers including proteins associated with neuroendocrine granules (chromogranin A) or presynaptic vesicles (synaptophysin)
- Paneth cells
  - Reside in the crypt basis
  - Are involved in innate immunity of the gut
  - Are characterized by cytoplasmic granules which contain lysozyme and defensins, proteins involved in the innate defense against bacteria, which can be used to immunohistochemically stain them
  - Elaborate peptide hormones, which exactly depending on the cell type and the specific localization of the cell in the gut

- The crypt stem cell is morphologically indistinct. Recently, markers have been identified which can be used to identify crypt stem cells
  - Lgr5, an intestinal specific transcription factor, appears to be a reliable crypt stem cell marker
- It is likely that the Lgr5 expressing cell represents the stem cell responsible for regular renewal of intestinal and gastric epithelium. In case of increased need for epithelial cell renewal, such as in inflammatory conditions, additional stem cells are recruited. These facultative stem cells are less numerous and as yet ill characterized

Molecules Involved in Growth and Differentiation of (Transformed) Intestinal Epithelial Cells

- Two signaling pathways play an important role in the differentiation of (normal or transformed) intestinal epithelial cells. Wnt signaling is of crucial importance in maintaining normal mucosal architecture and differentiation and it is no surprise that disturbance of Wnt signaling is a key element in colorectal carcinogenesis. How Wnt maintains mucosal architecture is schematically illustrated in Fig. 1.2
- Wnt signaling

  - Wnt signaling is active in the crypt stem cell
  - Active Wnt signaling leads to an expansion of the populations of undifferentiated and Paneth cells and a complete loss of goblet and enterocyte progenitors
  - Silencing of the Wnt pathway occurs with migration of the proliferative cell upward in the crypt, the phenotype of the cell switching from a proliferative to a specific functional state
  - Absence of Wnt signaling induces a complete loss of undifferentiated and Paneth cells
  - An *APC* gene mutation in the Wnt pathway is responsible for familial adenomatous polyposis (FAP)
  - In about 90% of colorectal carcinomas the Wnt pathway is abnormally active, either through a mutation in *APC* or to a mutation in the β-catenin gene
  - β-catenin purportedly accumulates in the nucleus of clonogenic cells in the invasion front of the tumor, from where tumor metastases arise
- Notch signaling
  - Stimulates the proliferation of crypt stem cells
  - Stimulates differentiation of the absorptive lineage
  - Appears to be constitutively activated in CRC but the mechanisms involved are insufficiently explored

Clinical and Molecular Features of Colorectal Cancer

General Principles of the Molecular Biology of Colorectal Cancer

- Colorectal cancer is one of the cancer types following the principles of stepwise progression, which is illustrated in Fig. 1.3
  - Initially a benign precursor lesion (adenoma)
  - Progression to an invasive lesion (adenocarcinoma)
  - With finally metastatic potential (metastatic adenocarcinoma)
- In colorectal cancer, this arises
  - In an intestinal clonogenic precursor cell (crypt base stem cell)
  - Through the accumulation of a combination of abnormalities in the genome allowing uncontrolled proliferation, incapacity for apoptosis and ultimately invasive and metastatic propensity
- Early key roles are played by
  - An inactivating mutation in the *APC* gene with subsequent silencing (epigenetic or through allelic loss) of the second allele
  - Activating mutations in the *KRAS* gene
  - Mutations in the *TP53* gene and in the TGFβ pathway, which are important in progression towards malignancy

Colorectal Cancer Develops in Several Contexts

- The term CRC suggests that we are dealing with a single disease. In reality CRC consists of many different conditions, which differ in genetic background, associated conditions, molecular profile, clinical behavior, and response to therapy

Sporadic Colorectal Cancer

- This is the prototypical cancer type in the colorectum. Histologically, these are adenocarcinomas which as a rule develop from a benign precursor, the adenomatous polyp, whatever the context of the disease may be. Exception to the latter rule are the CRCs developing in the context of inflammatory bowel disease, which frequently develop in areas of flat dysplasia
- Clinical
  - Constitute the second most frequent cancer worldwide
  - Have a typical age of onset in the seventh decade, the prevalence increasing with age
  - Are most frequent in the left (sigmoid and rectum) and right colon
  - Are lethal in about 50% of the cases
  - Are prognostically largely dependent on the extent of spread (stage)
    - Depth of invasion into the bowel wall

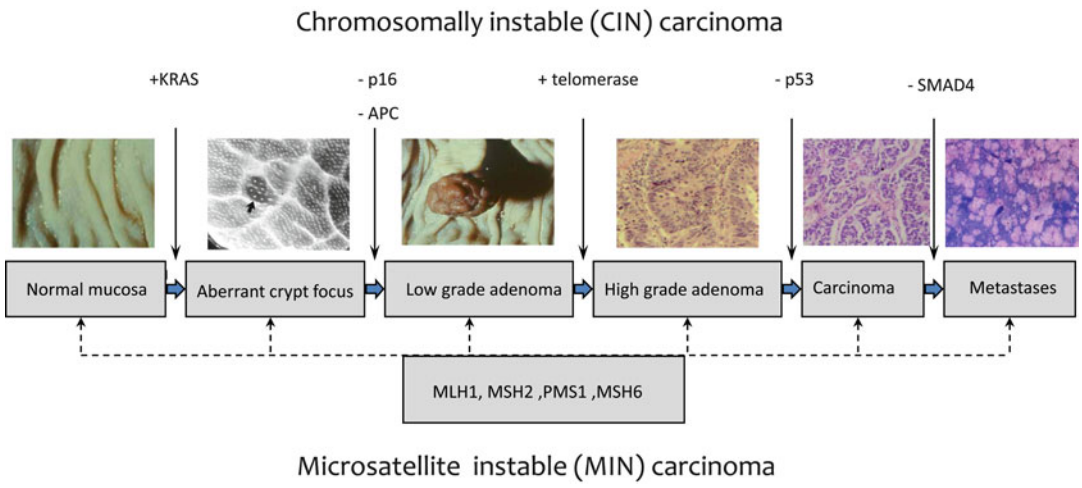

Fig. 1.3 The two major pathways in the development of colorectal cancer (after Vogelstein). The adenoma–carcinoma sequence starts with disturbance in the crypt architecture, endoscopically visible as ACF. Activating (+) *KRAS* mutations may occur already in these lesions. Some of these may show features of epithelial dysplasia; these might go on to develop into adenomas. If this is the case, often inactivating (−) *APC* mutations are found. Low grade adenomas are small, mostly tubular, and have limited cytonuclear and architectural features of dysplasia. Often telomerase is not activated at this stage. Once telomerase is activated (+) the lesions often show high grade features: large, villous architecture, and cytonuclear features of high grade dysplasia. Progression towards invasive carcinoma is often accompanied by a TP53 mutation. Progression towards metastasis is accompanied by further molecular events, such as inactivation of SMAD4. This main sequence of events is characteristic of both the chromosomal instability (CIN) and the MIN pathways. In the CIN pathway mismatch repair remains intact but CIN leads to accumulation of CIN with a high level of aneuploidy. In the MIN pathway often early on (due to a germline mutation in one of the mismatch repair genes or to MLH1 promoter methylation) microsatellite instability (MSI) occurs with accumulation of gene mutations but far fewer chromosomal aberrations

    - Presence or absence of lymph node metastases
  - Can be detected by the fecal occult blood (FOB) test (reasonable sensitivity, low specificity) or colonoscopy; colonoscopic polypectomy in principle would allow eradication of the disease
  - Are treated typically by surgery (hemicolectomy), (adjuvant) chemotherapy depending on the disease stage; rectal cancer is often treated with a neoadjuvant chemo/radiotherapy protocol, followed by surgery
  - Are recently amenable to targeted therapies, targeting the EGFR
- Molecular pathology
  - CRCs are characterized by mutations in the Wnt pathway genes *APC* (70%) or *CTNNB* (75%), pointing towards the Wnt pathway as a crucial element in the molecular carcinogenesis
  - *KRAS* mutations usually in codons 12 and 13 (45%)
  - *BRAF* mutations (5%)
  - *TP53* mutations (70%), in frequency going up along the progression from adenoma to carcinoma
  - Telomerase activation (70%)
  - SMAD4 loss
  - Frequent allelic imbalance (85%) constituting the chromosomal instability type (CIN)
  - Microsatellite instability (MSI) (15%) due to promoter methylation of the *MLH1* gene (MIN)

Sporadic Colorectal Cancer in the Context of Inflammatory Bowel Disease

- Crohn disease (CD) and ulcerative colitis (UC), generally known under the generic term IBD, are relatively frequent conditions

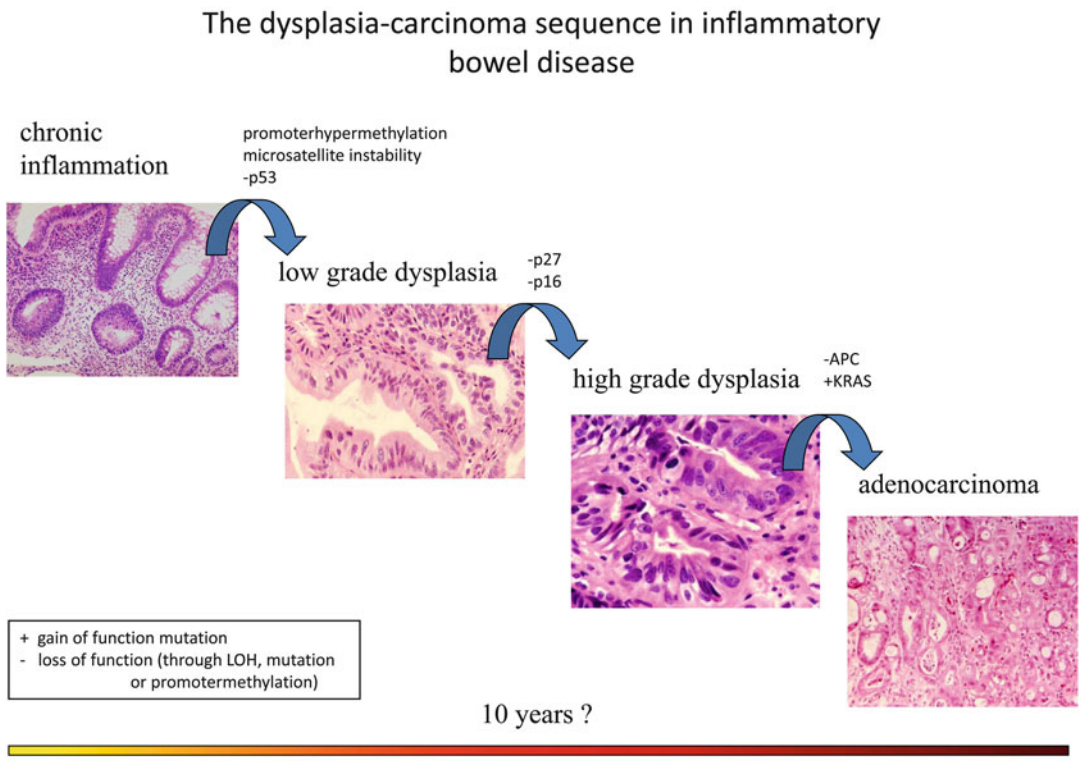

Fig. 1.4 The dysplasia–carcinoma sequence in colorectal carcinogenesis in inflammatory bowel disease. Chronic inflammation with the continuous liberation of oxygen radicals and inflammatory cytokines creates an environment that is both mutagenic and growth stimulating. *TP53* mutations occur early in this pathway, associated with the development of low grade dysplasia. In addition, hypermethylation of CpG islands leads to promoter silencing, among others of p16 and p27. Accumulation of such events drives progression towards high grade dysplasia. Additional events, such as *KRAS* mutations and activation of the Wnt pathway, for example through *APC* mutation, are involved in the progression towards invasive carcinoma

  - CD can affect any part of the gastrointestinal tract and most commonly affects the terminal ileum
  - CD is characterized by granulomas and transmural inflammation
  - UC is restricted to the colon and/or rectum
  - UC-related inflammation is restricted to the mucosa and submucosa
- Chronic inflammatory damage to the colon and rectum in both types of IBD incur an increased risk of CRC, which is more pronounced with
  - Longer duration of disease
  - Greater extent and severity of colitis
- In the connection between inflammation and carcinogenesis, the transcription factor NF-κB is a key mediator, inducing expression of the proinflammatory mediators, COX2 and TNFα, of which a role in colorectal carcinogenesis has also been proven
- Genome abnormalities in CRC in the context of IBD are similar to those in sporadic CRC
  - *TP53* mutations often occur early
  - *APC* mutations are less frequent
- At variance with sporadic CRC, in IBD carcinomas develop through a dysplasia–carcinoma sequence, which is illustrated in Fig. 1.4. Endoscopic surveillance allows early detection of dysplasia, which justifies colectomy, to prevent the development of invasive cancer. Sensitive and specific molecular tests for dysplasia detection have not been identified

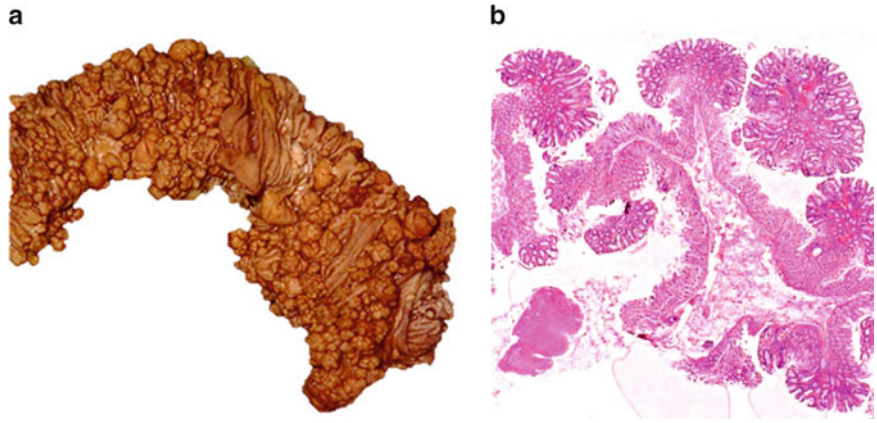

Fig. 1.5 (**a**) Colon mucosa of a familial adenomatosis polyposis (FAP) patient who underwent prophylactic total colectomy at the age of 21. (**b**) H&E section, note the mucosa extensively colonized with adenomatous polyps

Familial Clusters of Colorectal Cancer

- Familial clusters of CRC account for almost 20% of CRCs but less than half of those fall within the defined familial CRC syndromes. A family history of colorectal cancer (i.e., one or more relatives with CRC) confers a two to sixfold increased CRC risk, which increases with
  - The number of affected family members
  - Age at diagnosis of affected relatives
- These point to the existence of other less penetrant genes and/or gene environment interactions explaining these familial CRC aggregates. For this phenomenon the term familial colorectal cancer is used. Approaches to identify loci that might contribute to familial CRC, include
  - Family linkage
  - Affected relative pair studies
  - Genome-wide association studies
- Although a variety of chromosomal loci and more recently SNPs have been identified associated with increased CRC risk, the mechanisms responsible for these familial clusters remain ill understood

Familial Colorectal Cancer Syndromes

- Around 10% of all colorectal cancers arise in the context of a familial cancer syndrome

The Familial Adenomatous Polyposis Syndrome

- FAP syndrome accounts for about 1% of all CRCs
- Is characterized by the development early in life of numerous (hundreds to thousands) of adenomatous polyps in the colon (Fig. 1.5)
- Also develops mucosal growths in the higher tubal gut, notably in the stomach
- Genetics
  - Caused by mutations in the *APC* gene; depending on the genotype the phenotype varies in terms of number of polyps and age of onset of polyps and of carcinomas, as well as the occurrence of neoplasms in other sites
  - Mutations between codons 1250 and 1464 are associated with severe polyposis (>1,000 adenomas)
  - Mutations before codon 157, after codon 1595, and in the alternatively spliced region of exon 9 are associated with attenuated adenomatous polyposis coli (AFAP, usually 10–100 adenomas, 70% lifetime risk of CRC); in addition association with duodenal/periampullary adenomas and thyroid carcinomas
  - Mutations in the remainder of the APC gene cause an intermediate phenotype (hundreds to thousands of adenomas)

  - Mutations between codons 311 and 1444 and after codon 1444 are associated with congenital hypertrophy of the retinal pigment epithelium and desmoid tumors respectively
- Molecular mechanisms
  - Cause of the disease is a germ line mutation in the *APC* gene
  - For a carcinoma to develop, both copies of the gene have to be inactivated through loss of the other allele due to a chromosomal abnormality or hypermethylation of the gene promoter
  - APC negatively regulates β-catenin, a component of the E-cadherin-catenin cell adhesion complex but also a component of the Wnt signaling pathway
  - In a normal cell the APC protein binds to β-catenin which leads to its degradation in the proteasome (Fig. 1.6)
  - Loss of function of APC leads to accumulation and translocation of β-catenin to the nucleus where it acts as a transcription factor, promoting transcription of gene products favoring proliferation, such as MYC and cyclin D1
  - This gives rise to further mutations and activation of telomerase, conferring unlimited life span to the transformed cell
  - Neoplastic progression due to the continued accumulation of additional mutations affects TGFβ signaling, including the downstream signaling molecules SMAD2 and SMAD4
  - *TP53* is mutated in 70–80% of colon cancers, commonly at late stages of tumor progression
  - Loss of function of other tumor suppressor genes is often caused by chromosomal deletions, due to CIN, a hallmark of the APC/β-catenin pathway

Gardner Syndrome

- A variant of FAP characterized by numerous gastrointestinal polyps and
  - Osteomas
  - Dental anomalies
  - Desmoid tumors
  - Epidermoid cysts
- Mutations in *APC* causing Gardner syndrome are clustered in a region encoding a series of amino acid repeats responsible for the binding to β-catenin

The Deficient Mismatch Repair Syndrome

- Mismatch repair syndrome (MMR) is better known as hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome
- The most common hereditary CRC predisposition syndrome
- Responsible for around 3% of all CRCs
- Carries a lifetime risk of developing CRC for carriers of a MMR gene mutation of about 80%
- Develops CRC's through the adenoma–carcinoma sequence
- Not associated with polyposis
- Develops colon cancers in the right colon, often mucinous or medullary type, with lymphoid aggregates around the fields of tumor cells but also diffuse tumor infiltrating lymphocytes, the latter often associated with the medullary type
- Has a better prognosis than the MMR competent cancers
- MMR deficient cancers respond less well to adjuvant chemotherapy
- In Lynch syndrome families not only CRC prevalence is increased but also other types of cancer occur, including:
  - Endometrium
  - Stomach
  - Pancreas
  - Ovary
  - Biliary tree
  - Urinary tract
  - Small bowel
  - Brain
- Genetics
  - Autosomal dominant
  - Caused by mutations in one of the DNA mismatch repair (MMR) genes
    - *MLH1* (mutL homolog 1) on chromosome 3p21
    - *MSH2* (mutS homolog 2) on chromosome 2p16

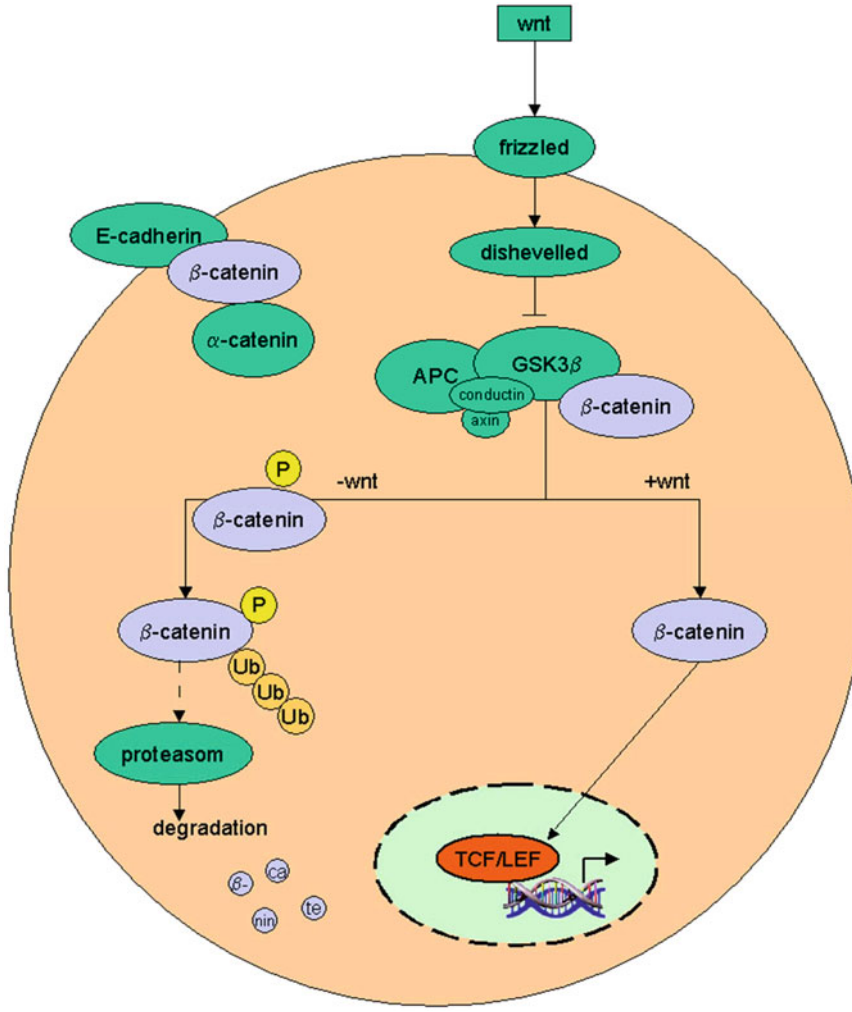

Fig. 1.6 Schematic of the role of activation of the Wnt pathway in colorectal cancer. In a normal cell (left arm) Wnt is off: β-catenin is part of the cell membrane-associated E-cadherin-catenin complex and cytoplasmic β-catenin is captured in the destruction complex of which APC is a component. When Wnt is on (right arm), normally through interaction of Wnt ligand with the Wnt receptor (a G-protein coupled receptor of the Frizzled family) β-catenin is released from the destruction complex, migrates to the nucleus and associates with other proteins (such as LEF1 and TCF4) to form a transcription complex which leads to the expression of proteins favoring cell proliferation. In case of an APC mutation, APC is no longer functional in the destruction complex. As a result β-catenin accumulates in the cytoplasm, migrates to the nucleus, and functions as an uncontrolled driver of cell proliferation

  - *MSH6* (mutS homolog 6) on chromosome 2p16
  - *PMS2* (postmeiotic segregation 2) on chromosome 7p22
- These genes encode for MMR proteins that correct base mismatches or small insertions or deletions occurring during DNA replication
- Recurrent or founder mutations have been identified in *MLH1* and *MSH2*
  - A genomic deletion of exon 16 of MLH1 (*MSH2* 1906G>C), a founder mutation in Ashkenazi Jews, likely dates back more than 1,000 years and is responsible for more than half of all Lynch syndrome cases in Finland

    - A genomic deletion of exons 1–6 of *MSH2* and the recurrent A → T transversion in the donor splice site of intron 5 of *MSH2* (c.942 + 3A → T) occur worldwide and are involved in 5–10% of all cases of Lynch syndrome
    - Constitutional 3′ end deletions of the *TACSTD1* gene (encoding for epithelial cell adhesion molecule of EpCAM) can cause Lynch syndrome through epigenetic silencing of *MSH2* in EpCAM-expressing tissues, resulting in tissue-specific *MSH2* deficiency (mosaicism)
    - Muir–Torre syndrome (MTS, an autosomal dominant disorder associated with germline mutations in the *MSH2* and less frequently the *MLH1* gene) is a rare variant of Lynch syndrome, characterized by at least one sebaceous gland neoplasm (sebaceous adenoma or sebaceous carcinoma) and at least one visceral malignancy (most often colorectal cancer, followed by urinary tract cancer)
- Molecular mechanisms
  - The typical molecular characteristic is MSI
  - This implies mutations in the dinucleotide repeats that occur throughout the genome; these provide the hallmark of the syndrome but are not as such pathogenic
  - Genes affected by MMR incompetency include
    - Genes involved in cell proliferation including *TGFβ, GRB1, WISP3, TCF4*
    - Genes involved in apoptosis including *BAX*, caspase 5, *PTEN, BCL10*
    - Genes involved in DNA repair including *MBD4, RAD50, MSH3, MLH3*
  - Which of these are of functional significance in the context of CRC development remains to be established
  - MMR deficient cancers are characterized by gene point mutations and less by chromosomal rearrangements and allelic imbalances

The Mut Y Homolog (MUTYH)-Associated Polyposis (MAP) Syndrome

- MAP syndrome resembles FAP
  - Adenomatous polyps of the colorectum develop, resulting in a very high risk of colorectal cancer
  - Adenomatosis resembles attenuated FAP as the burden of adenomas ranges from very few to hundreds
  - Extracolonic manifestations as in FAP, including duodenal adeno(carcino)mas and increased risk for several extraintestinal neoplasms
  - Genetics
    - An autosomal recessive disorder
    - Caused by biallelic mutations in the *MUTYH* gene, located on chromosome 1p
    - Commonly Tyr165Cys and Gly382Asp
    - *MUTYH* encodes a protein in the DNA base excision repair pathway
    - Its impaired function is responsible for G:C to T:A transversions

Other Polyposis Syndromes

- Several other less frequent polyposis syndromes exist, all associated with a lower risk for CRC
- Peutz–Jeghers syndrome (PJS)
  - Has a prevalence of about 1 in 8,300 to 1 in 280,000 individuals
  - An inherited, autosomal dominant disorder
  - Characterized by hamartomatous polyps in the gastrointestinal tract
  - Pigmented mucocutaneous lesions typically presenting in childhood on the lips, and in and around the mouth
  - PJS subjects suffer from various malignancies (gastrointestinal, pancreatic, lung, breast, uterine, ovarian, and testicular tumors)
  - Genetics
    - Most patients have a causative mutation in the *STK11* gene, a tumor suppressor gene located at 19p13.3, encoding a serine threonine kinase (STK)
    - Other genes are likely involved in non-STK-mutated cases but this needs to be resolved
- Juvenile polyposis syndrome (JPS)
  - A rare disease, occurring in juveniles
  - Characterized by the presence of hamartomatous polyps throughout the gastrointestinal tract resembling inflammatory polyps

  - Lifetime CRC risk is about 40%
  - Genetics
    - 15–20% of JPS patients carry autosomal dominant mutations in the *SMAD4/DPC4* gene, on chromosome 18q21.1
    - SMAD4 is a cytoplasmic mediator in the TGFβ signaling pathway
    - 25–40% of the patients carry bone lesions, associated with autosomal dominant mutations in the gene encoding morphogenetic protein receptor 1A (*BMPR1A*), on chromosome 10q22–23
    - Sporadic cases occur
- Hereditary mixed polyposis syndrome (HMPS)
  - Shows consistent phenotypic overlap with JPS
  - Characterized by polyps of mixed adenomatous/hyperplastic/atypical juvenile histology
  - Inheritance is autosomal dominant
  - Eventually colorectal cancer may develop
  - Genetics
    - A germline *BMPR1A* mutation has been identified in Chinese HMPS families
    - Linkage between HMPS and chromosome 15q has also been reported
- Cowden syndrome (CS)
  - A rare autosomal dominant hamartomatous polyposis condition
  - 40% of CS patients have hamartomatous polyps in the gastrointestinal tract
  - Low CRC risk
  - Caused by a mutation of the tumor suppressor phosphatase and tensin homolog (*PTEN*) gene

Molecular Pathways in the Development of Colorectal Cancer

- As elucidated in the description of its familial forms, in the development of CRC several distinct pathways operate. These pathways are not entirely distinct in that the same gene abnormalities partially occur in several of them. Significant overlap in molecular mechanisms therefore occur
- After elucidation of the role of APC in CRC, now known as the chromosomal instability or CIN pathway, the discovery of the involvement of the mismatch repair system in HNPCC led to the recognition of the microsatellite instability (MIN) pathway. Recently, the recognition of the role of serrated lesions in the development of CRC along a distinct pathway has led to the recognition of the serrated pathway. Although in a strict sense the CpG island methylator phenotype (CIMP) is not a distinct pathway it is also briefly reviewed

The CIN Pathway

- The CIN pathway is found in about 85% of sporadic colorectal cancers and is prototypical for the molecular evolution of CRC. The sequence most likely starts
  - As an aberrant crypt focus (ACF), a small focus of mucosa in which the regular crypt architecture is disturbed
  - Harboring mutations in the *KRAS* gene or
  - Mutations in the *APC* gene
- ACF with *APC* mutations are accompanied by distinct morphological abnormalities of the crypt epithelium, for which the term dysplasia is used
  - Dysplastic cells have morphological characteristics of cancer cells including
    - Nuclear pleomorphism and piling up
    - Increased nucleus to cytoplasm ratio
    - Increased mitotic activity
  - Dysplastic cells have some genetic characteristics of cancer cells but have not yet acquired the capacity for invasive growth and metastasis
- After this starts, these early lesions in the adenoma–carcinoma sequence acquire multiple genetic abnormalities including
  - Telomerase activation, conferring unlimited lifespan to the adenoma cells
  - *TP53* mutations, involved in progression from a low grade adenoma to a high grade adenoma
  - SMAD4 loss, involved in the progression from a noninvasive adenoma to an invasive carcinoma
- This pathway is illustrated in Fig. 1.3, which is a modification of the original schematic as

proposed by Vogelstein in a landmark paper in 1990. This pathway of evolution of colorectal cancer is characterized by striking CIN. For this reason this is called the CIN pathway

The MIN Pathway

- The MIN pathway occurs in about 15% of sporadic colorectal cancers. In morphological terms, it is highly likely that the early phases of development are quite similar to those of the CIN pathway: an adenoma–carcinoma sequence. However, cancers that arose through this pathway behave differently from those in the CIN pathway
  - Have a better prognosis
  - Respond differently to standard chemotherapy
  - Have fairly characteristic morphology
    - Situated in the right colon
    - Mucinous or medullary histology
    - Lymphocytic infiltrate
  - Have characteristic genetic features
  - MSI due to an incompetent mismatch DNA repair system
  - Promoter methylation of *MLH1* (sporadic MIN CRC) or
  - Mutated *MLH1, MSH2, MSH6,* or *PMS2* (familial MIN CRC known as HNPCC or Lynch syndrome)
  - Relatively high frequency of *BRAF* gene mutations
  - The MS status has gained quite a bit of clinical interest lately. MSI-H carcinomas as a rule have a better prognosis than MSS carcinomas of equal stage and grade. In addition their response to adjuvant therapy is more favorable

The Serrated Pathway

- This is a recently discovered alternative pathway to the development of CRC. The lesions resemble (benign) hyperplastic polyps but with more irregular crypt architecture (Fig. 1.7) and occasionally features of dysplasia, known as sessile serrated adenomas or polyps (SSA/P). Features include:
  - Development of flat nonadenomatous mucosal lesions
  - Notably in the right colon
  - Due to the abnormal retention of surface epithelium as a result of inhibition of apoptotic cell loss
  - Accumulating epithelial cells in the crypt resulting in a serrated appearance
- SSA/P have been associated with increased risk for the development of CRC but the extent of the risk has not yet been adequately established
- The molecular events in the pathway are
  - Mutation of the *BRAF*, characteristically the V600E mutation
  - Methylation of the promoter of a variety of genes (CIMP)
  - Subsequent methylation of the *MLH1* gene promoter with silencing of the expression of MLH1 as a result
  - MSI leading to accumulation of additional abnormalities in the genome

Cpg Island Methylator Phenotype

- CIMP has been noticed for some time that some colorectal carcinomas show hypermethylation of a whole series of genes. Meanwhile it has become known that this phenomenon is not limited to CRC but that a variety of cancers suffers from an increased level of promoter methylation. This is not a nonspecific general phenomenon. First of all, cancer cells in general show a decrease in the overall level of DNA methylation. This concerns mostly CpGs in (non)coding regions. In gene promoter-associated CpG islands, involved in the regulation of transcription, often hypermethylation is found. Promoter hypermethylation has become one of the frequently encountered mechanisms responsible for silencing of tumor suppressor genes. What causes the CIMP phenotype and what exactly its consequences are remains elusive? CIMP is associated with the serrated pathway and in addition CIMP carcinomas often harbor *BRAF* mutations

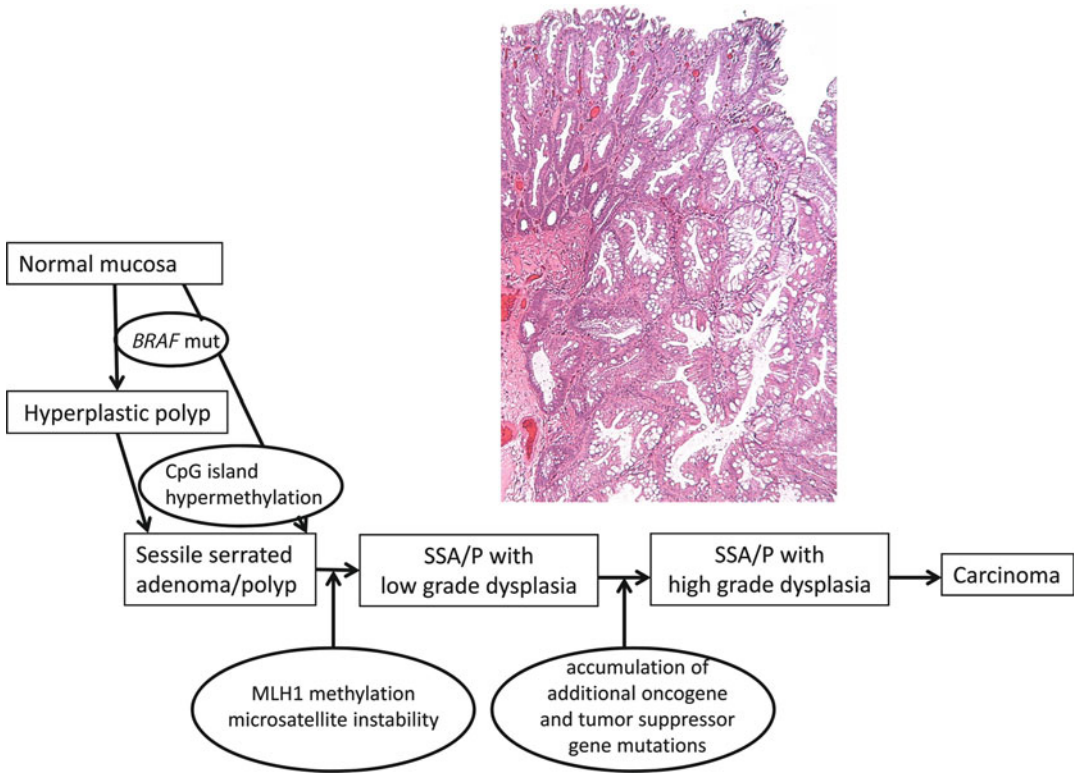

Fig. 1.7 Schematic of the serrated pathway of colorectal carcinoma. The *inset* photomicrograph shows such a serrated lesion, of which the characteristic feature is the sawtooth appearance of the colonic crypts. An important difference with the CIN and MIN pathways is the early occurrence of microsatellite instability together with *BRAF* mutations. Later on in the pathway, gene aberrations that frequently occur in the other pathways are also found

Practical Molecular Diagnostics of Colorectal Cancer

- Screening for CRC; apart from the classical FOB test several options are under development
  - Tests for abnormal circulating DNA targeting *KRAS* mutations or promoter methylation of a combination of target genes
  - Tests for abnormal DNA in stool targeting *KRAS* mutations or promoter methylation of a set of target genes
  - Molecular screening of blood or fecal matter still experimental due to low sensitivity
- Diagnosis of MSI carcinoma
  - Microsatellite instable carcinoma can be either sporadic or familial, the latter in case of HNPCC (Lynch syndrome)
  - MSI carcinoma is suspected if:
    - Arises in the right colon
    - Mucinous or medullary morphology
    - Accompanied by diffusely distributed tumor infiltrating lymphocytes or follicular peritumoral lymphocytic infiltrates
  - HNPCC is suspected if:
    - Before the age of 50
    - In a familial context of CRC and/or syn/metachronous presence of HNPCC-associated tumors
    - In a patient with a first degree relative with an HNPCC-associated tumor before the age of 50
    - In a patient with two first degree relatives with HNPCC-associated tumors, regardless of age

  - The diagnosis of either is important, in a familial case for making the diagnosis and for sporadic MSI cases as their prognosis is different, as well as their reaction to therapy
  - The diagnosis is made through MMR gene immunohistochemistry and/or MSI testing, in case of suspicion of HNPCC followed up by sequencing of the suspected gene. The following sequence of tests is cost-effective and highly sensitive:
    - MS analysis, using as markers the recommended panel of loci (BAT25, BAT26, D2S123, D5346, and D17S250) with as result
      - Instability of two or more markers MSI-H(igh)
      - Instability of one marker MSI-L(ow)
      - No instable marker MSS
    - Immunohistochemistry for MLH1, MSH2, MSH6, and PMS2; loss of MLH1 is usually accompanied by loss of PMS2
    - In case of loss of expression of MLH1: testing for gene promoter methylation through bisulfite sequencing
      - In case of methylation of the *MLH1* promoter a diagnosis of sporadic MMI carcinoma is made
      - In case of unmethylated *MLH1* promoter there is a high likelihood of HNPCC; gene sequencing to identify the mutation needs to be performed
    - Loss of expression of any one of the other genes indicates the likelihood of HNPCC; sequence the gene indicated by immunohistochemistry to identify the mutation is indicated
- In case of suspicion of FAP (polyposis, family history), sequence the *APC* gene to identify an eventual mutation
- In case of suspicion of MAP, sequence the *MUTYH* gene is indicated to identify an eventual mutation
- Prognostic factors
  - Currently used criteria for stratification of CRC patients in view of eventual adjuvant chemotherapy are primarily based upon classical TNM stage parameters tumor extension (T) and lymph node metastasis (N). However, these lack in precision and better parameters are urgently needed. A host of molecular (to a large extent immunohistochemically determined) parameters has been published but almost none of these has made it into clinical practice. New molecular parameters are
    - Allelic imbalance of 18q. This has been advocated for a decade as an important prognostic parameter notably in stage II patients. Recent data, however, indicate that its prognostic significance is not maintained when CRC is stratified for MSI
    - MSI; microsatellite instable carcinomas have a better prognosis than those that are MSS. In addition, patients with MSI carcinomas might not profit from 5-FU-based chemotherapy regimens but this remains controversial
    - *BRAF* V300E mutation status. This is an indicator of poor prognosis, notably for survival after relapse. A recently published BRAF mutated-like signature seems to identify BRAF wild type cases with similar behavior
    - *KRAS* mutation status. Recent data indicate that *KRAS* mutation status as such has no prognostic significance for overall survival of stage II/III patients, although this might be different for the G12D and G12A mutations, for which borderline significance was found
    - Gene expression profiling. Two recently published tests are Oncotype DX (multiplex RT-PCR-based) and Coloprint (array-based), which proclaim to provide essential additional prognostic value. As yet, these tests have not been independently validated
- Predictive molecular pathology of colorectal cancer
  - Ever since the introduction of targeted therapies, which as yet include for CRC anti-EGFR antibodies (Cituximab and Panitumumab) and anti-VEGFR (Avastin) the need for pretreatment testing of potential

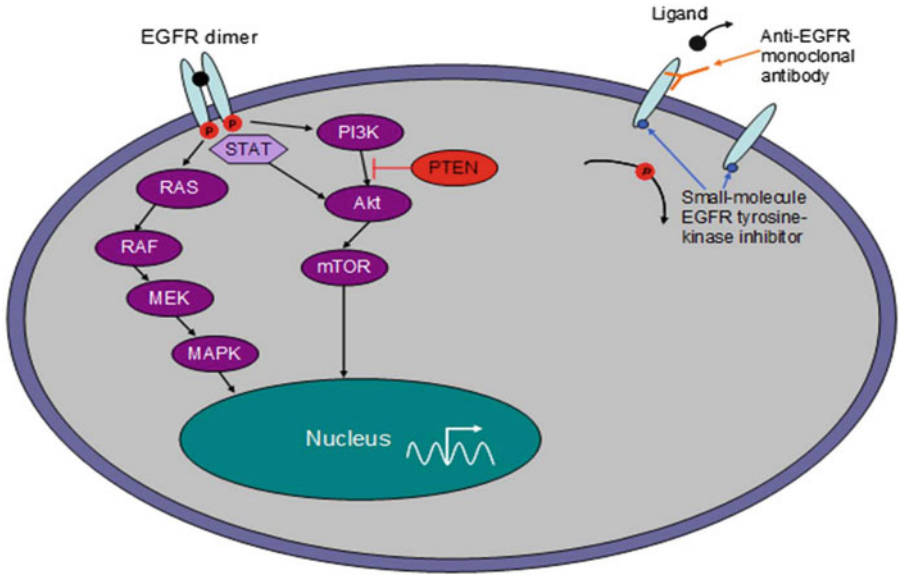

Fig. 1.8 Schematic of the role of KRAS mutations in the resistance of KRAS-mutated colorectal cancers to anti-EGFR therapy. KRAS functions downstream of EGFR signaling. In a KRAS wild type cancer, silencing of EGFR silences the signaling pathway. When KRAS is mutated, silencing of EGFR is without effect as the KRAS mutation continues to activate the pathway. Other proteins involved in the pathway may also be involved in resistance. This has been shown to be the case for NRAS, BRAF, and PIK3CA. Other pathways are also involved, such as PTEN

drug efficacy has become clear. For the anti-EGFR approach this has been found in

- EGFR amplification. This is done through FISH testing, although quantitative PCR of genomic DNA has been advocated. The latter has as disadvantage that noncancerous host cells cannot easily be separated from cancer cells and these reduce the sensitivity of the test. EGFR immunohistochemistry has proven to be insufficiently reliable as test
- *KRAS* mutation testing as overwhelming evidence indicates that KRAS-mutated carcinomas do not respond to these anti-EGFR therapies
  - This is due to the fact that KRAS is downstream of EGFR in the MAPK pathway (Fig. 1.8)
  - Mutations in *KRAS* consequently activate the pathway
  - Upstream elimination of EGFR activation is then no longer effective
- *KRAS* mutations identify cancers that do not respond to anti-EGFR therapy but not all *KRAS* wild type cancers do respond; additional factors are involved, notably the mutation status of
  - *PIK3CA*
  - *BRAF*
  - *NRAS*
  - *PTEN*
  - *Probably others*

Summary of Key Points in the Molecular Pathology of Colorectal Cancer

- Most CRC arise through an adenoma–carcinoma sequence; this holds true for sporadic and for familial CRC (CIN type end MIN type)
- Screening for adenomas (through colonoscopy) strongly reduces CRC risk; molecular screening approaches (feces, blood) are underway

- Exception to this rule are CRCs developing in the context of IBD which develop through a dysplasia–carcinoma sequence; specific and sensitive molecular tests for assessing cancer risk in IBD are not (yet) available
- SSA/P constitute a new pathway towards the development of CRC; this pathway is characterized by mutated *BRAF* and flat nondysplastic precursors known as SSA/P
- HNPCC is diagnosed through a combination of MSI testing, immunohistochemical testing for expression of MLH1, MSH2, MSH6, and PMS2, promoter methylation analysis of MLH1 and eventually gene sequencing
- *KRAS* mutation testing is essential for anti-EGFR therapies as *KRAS*-mutated cancers do not respond to this form of targeted treatment; better tests for positive response prediction are urgently needed
- MSI testing of CRC is becoming mandatory in view of its prognostic importance
- *BRAF*-mutated CRC have a poor prognosis; a *BRAF*-mutated signature identifies *BRAF* WT patients with a n equally poor prognosis
- New prognostic signatures (Genomic Health DsX and Coloprint) are promising but as yet insufficiently validated
- Stool or blood testing for abnormal DNA (*KRAS* mutations, gene promoter methylation) are promising but as yet insufficiently validated

Suggested Readings

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Molecular Pathology of Pancreatic Cancer

2

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Introduction to Normal Pancreas

- The exocrine and endocrine functions of the pancreas are regulated by distinct cell types:
  - Exocrine pancreas (enzymes secreted into ducts)—consists of enzyme-secreting acinar cells as well as mucin-secreting duct cells
  - Endocrine pancreas (hormones secreted systemically)—consists of multiple types of hormone-secreting cells
- Histology of the pancreas parallels this functional division, with cellular components divided into four compartments: acinar, ductal, endocrine, and interstitial
  - The exocrine pancreas (acinar and ductal components) consists of clustered acini that secrete into centrally located ducts which join to form progressively larger ducts
  - The endocrine compartment is composed of the islets of Langerhans scattered amongst the acini
  - These compartments are supported by interstitial stroma
- Neoplasms of the pancreas can be broadly divided based on the direction of differentiation of the neoplastic cells
- Neoplasms with distinct differentiation exhibit unique clinical, morphologic, and molecular features

L. Cheng and J.N. Eble (eds.), *Molecular Surgical Pathology*,
DOI 10.1007/978-1-4614-4900-3_2, © Springer Science+Business Media New York 2013

Neoplasms with Ductal Differentiation

Pancreatic Ductal Adenocarcinoma

Clinical Features

- Approximately 213,000 deaths/year in the world, 37,660 deaths/year in the United States (US)
  - Eighth leading cause of cancer death worldwide, fourth leading cause of cancer death in the U.S
  - Male predominance (male to female ratio of approximately 3:2); a disease of the elderly, as most patients are diagnosed between 60 and 80 years of age
  - Incidence of ductal adenocarcinoma is 50% higher in African Americans than in Caucasians
  - Most important risk factor is cigarette smoking, other known risk factors include chronic pancreatitis and diabetes mellitus
- Approximately 10% of pancreatic cancer has a familial basis (Table 2.1)
  - Family history of pancreatic cancer significantly increases an individual's risk of developing pancreatic cancer
  - Increased risk of pancreatic cancer is a feature of several genetic syndromes, but the genetic basis for the majority of familial pancreatic cancer remains unknown
  - *BRCA2* and other genes in the Fanconi anemia pathway
    - Proteins in this pathway are crucial for repair of DNA cross-linking damage
    - Germline mutations in *BRCA2* result in increased risk of breast, ovarian, and pancreatic cancer and account for a subset of patients with familial pancreatic cancer. Importantly, some patients with *BRCA2*-related familial pancreatic cancer do not report a family or personal history of breast or ovarian cancer
      - Cells with biallelic inactivation of *BRCA2* are exquisitely sensitive to therapies that target their DNA repair defect, such as mitomycin C and PARP inhibitors
    - Germline mutations in *PALB2*, also known as *FANCN*, whose protein product interacts with BRCA2, account for a subset (~3%) of patients with familial pancreatic cancer

Table 2.1 Inherited disorders with increased risk of pancreatic neoplasia

Syndrome	Gene	Chromosome	Neoplasm
Familial breast cancer	*BRCA2*	13	PDA
Familial breast cancer	*PALB2 (FANCN)*	16	PDA
Familial atypical multiple mole melanoma syndrome (FAMMM)	*p16/CDKN2A*	9	PDA
Peutz–Jeghers syndrome (PJS)	*STK11/LKB1*	19	PDA, IPMN
Hereditary pancreatitis	*PRSS1, SPINK1*	7, 5	PDA
Lynch syndrome/hereditary nonpolyposis colorectal cancer (HNPCC)	Multiple	Multiple	PDA (medullary variant)
Fanconi anemia pathway	*FANCC*, *FANCG*	Multiple	PDA (?)
Familial adenomatous polyposis (FAP)	*APC*	5	PDA (?)
von Hippel–Lindau syndrome (VHL)	*VHL*	3	SCA, PanNET
Multiple endocrine neoplasia type 1 (MEN1)	*MEN1*	11	PanNET
Tuberous sclerosis complex (TSC)	*TSC1, TSC2*	9, 16	PanNET
Neurofibromatosis type 1 (NF1)	*NF1*	17	PanNET
Beckwith–Wiedemann syndrome (BWS)	unknown	11	PB

PDA pancreatic ductal adenocarcinoma; *IPMN* intraductal papillary mucinous neoplasm; *SCA* serous cystadenoma; *PanNET* well-differentiated pancreatic neuroendocrine tumor; *PB* pancreatoblastoma; (?) weak or questionable association

    - Germline mutations in other Fanconi pathway genes (*FANCC*, *FANCG*) have been reported in young patients with pancreatic cancer, but their importance in familial pancreatic cancer has not been firmly established
    - The importance of *FANCA* in familial pancreatic cancer has also been investigated, but no significant contribution to cancer susceptibility could be established
    - Germline mutations in *BRCA1* may slightly increase the risk of pancreatic cancer in breast cancer kindreds; however, mutations in this gene are not a major cause of familial pancreatic cancer in the absence of breast or ovarian cancer
  - *p16/CDKN2A* and familial atypical multiple mole melanoma syndrome (FAMMM)
    - Germline mutations in *p16/CDKN2A* result in increased risk of both melanoma (with multiple nevi and atypical nevi) and pancreatic cancer
  - *STK11/LKB1* and Peutz–Jeghers syndrome (PJS)
    - PJS is an inherited syndrome caused by germline mutations in *STK11/LKB1* and characterized by gastrointestinal hamartomas as well as cancer predisposition. Patients with PJS have very high risk of pancreatic cancer, and carcinomas in PJS patients show somatic loss of the wild type *STK11/LKB1* allele
  - *PRSS1*, *SPINK1*, and hereditary pancreatitis
    - Germline mutations in *PRSS1* and *SPINK1* cause hereditary pancreatitis, characterized by continuing or relapsing pancreatic inflammatory disease. Patients with hereditary pancreatitis have a markedly increased risk of developing pancreatic cancer
  - *APC* and familial adenomatous polyposis (FAP)
    - Germline mutations in *APC* result in a markedly increased risk of adenomatous colorectal polyps as well as colorectal adenocarcinoma. In addition, an increased risk of pancreatic cancer has also been reported in these patients. However, some of this may reflect the increased risk of duodenal carcinomas, which frequently invade the pancreas and can mimic primary pancreatic adenocarcinoma
  - Mismatch repair gene defects and hereditary nonpolyposis colorectal cancer (HNPCC)
    - Germline mutations in *hMSH2*, *hMLH1*, *hPMS1*, *hPMS2*, and *hMSH6/GTB* cause HNPCC (also known as Lynch syndrome), in which patients demonstrate an increased risk of carcinomas of the colon as well as other sites
    - The neoplasms in these patients exhibit defects in the DNA mismatch repair machinery, resulting in microsatellite instability
    - Patients with HNPCC have a slightly increased risk of developing pancreatic cancer
      - Importantly, pancreatic cancers with microsatellite instability exhibit a distinct "medullary" morphology
- Symptoms of ductal adenocarcinoma are often nonspecific, including back pain, unexplained weight loss, and jaundice
- Aggressive neoplasm that is almost uniformly fatal, with a 5-year survival rate of only 5%
  - Mean survival for untreated patients is 3–5 months
  - Mean survival after surgical resection is 10–20 months
  - Resectability and stage are the most important determinants of prognosis

Gross and Microscopic Features

- Majority of ductal adenocarcinomas (60–70%) arise in the pancreatic head
- Usually form solitary masses
- Average size of carcinoma in pancreatic head is 3 cm in greatest dimension, while average size of carcinoma in pancreatic tail is 5 cm in greatest dimension

- On gross cut section, ductal adenocarcinomas are firm white-yellow poorly demarcated masses that obscure the normal lobular architecture of the pancreas
  - If the carcinoma obstructs the pancreatic duct, the pancreatic duct upstream from the carcinoma may be dilated, and the upstream pancreatic parenchyma may be firm and atrophic due to obstructive chronic pancreatitis
- Microscopically, pancreatic ductal adenocarcinoma is an invasive mucin-producing gland-forming neoplasm that elicits an intense stromal desmoplastic response
  - Histologic features of adenocarcinoma include haphazard arrangement of glands, nuclear pleomorphism, incomplete glandular lumina, luminal necrosis, neoplastic glands immediately adjacent to muscular vessels, perineural invasion, and lymphovascular invasion
  - These carcinomas are graded based on their degree of differentiation
    - Well-differentiated: well-formed neoplastic glands with lined by cuboidal to columnar epithelium with enlarged round to oval nuclei and eosinophilic cytoplasm
    - Moderately-differentiated: irregularly shaped and small glands with moderate nuclear pleomorphism, prominent nucleoli, and decreased mucin production
    - Poorly-differentiated: mixture of irregular glands, solid sheets, and single neoplastic cells with marked nuclear pleomorphism, minimal mucin production, and significant mitotic activity
- In addition to the morphology described, there are several morphological variants of ductal adenocarcinoma with unique prognostic and molecular features
- Pancreata with invasive ductal adenocarcinoma also frequently harbor noninvasive epithelial proliferations within the pancreatic ducts known as pancreatic intraepithelial neoplasia (PanIN)
  - These lesions are believed to be precursors to invasive adenocarcinoma and are graded based on architectural and cytologic atypia
    - PanIN-1A: flat lesion composed of uniform columnar cells with small round basal nuclei and abundant supranuclear mucin
    - PanIN-1B: papillary or micropapillary lesions composed of uniform columnar cells with small round basal nuclei and abundant supranuclear mucin (cytologically identical to PanIN-1A)
    - PanIN-2: flat or papillary lesions with moderate nuclear atypia (loss of polarity, pseudostratification, enlargement, hyperchromasia)
    - PanIN-3: flat or papillary lesions with severe architectural atypia (budding, cribriforming, luminal necrosis) and nuclear atypia (enlargement, hyperchromasia, loss of orientation, and polarity, prominent nucleoli). Mitoses are common
- Immunohistochemistry
  - Positive in neoplastic cells: cytokeratins (CK7, CK8, CK13, CK18, CK19), CEA, CA19–9, CA125, MUC1, MUC3, MUC4, MUC5AC
  - Variably positive in neoplastic cells: CK20, MUC2, MUC6
  - Negative in neoplastic cells: vimentin, chromogranin, synaptophysin, trypsin, chymotrypsin, lipase
    - Dpc4 (Smad4) expression is lost in approximately 55% of ductal adenocarcinomas, reflecting genetic inactivation of the *SMAD4/DPC4* gene (Fig. 2.1c)

Molecular Features

- See Table 2.2
- *KRAS* is the most frequently altered oncogene in ductal adenocarcinomas—somatic mutations in *KRAS* occur in >90% of ductal adenocarcinomas
  - These somatic mutations cluster in specific hotspots (most commonly codon 12), con-

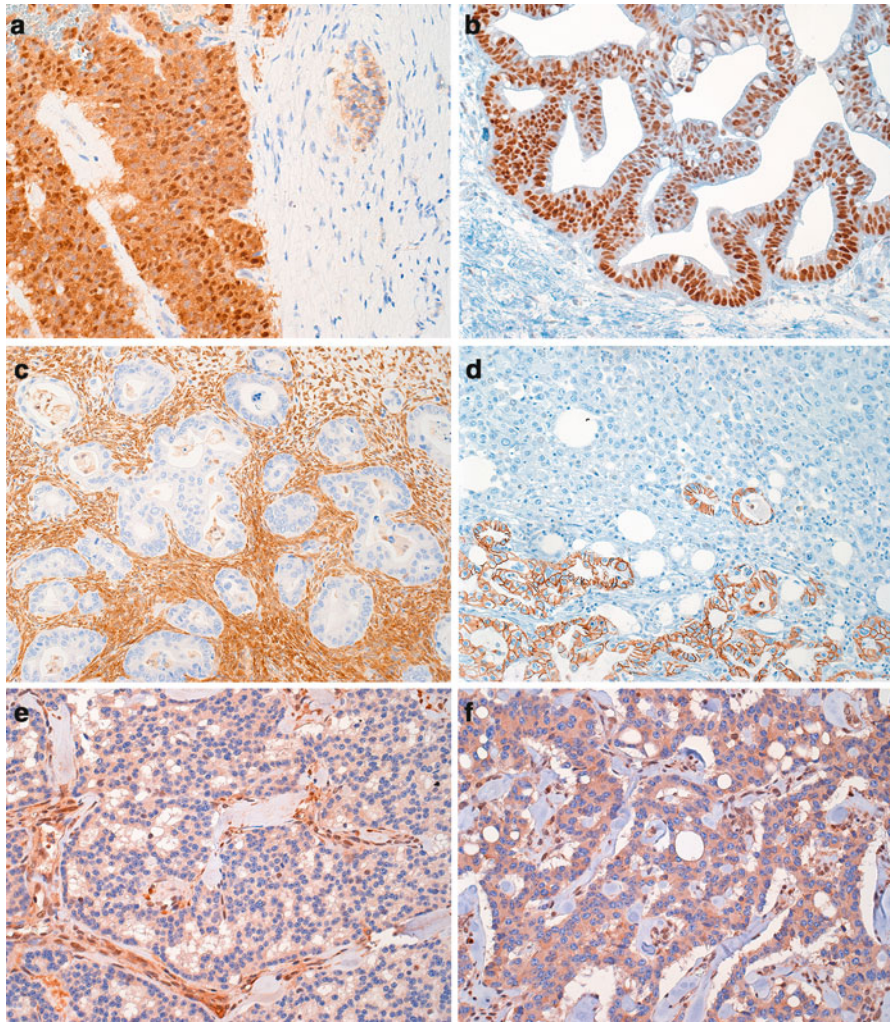

Fig. 2.1 Immunohistochemical labeling reflects genetic alterations in pancreatic neoplasms. (**a**) Solid-pseudopapillary neoplasms frequently contain somatic mutations in *CTNNB1*, leading to abnormal nuclear accumulation of β-catenin protein. Immunohistochemical labeling for β-catenin shows strong nuclear and cytoplasmic labeling in the neoplasm on the *left*. On the *right*, a nonneoplastic duct has normal membranous staining for β-catenin. (**b**) Somatic mutations in *TP53*, leading to abnormal nuclear accumulation of p53 protein, occur in a large proportion of pancreatic ductal adenocarcinomas. Immunoshistochemical labeling for p53 shows strong nuclear labeling in the neoplastic epithelium, while the reactive stroma is negative. (**c**) Pancreatic ductal adenocarcinomas frequently contain inactivating somatic mutations in *SMAD4/DPC4*, leading to loss of Dpc4 protein expression in neoplastic cells. Immunohistochemical labeling for Dpc4 shows retention of nuclear labeling in the reactive stroma, while the neoplastic glands are strikingly negative. (**d**) In contrast to well-differentiated pancreatic ductal adenocarcinoma, undifferentiated carcinomas frequently show loss of E-cadherin protein expression. Immunohistochemical labeling of a mixed carcinoma demonstrates retention of E-cadherin expression in the well-differentiated component (*lower half*), while E-cadherin is lost in the undifferentiated component (*upper half*). (**e**, **f**) Approximately 45% of well-differentiated pancreatic neuroendocrine tumors (PanNETs) contain inactivating somatic mutations in *DAXX* or *ATRXX*, leading to loss of protein expression in the neoplasm. Immunohistochemical labeling shows that although endothelial cells show strong nuclear labeling, neoplastic cells are negative for ATRX (**e**) and DAXX (**f**) in tumors with somatic mutations

Table 2.2 Somatic mutation prevalence of commonly altered genes in pancreatic neoplasms

Neoplasm	Gene	Chromosome	Alteration prevalence	Mechanisms of alteration
PDA	*KRAS*	12	95%	Missense mutation
	p16/CDKN2A	9	95%	Missense mutation with LOH, homozygous deletion, promoter methylation
	TP53	17	75%	Missense mutation with LOH
	SMAD4/DPC4	18	55%	Missense mutation with LOH, homozygous deletion
IPMN	*KRAS*	12	80%	Missense mutation
	RNF43	17	75%	Missense mutation or nonsense mutation with LOH
	GNAS	20	60%	Missense mutation
	p16/CDKN2A	9	Only in HGD/carcinoma	Missense mutation with LOH, homozygous deletion, promoter methylation
	TP53	17	Only in HGD/carcinoma	Missense mutation with LOH
	SMAD4/DPC4	18	Only in HGD/carcinoma	Missense mutation with LOH, homozygous deletion
	PIK3CA	3	10%	Missense mutation
MCN	*KRAS*	12	80%	Missense mutation
	RNF43	17	40%	Missense mutation or nonsense mutation with LOH
	p16/CDKN2A	9	Only in HGD/carcinoma	Missense mutation with LOH, homozygous deletion, promoter methylation
	TP53	17	Only in HGD/carcinoma	Missense mutation with LOH
	SMAD4/DPC4	18	Only in HGD/carcinoma	Missense mutation with LOH, homozygous deletion
SCA	*VHL*	3	>20%	Missense mutation with LOH
PanNET	*MEN1*	11	45%	Missense mutation with LOH
	DAXX/ATRX	6/X	45%	Missense or nonsense mutation with LOH
	mTOR pathway	Multiple	15%	Multiple
	VHL	3	25%	Promoter methylation
SPN	*CTNNB1*	3	95%	Missense mutation
ACC	*CTNNB1*	3	5%	Missense mutation
	APC	5	15%	Inactivating/truncating mutation with LOH
PB	*CTNNB1*	3	55%	Missense mutation
	APC	5	10%	Inactivating/truncating mutation with LOH
	Unknown	11	85%	Loss of heterozygosity

PDA pancreatic ductal adenocarcinoma; *IPMN* intraductal papillary mucinous neoplasm; *MCN* mucinous cystic neoplasm; *SCA* serous cystadenoma; *PanNET* well-differentiated pancreatic neuroendocrine tumor; *SPN* solid-pseudopapillary neoplasm; *ACC* acinar cell carcinoma; *PB* pancreatoblastoma; *HGD* high-grade dysplasia; *carcinoma* invasive carcinoma; *LOH* loss of heterozygosity

sistent with the role of *KRAS* as an oncogene
  - Rare mutations have been reported in other oncogenes, including *BRAF* mutations in *KRAS* wild type carcinomas
  - *KRAS* encodes a small GTPase that mediates downstream signaling from growth factor receptors, playing a crucial role in proliferation, survival, and differentiation. The protein encoded by *BRAF* is activated by KRAS and functions in the same cell signaling pathways
- *p16/CDKN2A* is the most frequently altered tumor suppressor gene in ductal adenocarci-

noma—loss of p16 function is seen in >90% of ductal adenocarcinomas
  - Mechanisms of functional alteration include intragenic mutation coupled with the loss of the second allele, homozygous deletion of the gene, and promoter methylation of *p16/CDKN2A*
    - The protein encoded by *p16/CDKN2A* plays a crucial role in cell cycle regulation. p16 blocks cell cycle progression by preventing the inactivation of Rb, another crucial cell cycle regulator
- Somatic mutations in *TP53* also occur frequently in ductal adenocarcinomas, reported in 75% of cases
  - Strong diffuse nuclear immunohistochemical labeling for p53 is associated with gene mutation (Fig. 2.1b)
  - *TP53* mutations occur most frequently through a small intragenic mutation, coupled with loss of the wild type allele
    - The protein encoded by *TP53* plays a key role in the cellular stress response, inhibiting cell growth and promoting cell death in the setting of cellular stress
- Somatic inactivation of *SMAD4/DPC4* occurs in approximately 55% of ductal adenocarcinomas
  - This gene is inactivated through either homozygous deletion or intragenic mutation coupled with loss of the wild type allele
    - The protein encoded by *SMAD4/DPC4* mediates cellular signaling downstream of the transforming growth factor β (TGFβ) receptor, playing a crucial role in the regulation of proliferation, migration, and apoptosis
    - Mutations in *SMAD4/DPC4* are associated with poor prognosis
    - Immunohistochemical loss of Dpc4 protein expression is correlated with the presence of genetic inactivation and can be used as a diagnostic tool to distinguish ductal adenocarcinoma from non-neoplastic pancreatic disease (Fig. 2.1c)
    - Less frequent somatic mutations have also been reported in other members of the TGFβ signaling pathway, including *TGFBR2* and *ALK5*
- Specific key genes (*KRAS*, *TP53*, *SMAD4/DPC4*, *p16/CDKN2A*) are crucial components of tumorigenesis in ductal adenocarcinoma that are preserved in different tumor subgroups
  - The prevalence of alterations in *KRAS*, *TP53*, and *SMAD4/DPC4* is similar in familial and sporadic ductal adenocarcinomas
  - Carcinomas from smokers contain significantly more somatic mutations than those from never smokers, but mutations in known driver genes such as *KRAS*, *TP53*, *p16/CDKN2A*, and *SMAD4/DPC4* do not differ between the two groups
- Studies of copy number alterations have revealed numerous areas of gains and losses in ductal adenocarcinomas, and cytogenetic analyses have revealed complex karyotypes
  - Some alterations target the known oncogenes and tumor suppressor genes discussed above
  - Others identify genomic regions that may contain loci involved in pancreatic tumorigenesis, but further characterization and validation are necessary
- MicroRNAs, small noncoding RNAs that negatively regulate gene expression, are also altered in ductal adenocarcinoma
  - Multiple microRNAs are differentially expressed in carcinomas compared to non-neoplastic pancreas and chronic pancreatitis
    - Specific microRNA expression profiles show prognostic significance
    - MicroRNAs possess diagnostic promise, as microRNA levels in fine needle aspirations can be an indicator of ductal adenocarcinoma
- PanINs, the noninvasive precursor lesions to pancreatic cancer, sequentially acquire the molecular changes common in invasive ductal adenocarcinoma
  - While some molecular changes reliably occur early in pancreatic tumorigenesis, others are limited to severely dysplastic and invasive lesions

    - Somatic *KRAS* mutations are present even in the low-grade PanINs (PanIN-1A)
    - Loss of p16 expression is also an early event, with loss in a subset of PanIN-1A lesions. However, the prevalence of detected p16 loss increases with increasing PanIN grade
    - Loss of Dpc4 expression (which occurs due to homozygous deletion or intragenic mutation coupled with loss of the wild type allele) is a late event, reported only in some PanIN-3 lesions and invasive carcinomas. Allelic loss may occur earlier than somatic mutation, with a subset of PanIN-2 lesions showing loss of one allele of *SMAD4/DPC4*
    - *TP53* displays a similar pattern to *SMAD4/DPC4*. Alteration of *TP53* is a late event, occurring only in PanIN-3 lesions and invasive carcinomas. Allelic loss may occur earlier than somatic mutation, with a subset of PanIN-2 lesions showing loss of one allele of *TP53*
    - Telomere shortening has been reported in a large proportion of PanINs including low-grade lesions, with shortening in approximately 90% of PanIN-1As
        - Thus, telomere shortening is one of the most frequently occurring early events in pancreatic tumorigenesis
    - In patients with familial pancreatic cancer due to germline *BRCA2* mutations, loss of the wild type *BRCA2* allele is a late event in tumorigenesis, occurring only in PanIN-3 lesions
- Sequencing of all protein-coding genes in pancreatic ductal adenocarcinoma reveals that although the individual genes altered in each tumor are markedly heterogeneous, there are 12 core cellular pathways that are genetically altered in the majority of carcinomas
    - Dysregulation of these core pathways, which include KRAS signaling, TGFβ signaling, DNA damage control, and cell adhesion, represents a shared feature of tumorigenesis in the vast majority of ductal adenocarcinomas
    - These studies identified an average of 48 nonsynonymous somatic mutations per tumor
- Studies of somatic mutations in metastases reveal a time period of approximately 15 years between the occurrence of the initiating mutation of ductal adenocarcinoma and the acquisition of metastatic ability, suggesting a broad time window for early detection

Variants of Ductal Adenocarcinoma and Their Molecular Features

Adenosquamous Carcinoma

- Uncommon morphologic variant of pancreatic ductal adenocarcinoma, accounting for 3–4% of malignant exocrine neoplasms
- Slight male predominance (male to female ratio of 1.5:1); mean age at diagnosis 63 years
- Aggressive neoplasm with poor prognosis, median survival of 6 months
- Microscopically, characterized by infiltrating carcinoma with both glandular and squamous differentiation
- Immunohistochemically, p63 is positive in squamous component and E-cadherin is frequently lost or reduced
- Molecular features similar to ductal adenocarcinoma, with frequent alterations in *KRAS*, *p16/CDKN2A*, *SMAD4/DPC4*, and *TP53*

Colloid Carcinoma

- Uncommon morphologic variant of pancreatic ductal adenocarcinoma, accounting for 1–3% of malignant exocrine neoplasms
- Slight male predominance, mean age at diagnosis 65 years
- Better prognosis than ductal adenocarcinoma, with a 5-year survival of approximately 55%
- Microscopically characterized by large extracellular pools of mucin containing suspended well-differentiated neoplastic cells—almost always arises in association with intestinal-type intraductal papillary mucinous neoplasm (IPMN)

- Immunohistochemically, the neoplastic cells are positive for MUC2 and CDX2, which are frequently positive in intestinal-type IPMNs but usually negative in ductal adenocarcinoma
- Lower prevalence of *KRAS* mutations (approximately 30%) and *TP53* mutations (approximately 20%) compared to ductal adenocarcinoma
  - In addition, colloid carcinomas often harbor somatic mutations in *GNAS*, an oncogene that is frequently mutated in IPMNs and associated adenocarcinomas

Hepatoid Carcinoma

- Very rare variant of pancreatic ductal adenocarcinoma, with only a few cases reported in the literature
- Too few cases reported to comprehensively determine clinical outcome
- Microscopically characterized by large polygonal neoplastic cells with abundant eosinophilic cytoplasm
- Immunohistochemically, neoplastic cells express markers of hepatocyte differentiation (HepPar1, polyclonal CEA, CD10, AFP)
- Poorly characterized at the molecular level

Medullary Carcinoma

- Uncommon morphologic variant of pancreatic ductal adenocarcinoma, accounting for <5% of ductal adenocarcinomas
- Age and sex distribution similar to that for pancreatic ductal adenocarcinoma
- More likely to report a family history of cancer than patients with pancreatic ductal adenocarcinoma
- Medullary carcinomas of the pancreas have been reported in patients with HNPCC or with synchronous colorectal adenocarcinomas (Table 2.1)
- Better prognosis than pancreatic ductal adenocarcinoma
- Microscopically characterized by poor differentiation, pushing borders, syncytial growth, and focal to extensive necrosis
- Medullary carcinomas have distinct molecular features
  - High prevalence of microsatellite instability
  - Lack somatic mutations in *KRAS*, though oncogenic *BRAF* mutations have been reported
- The diagnosis of a medullary carcinoma has therapeutic implications—though not studied thoroughly in the pancreas, patients with microsatellite-unstable medullary colorectal adenocarcinomas do not benefit from fluorouracil-based chemotherapy

Undifferentiated Carcinoma

- Uncommon morphologic variant of pancreatic ductal adenocarcinoma, accounting for <10% of ductal adenocarcinomas
- Male predominance (male to female ratio of 3:1), average age at diagnosis 63 years
- Aggressive neoplasm with poor prognosis, with an average survival of only 5 months
- Multiple possible microscopic patterns (can be mixed within a single tumor):
  - Anaplastic carcinoma—mix of noncohesive pleomorphic mononuclear cells and bizarre multinucleated giant cells
  - Sarcomatoid carcinoma—atypical spindle cells arranged in fascicles
- Immunohistochemically, the neoplastic cells express markers of epithelial differentiation (cytokeratins)
- Pattern of *KRAS* mutation is similar to pancreatic ductal adenocarcinoma
- Frequent loss of E-cadherin protein expression (Fig. 2.1d)
  - The loss of E-cadherin is a possible explanation for the neoplasm's discohesive morphology
  - Although *E-cadherin* promoter methylation has been reported in an undifferentiated carcinoma cell line, no somatic mutations in *E-cadherin* have been identified in neoplasms with loss of E-cadherin expression
  - Loss of E-cadherin is associated with decreased survival compared to well-, moderately-, and poorly differentiated carcinomas with intact E-cadherin expression

Undifferentiated Carcinoma with Osteoclast-Like Giant Cells

- Uncommon morphologic variant of pancreatic ductal adenocarcinoma
- Slight female predominance in reported cases, average age at diagnosis 62 years
- Aggressive neoplasm with poor prognosis, with a mean survival of only 12 months
- Microscopically, composed of at least two distinct cell populations:
  - Large multinucleated osteoclast-like giant cells with multiple round uniform nuclei
  - Undifferentiated discohesive pleomorphic mononuclear cells with marked nuclear pleomorphism and hyperchromasia
- Immunohistochemically, the osteoclast-like giant cells express histiocyte markers (CD68, CD45, KP1) while the atypical mononuclear cells variably express markers of epithelial differentiation (cytokeratin, EMA)
- Molecular analyses have clarified the nature of the two characteristic cell types
  - While the mononuclear cells contain frequent somatic mutations in *KRAS*, these mutations are less frequently present in the osteoclast-like giant cells
    - Moreover, while pleomorphic mononuclear cells variably overexpress p53, osteoclast-like giant cells are negative
  - The molecular and immunohistochemical findings support the conclusion that while the mononuclear cells are neoplastic, the osteoclast-like giant cells are reactive
    - The presence of *KRAS* mutations in these giant cells is most likely due to phagocytosis of tumor DNA by the nonneoplastic cells

Intraductal Papillary Neoplasms

Clinical Features

- Incidence and prevalence are difficult to estimate because most are asymptomatic
  - Account for <5% of exocrine pancreatic neoplasms but the majority of cystic neoplasms of the pancreas
  - May be more prevalent than currently appreciated—incidental pancreatic cysts are identified in approximately 3% of adults undergoing abdominal imaging, and many of these are IPMNs
- Slight male predominance (male to female ratio of 3:2), average age at diagnosis 63 years
  - Patients with noninvasive IPMNs are, on average, slightly younger than patients with an IPMN with an associated invasive carcinoma
- IPMNs occur in some patients with inherited cancer predisposition (Table 2.1)
  - IPMNs have been reported in patients with PJS, a cancer predisposition syndromes associated with germline mutations in the *STK11/LKB1* gene on chromosome 19p
  - In PJS, the IPMNs exhibit loss of heterozygosity at the *STK11/LKB1* gene locus, suggestive of a second somatic hit to the wild type allele in the neoplasm
  - In addition, an IPMN was reported in a patient with Lynch syndrome (HNPCC), with loss of expression of mismatch repair proteins in the neoplastic cells of the pancreas
    - This suggests the possibility of IPMN as a rare extracolonic manifestation of Lynch syndrome
  - Similarly, although the association is not well-established, IPMNs have been reported in patients with FAP
- Frequently discovered incidentally on abdominal imaging, also can present with symptoms due to pancreatic duct obstruction (abdominal pain, weight loss, recurrent pancreatitis)
  - Some patients report a history of symptoms for many years before diagnosis
- Approximately one-third of IPMNs have an associated invasive adenocarcinoma
- Prognosis depends almost entirely on the presence of an associated invasive carcinoma
  - Surgical resection is curative in most, but not all, patients with a noninvasive IPMN (90–95% 5-year survival)
    - Some patients with "noninvasive IPMN" eventually die of metastatic carci-

noma—this is due to metachronous multifocal disease
  - For patients with an associated invasive carcinoma, prognosis depends on stage, with an average 5-year survival of 40–50%, significantly higher than that of patients with pancreatic ductal adenocarcinoma not arising from an IPMN
    - Much of this improved survival appears to be due to the lower stage at which IPMN-associated invasive carcinomas are diagnosed

Gross and Microscopic Features

- Majority (70%) arise in the pancreatic head, with 20% in the body or tail and 10% diffusely involving the entire gland
- Multicentricity is common, occurring in up to 40% of cases
- By definition, IPMNs are >1 cm in greatest dimension, but can be quite large and involve the entire length of the pancreas
  - IPMNs with associated invasive carcinoma are usually larger than noninvasive IPMNs
- On gross cut section, the main pancreatic duct or one of its branches is dilated, containing papillary projections and thick mucin
  - The relationship of the dilated duct to the main pancreatic duct is used to classify the neoplasm:
    - Main duct-type—neoplasm involves the main pancreatic duct
    - Branch duct-type—neoplasm involves the secondary branches but not the main pancreatic duct
    - Combined type—neoplasm involves the main pancreatic duct and its branches (clinical behavior similar to main duct-type)
      - Main duct IPMNs are more likely to have high-grade dysplasia or an associated invasive carcinoma than are branch duct-type IPMNs
- Microscopically, IPMNs consist of mucin-secreting columnar epithelium lining the pancreatic duct system, variably forming papillary projections
  - Multiple histologic subtypes are recognized:
    - Intestinal type—papillae with long villous projections, lined by epithelial cells with apical mucin and cigar-shaped nuclei as well as scattered goblet cells
    - Gastric foveolar type—flat epithelium or small papillae lined by epithelium with small basal nuclei, eosinophilic cytoplasm, and apical mucin—often occurs in branch duct IPMNs with low-grade dysplasia
    - Pancreatobiliary type—complex papillary structures with bridging and cribriforming, lined by cuboidal epithelial cells with little extracellular mucin and round nuclei with open chromatin and variably prominent nucleoli—more likely than other types to harbor high-grade dysplasia
    - Oncocytic type—architecturally complex papillae with bridging and cribriforming, lined by cells with abundant granular pink cytoplasm and round nuclei
  - Noninvasive neoplasms are categorized based on the degree of dysplasia in their mucinous epithelium
    - Low-grade dysplasia: mild architectural and cytologic atypia
    - Intermediate-grade dysplasia: moderate architectural and cytologic atypia, with papillary projections, nuclear pseudostratification, and nuclear enlargement
    - High-grade dysplasia: marked architectural and cytologic atypia, with irregular papillae with cribriforming and budding, loss of nuclear polarity, significant nuclear pleomorphism, and mitotic figures
  - Approximately one-third of IPMNs have an associated invasive adenocarcinoma
    - In half the cases, the carcinoma is an invasive colloid carcinoma, characterized by large pools of extracellular mucin with free-floating neoplastic epithelial cells

        - These patients have a better prognosis than those with tubular carcinoma arising in association with an IPMN
        - Colloid carcinomas in the pancreas are almost universally associated with IPMNs of the intestinal subtype
    - In the remaining half of the cases, the carcinoma is a tubular carcinoma, morphologically indistinguishable from a pancreatic ductal adenocarcinoma arising without an IPMN, composed of an invasive gland-forming carcinoma with desmoplastic stroma and minimal extracellular mucin production
- Immunohistochemistry
  - Positive in neoplastic cells: cytokeratins and CEA
  - Variably positive in neoplastic cells: scattered basal neuroendocrine cells expressing chromogranin and synaptophysin
  - MUC expression varies based on histologic subtype
    - Intestinal type—positive for MUC2 and MUC5AC, negative for MUC1 (also positive for CDX2)
      - More likely to progress to invasive colloid carcinomas that are also MUC2 positive and MUC1 negative
    - Gastric foveolar type—positive for MUC5AC, negative for MUC2 and MUC1
    - Pancreatobiliary type—positive for MUC1 and MUC5AC, negative for MUC2
      - More likely to progress to invasive ductal adenocarcinomas that are MUC1 positive and MUC2 negative
- Far less common than IPMNs, intraductal neoplasms can also be nonmucinous
  - Intraductal tubulopapillary neoplasm—back to back tubular glands with cribriform architecture, prominent necrosis, minimal mucin production, and occasional papillary formations

Molecular Features

- See Table 2.2
- Frequent alterations in genes commonly mutated in pancreatic ductal adenocarcinoma (*KRAS*, *TP53*, *SMAD4/DPC4*, *p16/CDKN2A*)
  - Somatic mutations in *KRAS* occur frequently in IPMNs, involving 30–80% of neoplasms, with increasing mutation prevalence in neoplasms with high-grade dysplasia or associated adenocarcinoma
    - When extremely sensitive techniques are employed, 80% of IPMNs have been found to harbor *KRAS* gene mutations
      - Somatic *KRAS* mutations occur in all histologic subtypes of IPMN
      - Rare somatic mutations in *BRAF* have also been reported in IPMNs
  - p53 overexpression is most prevalent in areas of high-grade dysplasia and invasive carcinoma associated with IPMNs
    - Somatic mutations of *TP53* have also been reported, and these occurred only in cases with high-grade dysplasia
  - Dpc4 expression is retained in the vast majority of noninvasive IPMNs and lost in approximately one-third of IPMN-associated invasive carcinomas, suggesting a lower prevalence of Dpc4 loss in IPMN-associated invasive carcinomas compared to pancreatic ductal adenocarcinomas not arising in association with an IPMN
  - Loss of p16 protein expression occurs in both noninvasive IPMNs and invasive carcinoma arising in association with an IPMN, but loss is much more prevalent in invasive carcinomas (100% of invasive carcinomas vs. 10% of noninvasive IPMNs in one study)
    - Hypermethylation of the *p16/CDKN2A* promoter occurs in >50% of noninvasive IPMNs and IPMNs with associated adenocarcinoma
- Approximately 60% of IPMNs possess somatic mutations in *GNAS*, an oncogene with mutations in pituitary and other uncommon neoplasms
  - The mutations in IPMNs all occurred at a previously described oncogenic hotspot (codon 201), and in cases with an associated adeno-

carcinoma, *GNAS* mutations were detected in both in situ and infiltrating components
  - Mutations in *GNAS* are most prevalent in intestinal-type IPMNs
    - The protein encoded by *GNAS* couples transmembrane receptors to their downstream signaling proteins, such as adenylyl cyclase, playing a crucial role in numerous cell signaling pathways
- Approximately 75% of IPMNs possess somatic mutations in *RNF43*, which encodes an E3 ubiquitin ligase
  - The majority of these mutations lead to the insertion of stop codons, and there is frequent LOH at chromosome 17q (the location of *RNF43*), providing strong evidence that *RNF43* is a tumor suppressor gene
- Somatic mutations in *PIK3CA* (some at previously described oncogenic hotspots) occur in approximately 10% of IPMNs
  - The protein encoded by *PIK3CA* is the catalytic component of crucial cell signaling kinase that regulates numerous pathways involved in growth, proliferation, and apoptosis
- Rare somatic mutations in *EGFR* and *HER2* have been described in IPMNs
- In addition to reports of IPMNs in patients with germline alterations of *STK11/LKB1* (PJS), sporadic IPMNs also undergo somatic mutation (5%) and loss of heterozygosity (25%) at the *STK11/LKB1* locus
- Sequencing of all protein-coding genes in eight IPMNs identified the frequent alterations of *KRAS*, *GNAS*, and *RNF43*
  - In addition, two somatic alterations were also identified in *APC*. Although one mutation was a nonsense base substitution, in silico studies suggest that the other mutation is unlikely to alter protein function. Thus, the significance of *APC* mutations in IPMNs remains uncertain
  - These studies identified an average of 26 nonsynonymous somatic mutations per IPMN, approximately half as many as in invasive ductal adenocarcinoma
- Promoter hypermethylation occurs in several genes (including *APC*, *E-cadherin*, *hMLH1*, *MGMT*) in noninvasive IPMNs and IPMNs with associated adenocarcinoma, with more prevalent methylation in neoplasms with adenocarcinoma
  - Hypermethylation of multiple genes is also more prevalent in IPMNs with an associated invasive adenocarcinoma
- Large-scale chromosomal alterations have been identified in IPMNs with all levels of dysplasia, but copy number alterations are much more frequent in IPMNs with high-grade dysplasia
- Analyses of microRNA expression have revealed significantly higher expression of miR-21, miR-221, and miR-17–3p in IPMNs as compared to nonmucinous cysts
- Mutations in neoplastic cells can be detected in aspirated IPMN cyst fluid, indicating that molecular studies of cyst fluid represent a promising diagnostic tool to preoperatively classify cystic lesions in the pancreas
  - More than 95% of IPMNs contain a somatic mutation in either *KRAS* or *GNAS*, illustrating that molecular analyses are a highly sensitive assay for the identification of IPMNs

Mucinous Cystic Neoplasm

Clinical Features

- Uncommon neoplasms, accounting for <10% of all surgically resected cystic pancreatic lesions
- Vast majority, but not all, occur in women (female to male ratio of approximately 20:1), with a mean age at presentation of 40–50 years
- No known genetic predilection or association with particular genetic syndromes
- Frequently discovered incidentally on abdominal imaging, also can present with nonspecific symptoms due to compression of adjacent organs (abdominal pain or fullness)
- Associated invasive adenocarcinoma is present in 20–33% of mucinous cystic neoplasms (MCNs)
- Prognosis depends almost entirely on the presence of an associated invasive carcinoma

  - Surgical resection is curative in almost all patients with a noninvasive MCN
  - For patients with an associated invasive carcinoma, prognosis depends on stage, with an average 5-year survival of 25–50%

Gross and Microscopic Features

- Almost all (>95%) occur in body and tail of the pancreas
- Usually solitary, with a thick well-demarcated capsule
- Wide size range (2–35 cm), average 6–10 cm in greatest dimension
- On cut section, the neoplasm is most frequently a multiloculated thick-walled cyst with adherent thick mucin
  - Some locules may also contain hemorrhagic or necrotic debris admixed with mucin
  - Cyst walls can be smooth or may contain papillary excrescences or mural nodules, the latter associated with high-grade dysplasia or an associated invasive carcinoma. Importantly, the cysts do not communicate with the larger ducts of the pancreas
- Microscopically, by definition, two components are present—the cysts are lined by mucin-producing columnar epithelial cells with an underlying ovarian-type stroma
  - Architecturally, MCNs consist of a multiloculated cyst surrounding by a thick fibrous capsule, separating the cyst from the adjacent uninvolved pancreas
  - Cyst-lining epithelial cells are tall and columnar, with small basal nuclei and abundant apical mucin
    - Commonly observed directions of differentiation in the epithelium include pseudopyloric, gastric–folveolar, and intestinal
  - Associated with the mucinous epithelium, these neoplasms possess a characteristic ovarian-type stroma, consisting of dense spindle cells with elongated nuclei and sparse cytoplasm
    - This stroma is required for the diagnosis of MCN
  - Noninvasive neoplasms are categorized based on the degree of dysplasia in their mucinous epithelium
    - Low-grade dysplasia: mild architectural and cytologic atypia
    - Intermediate-grade dysplasia: moderate architectural and cytologic atypia, with papillary projections, nuclear pseudostratification, and nuclear enlargement
    - High-grade dysplasia: severe architectural and cytologic atypia, with irregular papillae, loss of nuclear polarity, and significant nuclear pleomorphism
  - Approximately one-third of MCNs have an associated invasive adenocarcinoma, most commonly conventional ductal adenocarcinoma though several less common carcinoma variants have been reported
    - The transition from low-grade to high-grade dysplasia, and even to invasive carcinoma, can be abrupt and focal, necessitating extensive histologic sampling of these neoplasms to assess for an invasive carcinoma
    - Rare cases of biphasic malignant neoplasms containing both carcinomatous and high-grade spindle cell ("sarcomatous") components have been reported in association with MCNs
- Immunohistochemistry
  - Epithelial cyst lining
    - Positive in neoplastic cells: cytokeratins and CEA
    - Variably positive in neoplastic cells: scattered basal neuroendocrine cells positive for chromogranin and synaptophysin
    - Negative in neoplastic cells: CDX2, CK20
    - Differing expression of mucin markers has been associated with the transition to invasive carcinoma—noninvasive MCNs are MUC5AC-positive and MUC1-negative, while invasive carcinomas are frequently MUC5AC-positive and MUC1-positive
  - Ovarian-type stroma

    - Positive in the stromal cells: vimentin, smooth muscle actin, desmin, calretinin, inhibin
    - Variably positive in the stromal cells: progesterone receptor (50–75% of neoplasms), estrogen receptor (25% of neoplasms)
    - Negative in the stromal cells: S100 protein, CD34

Molecular Features

- See Table 2.2
- Frequent alterations in genes commonly mutated in pancreatic ductal adenocarcinoma (*KRAS*, *TP53*, *SMAD4/DPC4*, *p16/CDKN2A*)
  - Somatic *KRAS* mutations are frequently detected in MCNs, with mutation prevalence correlated with degree of dysplasia
    - Typical *KRAS* mutations have been identified in approximately 30% of neoplasms with low-grade dysplasia and approximately 80% of neoplasms with high-grade dysplasia or invasive carcinoma
      - *KRAS* mutations can be detected in cyst fluid aspirated from MCNs, suggesting that mutational analysis of cyst fluid may become an important ancillary diagnostic test to characterize cystic lesions in the pancreas, including MCNs
  - p53 overexpression is limited to areas of high-grade dysplasia and invasive carcinoma in MCNs, and *TP53* mutation has been reported in neoplasms with high-grade dysplasia
  - Loss of Dpc4 protein expression, a surrogate for *SMAD4/DPC4* gene mutation, is associated with the transition to invasive carcinoma
    - Intact Dpc4 protein expression is reported in virtually all noninvasive neoplasms but only a subset of invasive carcinomas associated with MCNs
  - Mutation in *p16/CDKN2A* has also been reported in a neoplasm with high-grade dysplasia. Hypermethylation of the *p16/CDKN2A* promoter has been identified in approximately 15% of MCNs
  - These findings suggest a model of stepwise dysplasia and carcinogenesis in MCNs in which *KRAS* mutation is an early event and loss of Dpc4 is a late event
    - In a mouse model of pancreatic neoplasia, oncogenic *KRAS* mutation and haploinsufficiency of *SMAD4/DPC4* cooperatively led to the development of MCNs
    - Additional somatic genetic alterations occurred during the development of MCNs in this model, including loss of heterozygosity of *SMAD4/DPC4* and mutation of *TP53* or *p16/CDKN2A*
- Sequencing of all protein-coding genes in eight MCNs revealed frequent somatic alterations in *KRAS*, *RNF43*, and *TP53*
  - Most of the mutations in *RNF43* were nonsense substitutions, further confirming this gene's role as a tumor suppressor in mucin-producing cystic neoplasms of the pancreas
  - These studies identified an average of 16 nonsynonymous somatic mutations per MCN, fewer than in IPMN and invasive ductal adenocarcinoma
- Lack alterations in β-catenin, which is frequently altered in solid-pseudopapillary neoplasms of the pancreas
- No evidence of microsatellite instability. Aneuploidy has been reported in carcinomas associated with MCNs and is associated with poor prognosis
- Gene expression studies suggest different expression profiles in the epithelial and stromal components, with activation of the Notch pathway (*JAG1* and *HES1*) in the epithelial component and activation of estrogen metabolism (*STAR* and *ESR*) in the stromal component
  - The genetic or epigenetic underpinnings of these expression differences have not been elucidated
- In rare invasive carcinomas with both carcinomatous and "sarcomatous" components, the his-

tologically distinct components contain nearly identical patterns of loss of heterozygosity
  - These findings suggest a monoclonal origin for the two components with subsequent genetic and morphologic diversion in this rare subtype

Serous Cystadenoma

Clinical Features

- Uncommon neoplasm, accounting for 1–2% of all pancreatic neoplasms, but close to one-third of all cystic neoplasms of the pancreas
- Occurs primarily in adults (mean age 60–65 years) with female predominance (female to male ration of 7:3)
- Associated with von Hippel–Lindau syndrome (VHL), an autosomal dominant disorder characterized by clear cell neoplasms in multiple organs (Table 2.1)
  - VHL is caused by mutations in the *VHL* gene on chromosome 3p, which is involved in the regulation of the Hypoxia-inducible factor 1 (HIF1α) pathway
  - Up to 90% of patients with VHL develop serous cystadenomas (SCAs) of the pancreas, and these pancreatic lesions may be the first presentation in patients with VHL
  - The SCAs that arise in patients with VHL are often combined serous neuroendocrine neoplasms
- SCAs are frequently discovered incidentally on abdominal imaging but also can present with symptoms due to abdominal mass (abdominal pain, nausea, and vomiting)
  - Jaundice is rare
  - Patients with VHL may present with diabetes mellitus due to diffuse involvement of the pancreas by SCAs
- Excellent prognosis—neoplasms are slow growing (approximately 0.60 cm/year), and complete surgical resection is curative in almost all cases
  - Exceedingly rare malignant variant (serous cystadenocarcinoma), defined by distant metastasis, also has favorable prognosis, with most patients alive at the time their reports were published (average followup 36 months)

Gross and Microscopic Features

- Occur more frequently in the pancreatic body and tail (50–75% of cases) than in the pancreatic head
- In sporadic cases, most often solitary and well-demarcated mass
  - Patients with VHL frequently develop multiple SCAs, which may involve the pancreas diffusely
- Wide size range (1–25 cm), average 6 cm in greatest dimension
- On cut section, most commonly a well-circumscribed, slightly bosselated, sponge-like mass composed of numerous small thin-walled cysts filled with clear to straw-colored watery fluid
  - Often have a central scar with radiating fibrous septa
  - This common pattern is referred to as microcystic
  - However, uncommon variants exist, most of which are defined by their gross appearances, including:
    - Macrocystic variant—few or single large smooth-walled cyst(s)
    - Solid variant—soft, fleshy pink-tan solid mass mimicking a neuroendocrine tumor
    - VHL-associated variant—multiple lesions grossly similar to microcystic SCAs may diffusely involve the pancreas
    - Combined serous neuroendocrine neoplasm—the two components may be grossly distinct or intimately admixed. Many of these patients have VHL
- Microscopically, composed of cysts lined by uniform cuboidal cells with clear glycogen-rich cytoplasm and central round nuclei

– Most common architectural pattern (microcystic) is that of numerous small cysts lined by a single layer of cuboidal epithelial cells. Micropapillae may be present in cyst lining. Uncommon variants have distinct architectural features:

    - Macrocystic variant—few large cysts lined by a single layer of cuboidal epithelial cells
    - Solid variant—cuboidal cells arranged in small acini with minute central lumina
    - VHL-associated variant—microscopically similar to microcystic SCA
    - Combined serous neuroendocrine neoplasm—the serous areas form small thin-walled cysts, while the neuroendocrine areas form solid foci in which the neoplastic cells form nests or trabeculae
  - Neoplastic cells do not infiltrate adjacent pancreas, but atrophy of the adjacent pancreas is common, particularly when the neoplasm compresses the pancreatic duct
  - Cytoplasm is most often clear due to abundant glycogen, but rarely cytoplasm can have an oncocytic appearance
  - Nuclei are round and uniform—small, hyperchromatic, with inconspicuous nucleoli
- Immunohistochemistry
  - Positive in neoplastic cells: cytokeratins (including AE1/AE3, CAM5.2, CK7, CK8, CK18, CK19), inhibin, MUC6
  - Negative in neoplastic cells: CEA, chromogranin, synaptophysin, pancreatic hormones (insulin, glucagon, somatostatin), MUC2, MUC5
- Serous cystadenocarcinomas are defined by the presence of distant metastases and are microscopically indistinguishable from SCAs

Molecular Features

- See Table 2.2
- Somatic inactivating mutations in *VHL* gene have been reported in at least 20% of sporadic SCAs
  - Loss of heterozygosity of chromosome 3p at the *VHL* locus also occurs in a large proportion of sporadic neoplasms
    - Neoplasms in patients with VHL syndrome also consistently show loss of heterozygosity of chromosome 3p (*VHL* gene)
- Sequencing of all protein-coding genes in eight SCAs confirmed frequent somatic alterations in *VHL* (altered in 50% of SCAs studied)
  - No additional genes with frequent somatic alterations were identified
  - These studies identified an average of ten nonsynonymous somatic alterations per tumor, approximately half the number of alterations in IPMN and far fewer than in invasive ductal adenocarcinoma
- Lack alterations in genes frequently mutated in pancreatic ductal adenocarcinoma (*KRAS*, *TP53*)
- Aberrant methylation at a few loci reported in only a minority of cases
- Lack alterations in β-catenin, which is frequently mutated in solid pseudopapillary neoplasms of the pancreas
- Microsatellite instability has not been identified in sporadic neoplasms
- In addition to allelic loss of chromosome 3p in a large proportion of sporadic SCAs, allelic loss of chromosome 10q has been reported in 50% of cases
  - Losses in several other chromosomes have been reported in multiple SCAs
  - No target genes for these losses have been identified

Neoplasms with Neuroendocrine Differentiation

Pancreatic Neuroendocrine Tumors

Clinical Features

- Well-differentiated pancreatic neuroendocrine tumors (PanNETs) are uncommon neoplasms, accounting for 1–2% of all pancreatic neoplasms
  - High-grade neuroendocrine carcinomas are very rare, accounting for <1% of pancreatic carcinomas and 2–3% of PanNETs
- Can occur at any age, but are most common between 30 and 60 years (average age at diagnosis 50 years)—slight female predominance (female to male ratio of 1.15:1)

- PanNETs occur as key features of multiple inherited syndromes (Table 2.1)
  - Multiple endocrine neoplasia syndrome, type 1 (MEN1)—caused by germline mutations in tumor suppressor gene *MEN1* on chromosome 11q. MEN1 is an autosomal dominant clinical syndrome characterized by neuroendocrine lesions of the parathyroid, pituitary, pancreas, duodenum (gastrinomas), and adrenal
    - Pancreas is involved on 60–70% of patients with MEN1
  - VHL—caused by germline mutation in tumor suppressor gene *VHL* on chromosome 3p, which is involved in the regulation of the HIF1α pathway
    - VHL is an autosomal dominant clinical syndrome characterized by neoplasms in various organs including hemangioblastomas of the eye and cerebellum, pheochromocytomas, renal cell carcinomas, and serous cystadenonomas and PanNETs of the pancreas
    - Many of these neoplasms are composed of optically clear cells
    - PanNETs occur in 5–10% of patients, with somatic loss of wild type allele of *VHL* in neoplastic cells
- PanNETs rarely occur in other genetic syndromes (Table 2.1)
  - Tuberous sclerosis complex (TSC)—caused by germline mutations in tumor suppressor genes *TSC1* on chromosome 9q or *TSC2* on chromosome 16p
    - TSC is an autosomal dominant clinical syndrome characterized by hamartomas and rare malignant neoplasms
    - PanNETs have rarely been reported in children with TSC
  - Neurofibromatosis type 1 (NF1)—caused by germline mutations in tumor suppressor gene *NF1* on chromosome 17q
    - NF1 is an autosomal dominant clinical syndrome characterized most prominently by nervous system abnormalities
      - PanNETs expressing somatostatin (somatostatinomas) are a rare finding in NF1
      - Although pancreatic tumors have been reported, somatostinomas arise more frequently in the duodenum or ampulla of Vater of patients with NF1
- Presenting symptoms vary based on size and type of PanNET
  - PanNETs that secrete hormones frequently present with a characteristic clinical syndrome due to hormone excess (functional PanNETs)
    - The presence of a clinical syndrome of hormone excess is required to classify a PanNET as functional
  - Small nonfunctional PanNETs are usually found incidentally on abdominal imaging or in pancreata resected for other reasons
  - Larger nonfunctional PanNETs may present with nonspecific symptoms related to a large abdominal mass (abdominal pain, nausea)
    - Jaundice infrequently occurs in patients with PanNETs
- Prognosis depends on size, grade, and stage of the PanNET
  - Small nonfunctional microadenomas (<0.5 cm) are considered clinically benign and are completely cured by surgical resection
  - PanNETs are graded based on their proliferative rate, as assessed by mitotic count or Ki67 labeling index (see below)
    - However, all PanNETs except microadenomas are regarded as having malignant potential, with an average 5-year survival of 65%
  - High-grade neuroendocrine neoplasms (defined by mitotic count or Ki67 labeling index—see below) are designated pancreatic neuroendocrine carcinomas
    - They are highly aggressive neoplasms with mortality of almost 100% (survival of 1 month to 1 year)

Gross and Microscopic Features

- Can occur anywhere in the pancreas, with some specific localization for functional types
  - For example, gastrinomas and somatostatinomas tend to be in the duodenum

- Sporadic PanNETs are usually solitary and well-demarcated. Patients with MEN1 are frequently diagnosed with multiple synchronous PanNETs
- Functional PanNETS, particularly insulinomas, are frequently small at diagnosis (<2 cm) due to early clinical detection from symptoms of hormone excess
  - Nonfunctional PanNETS are generally larger (frequently >5 cm), at least partially due to later detection in absence of a specific clinical syndrome
- On gross cut section, most PanNETs are solid pink-tan masses with well-defined borders, varying in consistency from soft and fleshy to densely fibrotic
  - Infrequently, areas of hemorrhage, necrosis, and cystic degeneration can occur, particularly in larger neoplasms
  - In contrast, neuroendocrine carcinomas are usually large firm masses with ill-defined borders
- Microscopically, PanNETs consist of an organoid proliferation of uniform cells with neuroendocrine features
  - Numerous architectural patterns have been described, including trabecular, nested, and gyriform
    - Many neoplasms have a mixed architectural pattern
  - Some PanNETs have a fibrotic capsule
  - Individual cells have a distinct neuroendocrine morphology, with a moderate amount of finely granular cytoplasm, round nuclei with coarsely clumped ("salt and pepper") chromatin, and occasional nucleoli
    - Cytoplasmic hyaline globules (similar to those seem in solid-pseudopapillary neoplasm) may rarely be present
  - Some morphologic findings are suggestive of specific functional PanNETs
    - Amyloid deposition is common in insulinomas
    - Psammoma bodies occur most commonly in somatostatinomas
  - PanNETs are graded based on proliferative rate, as assessed by mitotic count or Ki67 labeling index
    - Grade 1 (low grade) PanNET—0–1 mitosis per 10 high-power fields (hpf), Ki67 index of 0–2%
    - Grade 2 (intermediate grade) PanNET—2–20 mitoses per 10 hpf, Ki67 index of 3–20%
    - Grade 3 (high grade) pancreatic neuroendocrine carcinoma—>20 mitoses per 10 hpf, Ki67 index >20%
  - Two histologic subtypes of neuroendocrine carcinoma
    - Small cell carcinoma—highly cellular infiltrative neoplasm of small-to medium-sized cells with scant cytoplasm, nuclear molding, and finely granular chromatin
    - Large cell variant of neuroendocrine carcinoma—nested pattern of large cells with amphophilic cytoplasm and large oval nuclei with coarsely clumped chromatin and prominent nucleoli
- Immunohistochemistry
  - Positive in neoplastic cells: synaptophysin (diffuse), chromogranin (diffuse or focal), CD56 (less specific), CD57 (less specific), cytokeratins 8 and 18
    - Functional PanNETs are frequently positive for their secreted hormone (insulin, glucagon, etc.)
      - However, immunohistochemical evidence of hormone expression is not sufficient to classify a PanNET as functional in the absence of specific clinical symptoms
    - Neuroendocrine carcinoma may have variable or absent expression of neuroendocrine markers (chromogranin, synaptophysin)
    - If >25% of neoplastic cells express acinar markers (trypsin, chymotrypsin), the neoplasm should be classified as a mixed acinar neuroendocrine carcinoma

Molecular Features

- See Table 2.2
- Somatic mutations in *MEN1* occur in approximately 45% of sporadic PanNETs
  - Loss of heterozygosity at the *MEN1* locus also occurs in 30–70% of PanNETs, includ-

ing some PanNETs without somatic *MEN1* mutation
  - Mutations in *MEN1* are associated with better prognosis
  - Neoplasms with *MEN1* mutation show loss of expression or aberrant localization of the MEN1 protein
    - In addition, some neoplasms without *MEN1* mutation also exhibit aberrant MEN1 protein localization, suggesting that mechanisms in addition to *MEN1* mutation contribute to MEN1 protein dysfunction in PanNETs
- Genes involved in a chromatin remodeling complex (*DAXX* and *ATRX*) are somatically mutated (including numerous inactivating mutations) in approximately 45% of sporadic PanNETs
  - These genes are part of a complex that is important for telomere maintenance, and inactivation of these genes in PanNETs is associated with the telomerase-independent telomere maintenance mechanism known as ALT (alternative lengthening of telomeres)
  - Mutations in *DAXX* or *ATRX* are associated with better prognosis
  - Neoplasms with somatic mutations in *DAXX* or *ATRX* show loss of protein expression by immunohistochemistry (Fig. 2.1e, f)
- Somatic mutations of genes in the cell signaling pathway of mammalian target of rapamycin (mTOR), including *PIK3CA*, *PTEN*, and *TSC2*, occur in approximately 15% of sporadic PanNETs
  - In addition to somatic mutation of the *TSC2* gene, loss of heterozygosity at the *TSC2* locus on chromosome 16p occurs in approximately one-third of PanNETs
  - The alterations in the mTOR pathway may carry clinical significance, as drugs targeting this pathway have been developed for clinical use
- Sequencing of all protein-coding genes in ten PanNETs revealed an average of 16 nonsynonymous somatic mutations per PanNET, far fewer than in invasive ductal adenocarcinoma
- Lack alterations in genes commonly mutated in pancreatic ductal adenocarcinoma (*KRAS*, *TP53*, *SMAD4/DPC4*, *p16/CDKN2A*)
  - Somatic mutations in *KRAS* and *BRAF* have not been identified in PanNETs
  - Rare *TP53* mutations occur in approximately 3% of PanNETs, significantly lower mutation prevalence from that reported in invasive ductal adenocarcinomas (approximately 85%)
    - However, copy number alterations of p53 regulators such as *MDM2* have been reported in PanNETs, suggesting other possible mechanisms for alteration of the p53 pathway in these neoplasms
  - Although rare *SMAD4/DPC4* alterations have been reported in a small set of PanNETs, no alterations were identified in a recent whole-exome sequencing study of PanNETs
  - Promoter hypermethylation of in *p16/CDKN2A* occurs in approximately 50% of gastrinomas, but somatic mutation or homozygous deletion of in *p16/CDKN2A* has not been reported in PanNETs
- Promoter methylation and deletion of *VHL* occurs in up to 25% of sporadic PanNETs and is associated with activation of the HIF1α hypoxia signaling pathway
- Large-scale chromosomal gains and losses (including some recurrent alterations) are also present in PanNETs
  - Moreover, PanNETs with multiple chromosomal abnormalities are more likely to present with metastatic disease and possess a worse prognosis
- The prevalence of microsatellite instability in PanNETs is not known, although none of the tumors in a recent large-scale PanNET sequencing study showed defects in mismatch repair

Neoplasms with Ambiguous Direction of Differentiation

Solid Pseudopapillary Neoplasm

Clinical Features

- Rare pancreatic neoplasm, accounting for <5% of all pancreatic malignancies
- Occur predominantly in young women (mean age 28 years), with a female to male ratio of 9:1
- No known genetic predilection or association with particular genetic syndromes, although one case has been reported in a patient with FAP
- Frequently discovered incidentally on abdominal imaging, also can present with symptoms due to abdominal mass (abdominal pain, nausea, early satiety)
  - Rarely rupture of the tumor can cause an acute abdomen
- Overall prognosis is very favorable, with 85–95% of patients cured after complete surgical resection
  - Even recurrences and metastases are frequently surgically resectable, with only rare patients dying of disease
  - Stage is the best predictor of outcome in patients with solid pseudopapillary neoplasm

Gross and Microscopic Features

- Not localized to a specific part of the pancreas (evenly distributed throughout head, body, and tail)
- Almost all are solitary. Most are grossly well-demarcated and often appear grossly encapsulated
- Wide size range (0.5–25 cm), but on average tumors are large (9–10 cm in greatest dimension)
- On gross cut section, most have both solid areas (soft white-gray to yellow) and cystic degenerative areas (irregularly shaped with friable material and hemorrhage)—proportion of individual components is variable among tumors
- Microscopically, consist of uniform poorly cohesive cells supported by delicate small blood vessels. The neoplastic cells have no known counterpart in the normal pancreas
  - Architecture is often a mix of solid areas and areas with degenerative changes
    - In the degenerative areas, characteristic pseudopapillae are formed when some poorly cohesive neoplastic cells drop away, leaving a thin layer of neoplastic cells surrounding a small blood vessel
    - Cystic degeneration may also occur
  - Although grossly well-demarcated, neoplastic cells often microscopically delicately infiltrate into the adjacent nonneoplastic pancreas
  - Foamy macrophages, cholesterol clefts, and hemorrhage are common
  - Cytoplasm of the neoplastic cells is frequently eosinophilic, though clear or foamy change can occur
    - Vacuoles may be present, and hyaline globules can be a clue to the diagnosis
  - Nuclei are round with stippled chromatin and frequent nuclear grooves
- Immunohistochemistry
  - Abnormal nuclear labeling with β-catenin (Fig. 2.1a)
  - Diffusely positive in neoplastic cells: β-catenin (nuclear and cytoplasmic), CD10, α-1 antitrypsin, progesterone receptor
  - Variably positive in neoplastic cells: synaptophysin, cytokeratin
  - Negative in neoplastic cells: chromogranin, pancreatic hormones (insulin, somatostatin, glucagon), lipase, trypsin, chymotrypsin, estrogen receptors

Molecular Features

- See Table 2.2
- Activating somatic mutations in β-catenin gene (*CTNNB1*) occur in 95% of cases. These alterations lead to abnormal nuclear accumulation of β-catenin protein in almost 100% of cases (Fig. 2.1a)
  - The β-catenin protein has diverse functions in normal cells
    - β-catenin is involved in cell adhesion through its interactions with E-cadherin

    - When not associated with E-cadherin, β-catenin is normally targeted for degradation
    - When degradation of β-catenin is inhibited by Wnt signaling, β-catenin translocates to the nucleus and leads to transcription of target genes
  - Mutations in *CTNNB1* frequently affect key phosphorylation sites, preventing β-catenin degradation
  - *CTNNB*1 mutations lead of overexpression of cyclin D1 protein, a key cell cycle regulator and downstream target of β-catenin, in the majority of solid pseudopapillary neoplasms
  - *CTNNB1* mutations also are associated with loss of E-cadherin in the cell membrane, sometimes with mislocalization to the cytoplasm or nucleus, explaining the tumor's discohesive morphology
    - As a result, solid-pseudopapillary neoplasms do not label with antibodies to the extracellular domain of E-cadherin, while immunolabeling with antibodies to the cytoplasmic domain of the protein produce an abnormal nuclear pattern of labeling
    - No mutations in E-cadherin have been reported
  - Activation of β-catenin signaling (with the overexpression of expression of AXIN2, TBX3, SP5, and NOTUM) and Notch signaling (with the overexpression of HEY1, HEY2, and NOTCH2) pathways has been documented by gene expression profiling
  - Discovery of the genetic underpinning of this tumor (*CTNNB1* mutation) has lead to a crucial diagnostic test (β-catenin immunolabeling) to distinguish solid pseudopapillary neoplasm from other solid cellular neoplasms of the pancreas (Fig. 2.1a)
- Sequencing of all protein-coding genes in eight SPNs revealed very few alterations, with only *CTNNB1* altered in more than one SPN
  - These studies identified an average of three nonsynonymous somatic alterations per SPN, the lowest number of any neoplasm sequenced to date. Several of the SPNs studied contained only one or two somatic mutations
- Lack alterations in genes frequently mutated in pancreatic ductal adenocarcinoma (*KRAS*, *TP53*, *SMAD4/DPC4*)
  - Conversely, pancreatic ductal adenocarcinomas and PanNETs lack mutations in *CTNNB1* and nuclear localization of the β-catenin protein
- Role of large-scale chromosomal gains, losses, and rearrangements remains controversial, with conflicting reports in the literature
  - Multiple case reports of translocations and complex chromosomal abnormalities in single patients, but none have been found to be recurrent in multiple tumors
  - While some studies show recurrent chromosomal gains and losses in small case series, other studies fail to identify any chromosomal gains or losses at all

Neoplasms with Acinar Differentiation

Acinar Cell Carcinoma

Clinical Features

- Rare pancreatic neoplasm, accounting for <2% of all pancreatic malignancies
- Most occur in adults (mean age, 58 years), though a small proportion (5–10%) occur in children
- Male predominance (male to female ratio of 3.6:1)
- No known genetic predilection or association with genetic syndromes
  - Acinar cell carcinomas in patients with germline *BRCA2* gene mutations have been reported to exhibit loss of heterozygosity of the *BRCA2* allele, suggesting biallelic inactivation of the gene in these tumors
- Most frequently present with nonspecific abdominal symptoms (pain, vomiting, weight loss)—jaundice is rare due to growth pattern of tumor

  - Approximately 15% develop lipase hypersecretion syndrome, characterized by subcutaneous fat necrosis, eosinophilia, and polyarthralgia—more common in metastatic disease
- Prognosis is relatively poor, with a 5-year survival of only 25%
  - Outcome for acinar cell carcinoma is better than for stage-matched pancreatic ductal adenocarcinoma
  - Stage is the best predictor of outcome in acinar cell carcinoma

Gross and Microscopic Features

- Occur slightly more often in the head of the pancreas, but can occur anywhere in the gland
- Usually forms solitary mass, but can be multilobulated
  - In contrast to the invasive growth of ductal adenocarcinoma, acinar cell carcinomas are frequently grossly well-circumscribed and may be encapsulated
- Usually large (average 10 cm in greatest dimension)
- Most often a solid mass with a soft, red-tan, and fleshy cut surface, but can have frank necrosis and cystic degeneration
- May invade into adjacent organs as well as into the pancreatic ductal system
- Rarely, a multicystic form can occur (acinar cell cystadenocarcinoma)
- Microscopically composed of neoplastic cells with architectural, histologic, or immunohistochemical evidence of acinar differentiation
  - Multiple possible architectural patterns, most commonly acinar (with pyramidal-shaped cells forming small lumina) or solid (with sheets of cells not forming well-defined structures), though glandular and trabecular patterns can occur
  - Microscopically invasive growth is common (vascular invasion, perineural invasion)
  - Cytoplasm typically contains amphophilic or eosinophilic zymogen granules, though in some cases these granules are not well developed and can be difficult to appreciate on routinely stained sections
  - Nuclei are round to oval, characteristically with a single prominent nucleolus
- Immunohistochemistry
  - Diffusely positive in neoplastic cells: trypsin (most sensitive), chymotrypsin, lipase, elastase, cytokeratin (CK8 and CK18)
  - Variably positive in neoplastic cells: chromogranin, synaptophysin, β-catenin (nuclear and cytoplasmic in a minority of neoplasms)
    - If chromogranin or synaptophysin is positive in >25% of neoplastic cells, the neoplasm should be classified as a mixed acinar neuroendocrine carcinoma

Molecular Features

- See Table 2.2
- Somatic alterations in the APC/β-catenin pathway occur in 20–25% of acinar cell carcinomas, including activating mutations in *CTNNB1* as well as truncating mutations in *APC*
- Lack frequent alterations in genes commonly mutated in pancreatic ductal adenocarcinoma (*KRAS*, *TP53*, *SMAD4/DPC4*)
  - Only rare mutations in *KRAS* and *TP53* as well as rare loss of Dpc4 expression have been reported. Most studies report no alterations in these genes
- Subset of carcinomas exhibit microsatellite instability
- Promoter methylation of several tumor suppressor genes has been reported in few tumors, though the functional consequences of this methylation remain to be explored
- Large-scale chromosomal losses and gains have been reported, including several chromosomal regions that are altered in multiple carcinomas
  - Specific target genes of these alterations have not been identified, and the regions altered are different from those frequently altered in pancreatic ductal adenocarcinoma
  - In addition, most acinar cell carcinomas harbor numerous chromosomal gains and losses

Pancreatoblastoma

Clinical Features

- Rare pancreatic neoplasm, accounting for very small proportion of adult pancreatic malignancies but approximately 25% of pancreatic neoplasms in the first decade of life
- Majority occur in children <10 years of age, but can occur in adults (median age in children, 2.4 years; median age in adults, 40 years)
- Slight male predominance (male to female ratio 1.3–2:1)
- Several cases of pancreatoblastoma reported in patients with Beckwith–Wiedemann syndrome, a disorder associated with imprinting dysregulation on chromosomal 11p, leading to overgrowth of various organs and predisposition to embryonal tumors (Table 2.1)
  - One case reported in a patient with FAP
- Often diagnosed due to palpable abdominal mass in young patients, but can also cause vague abdominal symptoms (pain, vomiting, weight loss)
  - Jaundice is uncommon
- Up to one-third of patients with pancreatoblastoma have elevated serum levels of α-fetoprotein (AFP), due to production of AFP by the tumor
- Prognosis is poor, with an overall survival of approximately 50%
  - Cure by surgery can occur in young patients without metastases, but many patients with an initially complete resection will develop local recurrence (20%) or metachronous metastasis (25%)
  - Stage is the best predictor of outcome in patients with pancreatoblastoma

Gross and Microscopic Features

- Not associated with specific location in the pancreas (evenly distributed throughout head, body, and tail)
- Similar to acinar cell carcinoma, usually forms a solitary mass but can be lobulated, usually grossly well-demarcated, and often encapsulated
- Wide size range (1.5–20 cm), but on average are large (average 10.6 cm in greatest dimension)
- On cut section, usually similar to acinar cell carcinomas—solid, soft, and fleshy, with color varying from gray to tan-yellow
  - Cystic degeneration is common in patients with Beckwith–Wiedemann syndrome
- Microscopically, composed of multiple different components including, at a minimum, cells with acinar differentiation and squamoid nests
  - Acinar component is usually predominant, consisting of relatively uniform cells with round nuclei, prominent nucleoli, and granular cytoplasm, often polarized around small lumina
  - Squamoid nests are a characteristic feature and required to establish the diagnosis
    - They consist of whorled groups of plump squamoid cells with eosinophilic cytoplasm. Focal keratinization can be present
    - The nuclei in these nests are often clear
  - Subset of pancreatoblastomas exhibits a focal neuroendocrine component, consisting of uniform round cells with characteristic finely stippled chromatin
    - Neuroendocrine cells are usually diffusely scattered among cells with acinar differentiation, but may focally form solid nests or trabeculae
  - Minority of pancreatoblastomas also have a ductal component, consisting of columnar cells (often mucin producing) that surround larger lumina
  - Nests of neoplastic cells of all types are separated by variably cellular stroma which can exhibit cartilaginous or even osseous differentiation
  - A primitive component of immature monotonous small round blue cells may also be present
- Immunohistochemistry
  - Acinar component—positive for cytokeratins (CK8 and CK18), trypsin, chymotrypsin, and lipase
  - Neuroendocrine component—positive for chromogranin, synaptophysin, NSE; usually negative for pancreatic hormones (insulin, glucagon, somatostatin)

  - Ductal component—positive for cytokeratins (CK7 and CK19), CEA
  - Squamoid nests—largely negative for most immunohistochemical markers, but the optically clear nuclei contain biotin and may nonspecifically label with a variety of antibodies
    - This nonspecific labeling sometimes highlights the squamoid nests and makes them easier to identify

Molecular Features

- See Table 2.2
- Frequent allelic loss of chromosome 11p (86% of cases in one study)
  - Loss of heterozygosity of 11p has also been reported in other embryonal neoplasms, such as hepatoblastoma and Wilms' tumor, suggesting the possibility of a common genetic pathway in embryonal tumors
- Majority of cases have somatic alterations in the APC/β-catenin pathway, including activating mutations in *CTNNB1* as well as inactivating mutations in *APC*, leading to abnormal nuclear accumulation of β-catenin protein
  - β-catenin localization can be patchy in individual tumors, with nuclear β-catenin most frequently found in squamoid nests—cyclin D1 overexpression is also most frequently present in squamoid nests
- Lack the alterations in genes commonly mutated in pancreatic ductal adenocarcinoma (*KRAS*, *TP53*, *SMAD4/DPC4*)
  - Rare loss of Dpc4 expression has been reported, but no *KRAS* gene mutations or altered p53 expression have been identified
- Several reports describe cases with complex karyotypes with multiple regions of chromosomal loss and gain
  - Trisomy 8 has been reported in a single case
  - No target genes for large-scale chromosomal alterations have been identified

Summary of Molecular Pathology of Pancreatic Cancer

- Neoplasms of the pancreas can be classified morphologically and immunohistochemically. These classifications frequently parallel distinct molecular alterations
- Ductal adenocarcinoma is the most common pancreatic neoplasm
  - Sequential accumulation of somatic alterations in key oncogenes and tumor suppressor genes transform normal cells through noninvasive dysplastic precursors into invasive ductal adenocarcinoma
- Neoplasms with other directions of differentiation (PanNETs, SPNs, acinar cell carcinomas) exhibit distinct sets of molecular alterations not shared with ductal adenocarcinoma
- Knowledge of molecular alterations has led to key diagnostic tests currently in use in pancreatic cancer, such as immunohistochemical labeling for Dpc4 in ductal adenocarcinoma and β-catenin in SPNs (Fig. 2.1)
- Molecular analyses of clinical samples, such as DNA sequencing of pancreatic cyst fluid to distinguish precancerous from benign cysts, show promise in augmenting current diagnostic practice to provide the best possible clinical care

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Molecular Pathology of Liver Tumors

3

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Contents

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Hepatocellular Adenoma

Definition

- Hepatocellular adenoma (HCA) is a benign liver neoplasm of hepatocellular differentiation.

Epidemiology

- The incidence is 1–4/100,000 people in Europe and North America, but lower in Asia.
- Most cases (85%) occur in young women.

L. Cheng and J.N. Eble (eds.), *Molecular Surgical Pathology*,
DOI 10.1007/978-1-4614-4900-3_3,

Etiology

- Exposure to estrogenic (e.g., contraception >2 years) or androgenic (e.g., bodybuilding) steroids including Klinefelter syndrome or steroid hormone-producing lesions.
- Androgen therapy of Fanconi anemia or acquired aplastic anemia.
- Metabolic disease (e.g., maturity-onset diabetes of the young (MODY) type III, hereditary HNF1α mutation, glycogenosis type 1 (von Gierke disease), or type 3 Forbes disease).
- Familial adenomatosis polyposis coli (adenomatosis polyposis coli (APC) mutation).
- β-thalassemia with iron overload.

Clinical

- Symptoms develop in 90% of patients and include mild chronic or acute abdominal pain due to intratumoral or intraperitoneal hemorrhage.
- Adenoma may present with elevated levels of liver enzymes, or as incidental finding of a liver mass by imaging techniques.
- Intraperitoneal hemorrhage may occur in up to 20–25%; the risk is increased in HCA >5 cm.
- Malignant transformation is rare, but has been repeatedly observed in tumors >6 cm.
- Risk for transformation varies between HCA subtypes (see below) and etiology (higher in glycogenoses and drug anabolic abuse). In case of drug-induced HCA, discontinuation of the drug may result in spontaneous regression.

Histopathology

- HCA shows a solid, clonal growth pattern consisting of liver cell plates, which are up to two cells wide and are arranged in sheets and cords with compression of the sinusoids. HCA typically lacks a capsule (↔progressed hepatocellular carcinoma (HCC)).
- The neoplastic hepatocytes of HCA are usually uniform with regular nuclear:cytoplasmic ratio (↔HCC).
- Cell size is typically mildly increased compared to the normal hepatocytes of the surrounding liver.
- Cytoplasm may be normal or paler compared to normal hepatocytes due to glycogen or fat storage.
- HCA may show some nuclear atypia as well as pseudogland formation with bile plugs within canaliculi (especially in setting of anabolic steroids or glycogenoses), which requires careful differentiation from HCC (see below).
- Hepatocytes of HCA are usually surrounded by a regular reticulin framework.
- HCA (except inflammatory HCA, see below) lack ductular proliferations and portal tracts, but preexisting portal tracts may be entrapped in the periphery of the lesion.
- HCA is supplied by (a few) solitary arteries (e.g., unaccompanied by bile ducts).
- Areas of infarction, hemorrhage, or regression indicate an increased risk of spontaneous rupture.

Differential Diagnosis

- Capillarization of sinusoids (CD34 staining), atypia, pseudogland formation, and mitosis should raise the differential diagnosis of highly differentiated HCC (immunohistochemistry (IHC): glypican 3 (GPC3), heat shock protein 70 (HSP70), glutamine synthetase (GS) positive).
- Presence of ductules and fibrosis require separation of inflammatory HCA from focal nodular hyperplasia (IHC: map-like GS expression) and macroregenerative nodule (comparison to surrounding liver tissue).
- Epithelioid angiomyolipoma may superficially resemble HCA, but is HMB45 and Melan A positive.

Molecular Pathology

- HCAs are heterogeneous, clonal lesions that in up to 20% of cases show a few chromosomal alterations.

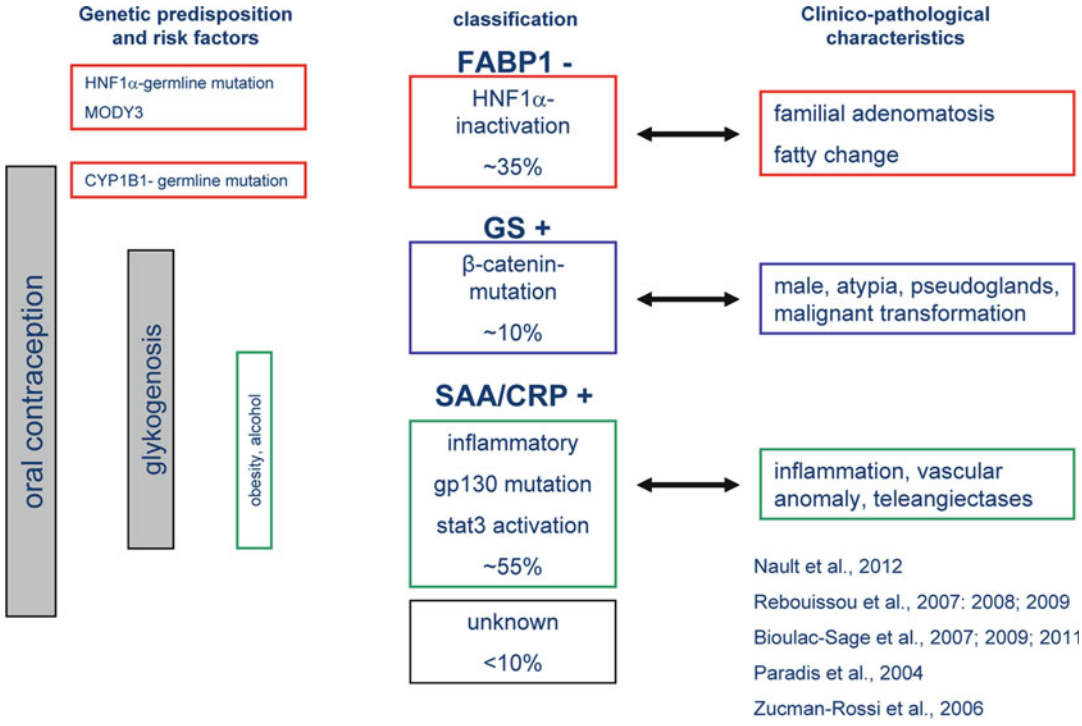


Fig. 3.1 Classification of HCA by genotype and phenotype (material adapted from Rebouissou et al., 2008). *GS* glutamine synthetase; *SAA/CRP* serum amyloid A/c-reacting protein; *L-FABP1* liver-fatty acid binding protein1; *HNF1A* hepatocyte nuclear factor 1-alpha; *CYP1B1* cytochrome P450 family 1, subfamily B, polypeptide 1.

- Genomic aberrations include gains at 1p, 1q, 7p, 11q, 17q, and 20, but the detection of more than two alterations in one tumor favors a different diagnosis (e.g., early HCC).
- The identification of the transcription factor 1 (TCF1) encoded hepatocyte nuclear factor 1α (HNF1α) as a frequently inactivated gene in HCA was the first step to our current understanding of the pathogenesis of HCA, later on complemented by the identification of β-catenin mutations in some HCA and molecular definition of inflammatory HCA.
- Typical histological changes and detection of mutations or their consequences are the basis for our current HCA subtyping. The subtypes described below differ with respect to clinical, genomic, pathological, and radiological features.
- Although epigenetic changes (e.g., aberrant p14(ARF)/p16(INK4a) methylation) have been reported in about 20% of HCA, these features are not included into the current HCA classification that has been independently validated. The genetic predisposition, risk factors, diagnostic marker, and clinicopathological characteristics of HCA subtypes are summarized in Fig. 3.1.

HNF1α-Inactivated Hepatocellular Adenoma

- HNF1α is a transcription factor involved in hepatocellular differentiation.
- Mutational inactivation of HNF1α is found in 35% of HCA.
- 90% of HNF1α mutations are somatic, the remaining ones are inherited.
- Heterozygous HNF1α germline mutations are responsible for maturity-onset diabetes of the

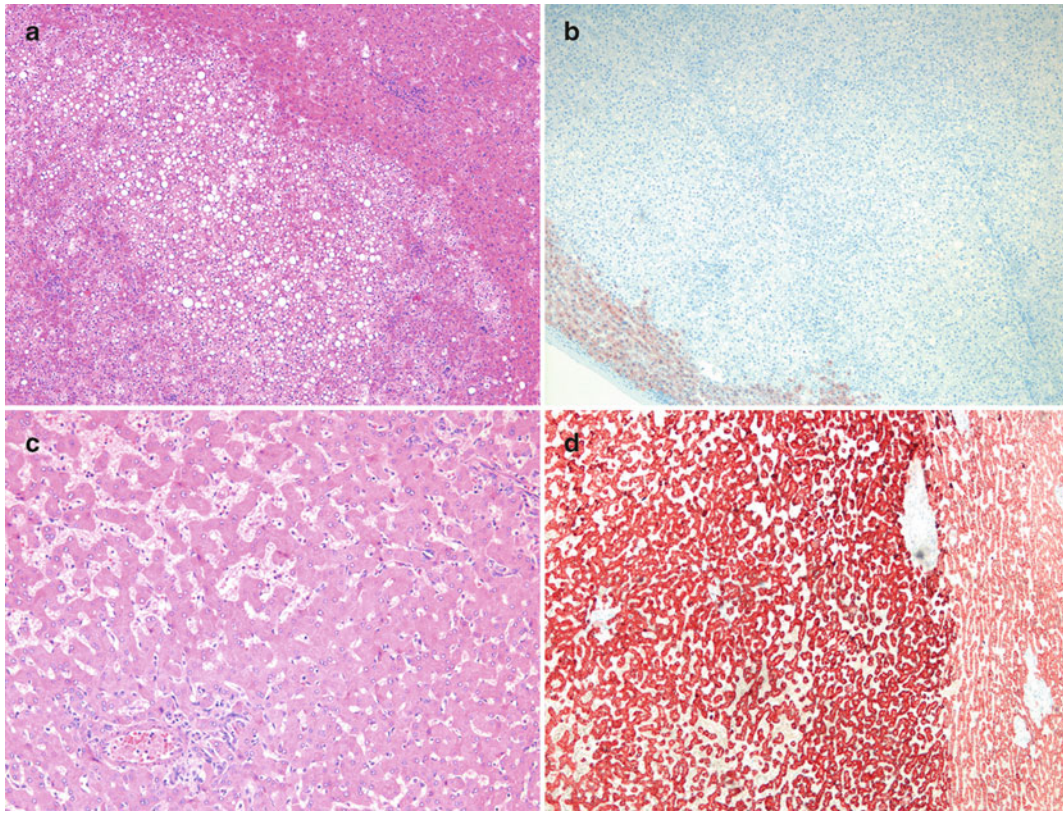


Fig. 3.2 (A+B) HNF1α-inactivated HCA (original magnification 40-fold). (**a**) Note the prominent fatty change and expansive growth. (**b**) The tumor cells show loss of FAPB1 expression. (C+D) Inflammatory HCA. (**c**) Focal sinusoidal dilatation and presence of ductular structures within the tumor (original magnification 100-fold). (**d**) Strong SAA expression of tumor cells in inflammatory HCA (original magnification 40-fold)

young type 3 (MODY3); an autosomal dominant type of diabetes.

- HCA in MODY3 patients carry a second somatic HNF1α-inactivating mutation.
- Germline mutations of CYP1B1 may predispose to the development of somatic HNF1α mutation.
- Histologically, HNF1α-inactivated HCA show severe steatosis, but lack significant inflammatory infiltrates and atypia.
- Liver-fatty acid binding protein (L-FABP) represents an HNF1α target gene. Thus lack of immunohistological L-FABP expression represents as a diagnostic biomarker for HNF1α-inactivated HCAs with the surrounding liver tissue serving as an internal positive control (Fig. 3.2a, b). The down-regulation of L-FABP may contribute to the steatotic phenotype through impaired fatty acid trafficking.
- HNF1α-inactivated HCA occur almost exclusively in women and carry no malignant transformation risk even in the case of adenomatosis.

Inflammatory Hepatocellular Adenoma

- Inflammatory HCA has formerly been termed telangiectatic adenoma or telangiectatic focal nodular hyperplasia; the latter term is now obsolete since the clonal nature of these lesions has been proven. It constitutes the largest subgroup of HCA (55%).
- Histologically inflammatory HCA is characterized by focal or diffuse inflammation and

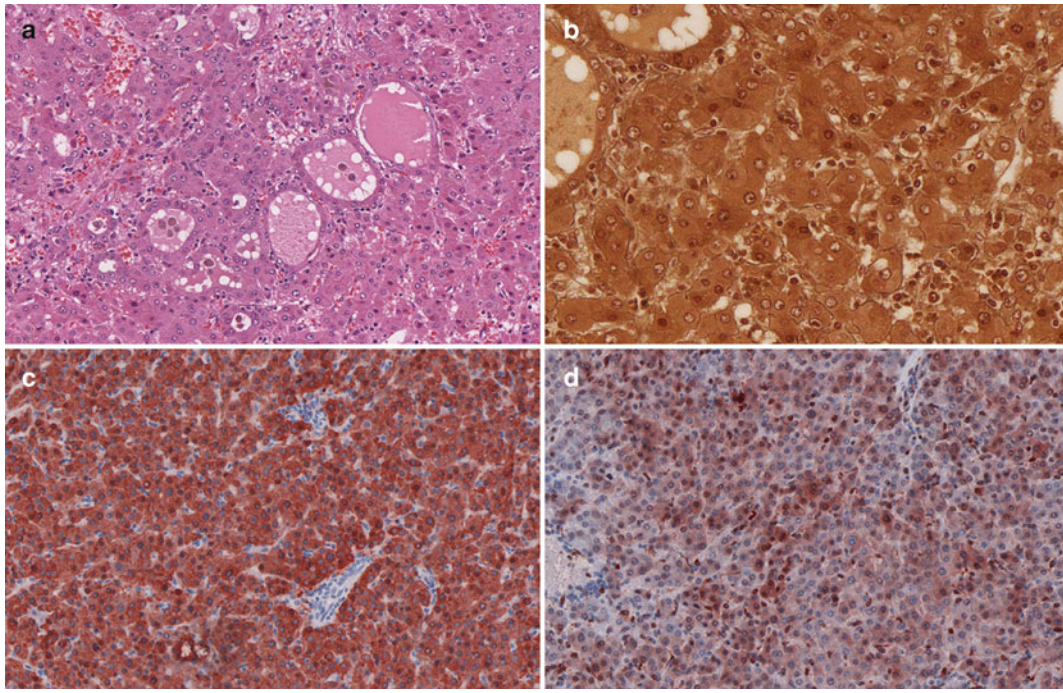


Fig. 3.3 Atypical adenoma detected in a 25-year-old female patient without know liver disease. (**a**) The highly differentiated hepatocellular tumor shows areas of pseudoglandular differentiation (H&E, original magnification 200-fold). (**b**) The reticulin network is lost (modified Gomori stain, original magnification 400-fold). (**c**) Overexpression of glutamine synthetase and (d) HSP70 (original magnification 200-fold)

prominent vascular changes (sinusoidal dilatation, congestion, and thick-walled arteries). A ductular reaction along thin fibrotic septa can be found in inflammatory HCA.

- Increased expression of inflammatory-associated proteins (e.g., serum amyloid A (SAA) and C-reacting protein (CRP)) can be used as immunohistological biomarkers for the differentiation of this subtype (Fig. 3.2c, d).
- Signs of systemic inflammation (e.g., elevated CRP level in serum, increased erythrocyte sedimentation rate) can be found in association with inflammatory HCA.
- About 60% of inflammatory HCA contain mutations in gp130, a coreceptor of IL-6 signaling leading to constitutive activation of IL-6/Stat3 signaling.
- β-catenin and gp130 mutations may occur together indicating that these HCA also carry an increased risk for malignant transformation.
- Inflammatory HCA occurs more likely in women and is associated with obesity and fatty liver disease.

β-Catenin-Activated Hepatocellular Adenoma (Atypical Adenoma)

- Activating mutations of β-catenin are present in about 10% of HCA.
- Glutamine synthetase (GS) is a β-catenin target gene.
- Diffuse and strong immunohistological GS overexpression is a marker of β-catenin-activated HCA, whereas nuclear β-catenin staining is less sensitive and may be seen only in the minority of tumor cell nuclei (Fig. 3.3).
- In equivocal cases β-catenin inactivation can be demonstrated through molecular pathological techniques (e.g., demonstration of

β-catenin mutations using DNA from formalin-fixed, paraffin-embedded tissues).
- β-catenin-activated HCA are preferentially associated with male sex, administration of androgenic anabolic steroids, or glycogenoses, and carry an increased risk for malignant transformation (~50%).
- Distinguishing β-catenin-activated HCA from well-differentiated HCC may be difficult and in some cases arbitrary, since both lesions show pseudogland formation and atypia, whereas this subtype is not featured by steatosis and significant inflammation.
- β-catenin-activated HCC is a clear indication for resection.

Unclassified Hepatocellular Adenoma

- About 10% of HCA have neither HNF1α-inactivation, β-catenin-activation, nor gp130 activation and remain molecularly unclassified so far.
- These HCA do not carry an increased risk for malignant transformation.

Diagnosis

- Radiological diagnosis is difficult due to the variable features (especially vascularization) of HCA, but may be possible for HNF1α-inactivated and inflammatory HCA using magnetic resonance imaging.
- In equivocal cases liver biopsy will confirm diagnosis and allow reliable subtyping.

Prognosis and Predictive Factors

- The risk for rupture and intraperitoneal hemorrhage increases when the tumor diameter exceeds 5 cm and may result in hemorrhagic shock and death.
- Pregnancy is considered a risk factor for rupture.
- HCA >6 cm are at risk for malignant transformation, especially when detected in men with metabolic syndrome.
- HCA >5 cm in diameter should be treated by surgery or local intervention (e.g., embolization, radiofrequency ablation) independent of the subtype due to increased risk of rupture.
- Detection of β-catenin-activated HCA is important due to the increased risk of malignant transformation.
- In the case of adenomatosis liver transplantation can be a therapeutic option.

Hepatocellular Carcinoma

Definition

- Malignant primary liver tumor with hepatocellular differentiation.

Epidemiology

- HCC is the sixth most frequent cancer worldwide and the third most frequent cause of cancer-related death.
- HCC accounts for more than 90% of primary liver cancer with approximately 700,000 new case per year worldwide.
- Depending on the region, incidence ranges from <5/100,000 (e.g., in Northern Europe) to more than 15/100,000 (e.g., in the Far East). In all areas males have a significant higher prevalence than females ranging from 2:1 to 4:1.

Etiology

- In more that 80% of all cases a defined etiology causes chronic liver disease, which represents the basis for the development of cirrhosis and HCC. Most prevalent are infections with hepatitis B virus (HBV) and hepatitis C virus (HCV).
- In addition, chronic alcohol abuse, ingestion of mycotoxins (e.g., aflatoxin B1), as well as hereditary metabolic diseases (e.g., haemochromatosis, glycogen storage disease, tyrosinemia)

carry a high risk for the development of HCC while Wilsons disease, α1-AT deficiency, and exposure to chemicals (e.g., vinyl chloride) are low risk factors.

Clinical

- Symptoms either by tumor itself or by advanced stage of underlying chronic liver disease: abdominal pain, weight loss, nausea, ascites, jaundice, or (hepato-)splenomegaly.
- Raised α-fetoprotein (AFP) levels >400 ng/mL or continuous rising AFP >100 ng/mL are indicative of HCC, but AFP elevation is only found in less than 50% of patients and especially early HCCs (see below) are frequently AFP-negative.
- Other serological markers, including lectin-bound AFP, des-gamma carboxythrombin, golgi protein 73, are not universally accepted.

Histopathology

Classical Hepatocellular Carcinoma

- HCC consists of cells that cytologically more or less resemble hepatocytes and that are separated by a sparse stroma consisting mainly of sinusoid-like blood spaces. These are lined by a endothelial cell layer that, in contrast to the specialized normal liver sinusoids, shows a capillarization as determined by immunohistological expression of CD34, factor VIII-related antigen or Annexin A2.
- Progressed HCC is supplied by newly formed unpaired arteries (e.g., not accompanied by bile ducts) and lacks portal triads, but residual portal tracts may be found entrapped at the periphery of a nodule, whereas the blood supply of early HCCs mainly derives from the portal–venous flow.
- Immunohistologically HCC typically (90% of cases) express carbamoyl phosphatase synthetase 1 (as detected by the Hepar1 antibody), but especially poorly differentiated HCCs may be negative.
- A canalicular expression pattern is found for antibodies against polyclonal CEA (pCEA) and CD10. Other markers that support HCC diagnosis include AFP, fibrinogen, cytokeratin (CK) 8 and CK18, whereas CK7 and CK19 are only positive in a fraction of HCCs that potentially evolve from hepatic progenitor cells (see below).
- HCCs are distinguished from benign hepatocellular lesions through both architectural and cytological atypia, although the differences may be subtle in early HCC.
- Architectural atypia includes irregular trabecular growth pattern (more than two-cells-wide cords separated by capillarized sinusoids), pseudoglandular/acinar growth (pseudogland formed due to abnormal and dilated bile canaliculi between tumor cells), and solid growth. These pattern are frequently admixed, especially in less differentiated tumors.
- Cytologically HCC may be hepatoid, pleomorphic, or sarcomatoid (spindle cells), and may show a clear cytoplasm that requires differentiation from other clear cell tumors (e.g., kidney).
- Fatty change is frequently seen, especially in small tumors. Bile plugs may be seen in dilated canaliculi or in areas with pseudoglandular differentiation. Cytoplasmic inclusions are frequently seen and include Mallory–Denk bodies (aggregates of intermediate filaments), globoid bodies (α1-antitrypsin), and pale bodies (fibrinogen).
- Nodule-in-nodule growth (nodule of less differentiated HCC surrounded by a better differentiated component) is indicative of HCC.
- Histological grading is based on tumor differentiation: well-differentiated (<3 cm, mild nuclear atypia, thin trabeculae), moderately differentiated (more than three-cells-wide trabeculae, round nuclei with distinct nucleoli), poorly differentiated (solid growth, nuclear pleomorphism), and undifferentiated (spindle or round shaped tumor cells with sparse cytoplasm).

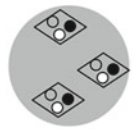
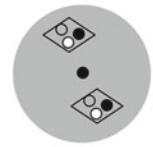
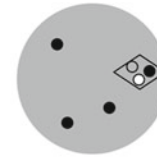
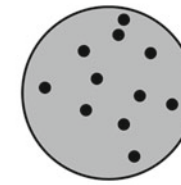
Special Hepatocellular Carcinoma Subtypes

- Fibrolamellar HCC.
  - Fibrolamellar carcinoma (FLC) differs from classical HCC by its clinical, histological, and molecular characteristics.
  - FLC accounts for 1% of all HCC.
  - It occurs mostly in patients younger than 35 years (85% of cases) and women tend to be more frequently affected than men.
  - FLC arises usually in noncirrhotic livers, but etiology and risk factors are not known.
  - Since there is no relation to chronic viral hepatitis, FLC are relatively more frequent in Europe and Asia compared to Asia and Africa.
  - Tumor cells of FLC are large cells with oncocytic cytoplasm (due to innumerable mitochondria), large vesicular nuclei, and large nucleoli. They are arranged in cords separated by (parallel) bands of lamellar fibrotic tissue. As in classical HCC pseudogland formation, pale and Mallory–Denk bodies can be found.
  - CK7 immunostaining is typically seen in FLC (↔ most classical HCCs).
  - There is no significant difference with respect to prognosis between FLC and classical HCC without concomitant cirrhosis, but its prognosis is significantly better than for HCC that developed in a cirrhotic liver.
  - Additional special HCC subtypes include scirrhous HCC (tumor trabeculae separated by marked fibrosis along the sinusoid-like blood spaces), undifferentiated HCC (primary liver tumor with epithelial differentiation lacking further lineage differentiation), lymphoepithelioma-like carcinoma (pleomorphic tumor cells intermixed with dense lymphocytic infiltrates, potentially associated with EBV infection), and sarcomatoid HCC (partially or fully comprised of malignant spindle cells).

Differential Diagnosis

- Well-differentiated HCC have to be differentiated from preneoplastic precursor lesions (so-called dysplastic nodules), HCA, and focal nodular hyperplasia.
- Dysplastic nodules (DN) are usually detected in cirrhotic livers.
- DNs show a clonal growth and mild cytological and structural atypia, but lack definite signs of malignancy, like severe trabecular or cellular atypia and interstitial or vascular invasion.
- They are subdivided into low grade dysplastic nodules (LGDN) and high grade dysplastic nodules (HGDN) depending on the degree of atypia.
- Compared to LGDN cell density and atypia is increased in HGDN (due to small cell change and increased nuclear to cytoplasmic ratio resulting in a nuclear crowding).
- Blood supply in LGDN is usually via the portal vessels, but there is a steady increase of newly formed unpaired arteriolar vessels from LGDN over HGDN to early HCC (Table 3.1).
- Early HCCs show more than twice increased cellular density compared to the surrounding liver tissue, trabecular disarray, pseudogland formation, unpaired arteries, and typically interstitial invasion. Portal tracts may be entrapped at the tumor periphery.
- The process of hepatocarcinogenesis represents a (morphological) continuum and the diagnostic features of a given lesion may not be present in the setting of a liver biopsy.
- Biopsy diagnosis of undefined nodules or minute biopsy specimen may need for additional immunohistological evaluation of transformation associated markers. Such a marker panels has been established and includes glypican 3 (GPC3), glutamine synthetase (GS), and heat shock protein 70 (HSP70).
- The oncofetal protein GPC3 is frequently reactivated in early HCCs. Immunohistological expression of GPC (≥10% of tumor cells) has a reported sensitivity of up to 77% and a specificity of up to 96% for the diagnosis of small HCCs.
- GS is expressed by hepatocytes of the pericentral acinus and in a periseptal localization in fibrosis/cirrhosis. A strong and diffuse staining (≥10% of tumor cells) without a zonal restriction is considered positive for the diagnosis of early HCC. Positive expression of HSP70 (≥10% of tumor cells) was reported in 80% of HCCs, but only occasionally in DN.
- Applying GPC3, GS, and HSP70 as a three-marker panel significantly increased accuracy

Table 3.1 Classification of small nodular lesions in cirrhotic liver according to International Consensus Group for Hepatocellular Neoplasia (2009)

Feature	Low-grade Dysplastic Nodule (LGDN)	High-grade Dysplastic Nodule (HGDN)	well-differentiated HCC	Moderately differentiated HCC
Anatomical change				
Gross appearance			Vaguely nodular	Distinctly nodular
Stromal invasion	(-)	(-)	+/-	+/-
Contrast-enhanced imaging:				
Arterial suppy	Iso-/ hypovascular	Iso-/ hypovascular	Iso-/hypo-/rarely hypervascular	hypervascular
Portal vein supply	+	+	+	-
Clinico-pathological	Premalignant lesion		Early HCC	Progressed HCC

 Intratumoral portal tract ● unpaired artery  Fibrous pseudocapsule

of HCC diagnosis (if at least two of the three markers were positive), particularly when diagnostic interstitial invasion was not seen in a biopsy specimen (Fig. 3.4).

- Further helpful markers include β-catenin, vascular markers (e.g., CD34, CD31, Annexin A2) to detect abnormal capillarization of the liver sinusoids, and CK7 to separate a ductular reaction from an early stroma invasion.

Molecular Pathology

Genomic Instability

- In general, HCC is a chromosomal instable cancer accumulating high numbers of macro- and microimbalances, in part responsible for the activation of oncogenes or inactivation of tumor suppressor genes. The most prominent amplifications of genomic material are present in 1q (57%), 8q (47%), 6p (22%), and 17q (22%), while losses are most prevalent in 8p (38%), 16q (36%), 4q (34%), 17p (32%), and 13q (26%).
- Distinct chromosomal imbalances (gains of 1q21–23 and 8q22–24) precede malignant transformation as they are detectable in a significant number of premalignant lesions.
- Specific chromosomal macroimbalances (e.g., gains of 1q32.1 and losses of 4q21.2–32.33) discriminate between HBV- and HCV-associated HCCs; however, the molecular reason for this observation is unknown, so far.

Epigenetic Changes

- In HCC global DNA hypomethylation has been associated with activation of oncogenes, loss of imprinting, and genomic instability, while hypermethylation of CpG islands located especially in gene regulatory sequences resulted in transcriptional silencing.

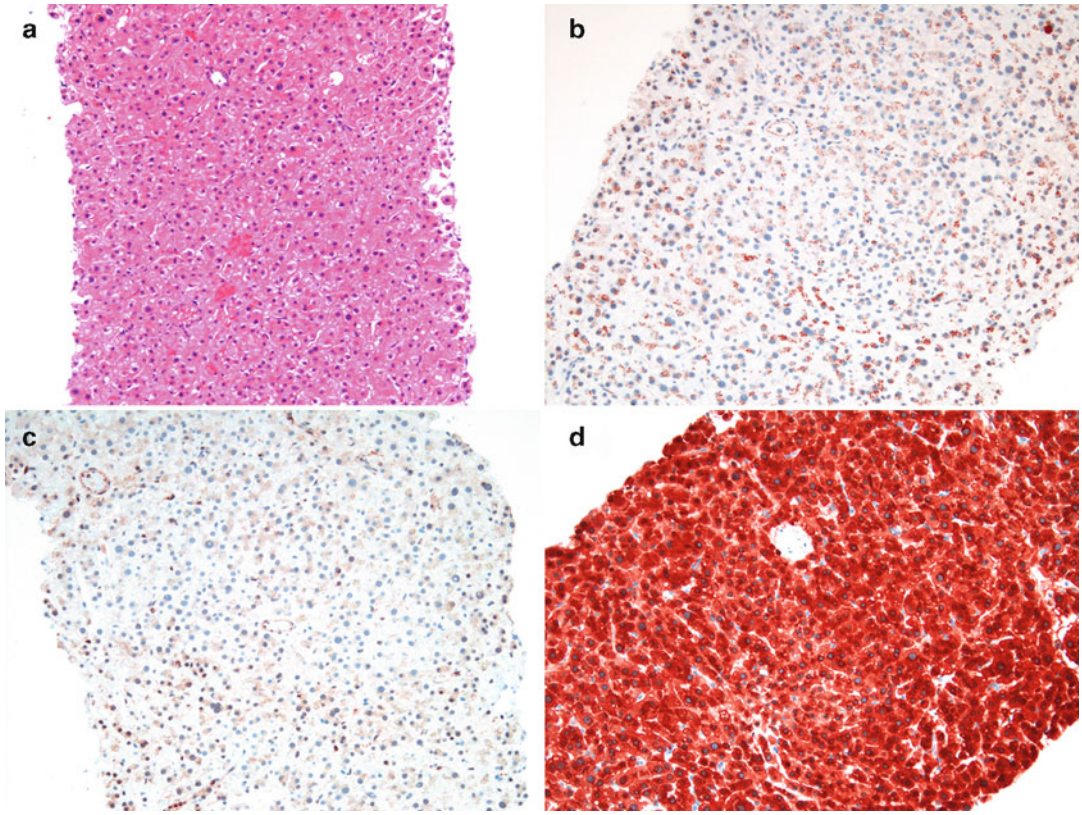


Fig. 3.4 (**a**) Early well-differentiated HCC showing areas of nuclear crowding and mild trabecular disarray. Additionally, a solitary artery can be seen. (**b**) GPC3 is expressed by the majority of tumor cells. (**c**) Predominantly nuclear HSP70 expression in areas with nuclear crowding. (d) Homogeneous overexpression of glutamine synthetase (original magnification 100-fold)

- Polycomb repressive complexes (PRC) target genes are prone to promoter hypermethylation in human HCC, which might be linked to a stem cell like chromatin pattern through de novo methylation in cancer.
- Genomic hypomethylation correlated with genomic instability in HCC while CpG promoter methylation was associated with poor prognosis.
- Methylation changes may occur early in the process of cancer development and CpG island hypermethylation of regulatory regions of tumor-relevant genes is a frequent event accumulating in multistep hepatocarcinogenesis.
- Hypermethylation of CpG islands is associated with the dysregulation of signaling pathway constituents (e.g., RASSF1A), adhesion molecules (e.g., E-cadherin), and cell cycle regulators (e.g., p16/CDKN2/INK4A).

Noncoding RNA/MicroRNA

- miRNA bind complementary sequences in the 3′ end of mRNAs and directly affect promoter activity through binding and or modifying DNA methylation. Therefore miRNAs represent effective posttranscriptional regulators of mammalian gene bioactivity.
- Different stages of hepatocarcinogenesis as well as liver tissues with HBV- or HCV-infection can be differentiated from each other based on their miRNA fingerprints.
- miRNAs such as miR-122a and miR-223 have recurrently been identified by independent screening approaches.

- miR-125b, miR-139, miR-181, and miR-221 are regulators of tumor-relevant proteins and processes in hepatocarcinogenesis.

High and Low Frequent Mutations in Specific Genes

- High frequency mutations in the cell cycle regulator and tumor suppressor gene TP53 can be found in 10–28% of all HCCs. In regions with high aflatoxin B1 exposure, mutations in codon 249 of TP53 can be detected in up to 48% of all HCC cases.
- Stabilizing point mutations and deletions in exon 3 of CTNNB1 (coding for the transcriptional regulator β-catenin) are present in 13–42% of all HCCs. Inactivation mutations lead to an accumulation and nuclear translocation of β-catenin, associated with the transcriptional activation of specific target genes relevant for mitosis and tumor development.
- Loss of heterozygosity at the IGF2R locus (coding for the growth factor receptor IGF2R) has been published for several HCCs and its premalignant lesions, whereas inactivating mutations of the second allele have been described in up to 25% of all cases. Additionally, missense mutations in the extracytoplasmic domain of IGF2R efficiently disrupt receptor/ligand interaction, which may then be followed by increased ligand bioavailability.
- Low frequency mutations (<20% of cases) have been described in AXIN1/2, TCF1/HNFα, PIK3CA, KRAS, p16/CDK2/INK4A, SMAD2/4, RB1, PTEN, ARID1A, ARID1B, and ARID2.

Frequent Aberrant Activation of Signaling Pathways

- High level expression of the insulin-like growth factor (IGF) 2 in up to 40% of all HCCs leads to an activation of the membrane-bound tyrosine kinase receptor IGF1R. Overexpression of IGF2 is predominantly mediated by epigenetic dysregulation of IGF2 gene promoters, IGF-binding proteins, and the presence of IGF2R which directs IGFs to proteasomal degradation. Activation of the IGF-signaling axis supports HCC proliferation, antiapoptosis, and migration.
- Mutations in wingless/β-catenin signaling axis components AXIN1/2 and CTNNB1 increase the nuclear enrichment of β-catenin in up to 40% of all cases. In addition, upregulation of different pathway ligands (e.g., Wnt3/4/5a), receptors (e.g., FZD3/6/7), and pathway modifiers (e.g., PIN1, HDPR1, DKK1) further supports nuclear accumulation of β-catenin. Activation of the Wnt/β-catenin pathway is associated with increased tumor growth and progression.
- The role of TGFβ signaling is controversially discussed, because elevated ligand levels have been detected in serum and urine of HCC patients, while most studies document a reduction of the respective receptors. Moreover, mutations in activating SMADs (SMAD2/4) and overexpression of the antagonistic SMAD7 have been demonstrated. TGFβ signaling may support bifunctional effects; although TGFβ has been suggested to inhibit hepatocyte proliferation, a proinvasive role has been shown, if resistance to its growth inhibitory effects occurs.
- HGF may show higher levels in HCC patients; however, it is not expressed by tumor cells themselves but by stellate cells and myofibroblasts. Its receptor, the tyrosine kinase c-MET, is activated in most HCCs (70%) based on genomic alterations, hypoxia, and growth factor-dependent stimulation. Activation of the HGF/c-MET signaling pathway supports tumor growth and tumor cell invasiveness.
- Several TGFα/EGF ligand family members are highly expressed in up to 80% of HCCs (e.g., TGFα, heparin-binding EGF) and may stimulate the group of EGF tyrosine kinase receptors. Furthermore, overexpression of these receptors (EGFR/HER1, HER2, HER3, and HER4) has been demonstrated in most HCCs (e.g., EGFR/HER1 in up to 70%). Stimulation of the TGFα/EGF signaling axis supports HCC cell proliferation
- Constitutive activation of growth factor pathways occurs at different levels: increased ligand and/or receptor bioavailability, mutational inactivation or constitutive activation of pathway constituents, and aberrant activity/expression of cytoplasmic downstream effectors.

Diagnosis

- Besides serum AFP level noninvasive HCC diagnosis is based on contrast-enhanced (CE) imaging studies.
- The standard techniques are CE computer tomography (CT), magnetic resonance tomography (MRT), and sonography.
- Typical findings in progressed HCCs are hypervascularity during arterial imaging phase followed by a rapid washout during portal venous phase.
- Early HCC cannot reliably diagnosed using these imaging techniques, since solitary arteries responsible for the blood flow characteristics of progressed HCC are rare in these lesions.
- New techniques like dynamic CE sonography with Kupffer phase imaging or MRT using hepatocyte-specific contrast media like gadolinium-ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB) may improve the noninvasive differentiation of early HCC from dysplastic nodules.
- Liver biopsy is the diagnostic technique with highest specificity and high sensitivity and is recommended in all suspicious lesions (especially less than 2 cm) without diagnostic features.
- Needle-tract seeding is a specific complication following biopsy of HCC and has been observed in 2.7% of cases.
- Since the risk for needle-tract implantation increases concurrent with the number of needle passes the use of guiding needles may significantly reduce this complication and needle track resection during surgery has been recommended.

Prognosis and Predictive Factors

- The overall 5-year survival rate of HCC patients with symptomatic HCC is less than 5%.
- Portal vein thrombosis is associated with worse outcome.
- The recurrence rate within 5 years after curative resection (e.g., partial hepatectomy) is 70% and 5-year survival after resection is 25–74%.
- Liver transplantation results in a 5-year survival rate of 75% in selected patients (e.g. (extended) Milan criteria).
- Local ablative treatment (e.g., transarterial chemoembolization, radiofrequency ablation, ethanol injection) can be used for local tumor control and bridging for liver transplantation.
- Molecular classification of HCC (predominantly based on genomic and transcriptomic analyses) revealed genetic signatures that correlate with differentiation, tumor size, prognosis, vascular invasion, and metastatic potential.
- Expression of e.g., FAS, AFP, Aurora kinase B, and the mitotic index as well as TP53 mutation and a progenitor cell phenotype (e.g., CK19) are known negative predictive factors.

Intrahepatic Cholangiocarcinoma

Definition

- Primary malignant epithelial liver tumor with biliary differentiation. Presumed to arise from epithelium of intrahepatic bile ducts (proximal of bile ducts of second order; more distal tumors (Klatskin tumors) are considered separately with extrahepatic cholangiocarcinoma).

Epidemiology

- Second most frequent malignant liver tumor (5–15% but variable depending on geographic region) but much less frequent compared to HCC.
- Highest incidence in Southeast Asia (liver fluke infestation); age-standardized incidence in Thailand: 88/100,000 males; 37/100,000 females.
- In US higher incidence in African–American population.
- Usually older patients, slight male predominance.
- Incidence increasing, even in nonendemic regions.

Etiology

- In most cases unknown.
- Defined etiologies for more central/hilar types are liver fluke infestation (primarily opisthorchiasis and clonorchiasis), hepatolithiasis (7%; about 50% of ICC associated with hepatolithiasis), primary sclerosing cholangitis (5–15%; highest incidence in Northern Europe), and biliary tree malformations.
- Defined etiologies for peripheral type are similar to HCC and include all causes of nonbiliary cirrhosis, especially viral etiology (HBV, HCV (1,000-fold increased risk in HCV cirrhosis in Japan)).

Clinical

- Nonspecific and depend on location and stage of disease as well as secondary consequences (e.g., cholangitis). General symptoms of malignant tumor disease may be present.
- Symptoms of preexisting/predisposing disease may dominate and mask tumor-related symptoms (e.g., PSC).
- Tumor-dependent hepatomegaly, portal hypertension, ascites are rarely or never present.
- Peripheral mass-forming tumors may grow to large size undetected; tumors close to the hilum may exhibit signs of biliary obstruction.

Histopathology

- Almost exclusively adenocarcinomas.
- Histologically no definitive distinction from other carcinomas of the pancreatobiliary system possible.
- Mainly tubular growth pattern; but basically all adenocarcinoma variants may occur, although at very low frequency; not infrequently tumors arising from the large bile ducts may show an intraductal papillary component; tumor cell anaplasia and sarcomatoid dedifferentiation may occur.
- Tendency to invade along portal tracts, infiltrate lymphatic vessels and perineural sheets (of large portal tracts) (preferentially periductal infiltrating or mixed types).
- Premalignant lesions identified
  - Biliary intraepithelial neoplasia (BilIN (1–3)) preferentially for periductal infiltrating type.
  - Intraductal papillary neoplasia of the bile duct (IPN-B; rare) for intraductal growing type (pancreatobiliary, intestinal, gastric, and oncocytic types).
  - Mucinous cystic neoplasia (MCN; rare; only females) for hepatobiliary cystadenocarcinoma.
- Grading follows standard UICC criteria.

Differential Diagnosis

- Differential diagnosis is aided by immunohistology for pancreatobiliary markers (e.g., CK7, CK19, Ca19-9) and markers for metastasis differentiation in question; ICC cannot be safely distinguished immunohistologically from other carcinomas of the pancreatobiliary system.
- Differential diagnostic problems may include:
  - Benign fibroinflammatory lesions in case of highly differentiated tubular adenocarcinoma (histology, proliferation marker).
  - Mixed hepatocellular–cholangiocarcinoma (for peripheral, mass-forming tumor) demonstration of hepatocellular differentiation (histology; e.g., Hepar1).
  - Metastases of extrahepatic adenocarcinomas.
  - Benign biliary lesions: bile duct adenoma, biliary adenofibroma, von Meyenburg complex, biliary cysts.
  - Premalignant lesions (BilIN, IPN-B, MCN) have to be evaluated meticulously for invasive growth.
- Cytological or biopsy diagnosis in PSC (dominant strictures) is difficult with a sensitivity of about 60–70%.

Molecular Pathology

- The value of most molecular analyses is restricted by the lack of consideration of the different cholangiocarcinoma topographies and types.

- Multiple pathogenetically relevant changes have been identified.
- Chronic inflammation is thought to contribute to cholangiocarcinogenesis; stroma cells as well as cholangiocytes may secrete cytokines (IL-6, IL-8, TGFß, TNFα). Cytokines may induce iNOS leading to increased formation of reactive oxygen species.
- Autocrine IL-6-mediated stimulation leading to enhanced tumor cell survival and promoter methylation of several oncogenes; activated JAK/STAT signaling was correlated with resistance to apoptosis.
- Resistance of cholangiocarcinoma cells to TGFß-mediated growth inhibition (by TGFßR- or SMAD4-mutations) may promote fibrous tissue deposition, neoangiogenesis (via VEGF), and tumor progression.
- EGFR-activation correlated with tumor recurrence and poorer prognosis; also HER2 expression is described to correlate to ICC progression; intracellular mechanisms may involve activation of RAS-RAF-MAPK or COX2; also activation of the HGF-MET axis has been described.
- Dysregulation of wnt-associated signaling (loss of membranous E-cadherin and ß-catenin expression) associated with invasiveness, poor differentiation, and potentially neoangiogenesis (via VEGF).
- Telomerase activation is present in almost all cholangiocarcinomas.
- Activation of the inducible COX2 is frequent in cholangiocarcinoma and has been linked to stimulation of proliferation in vitro and may reduce TRAIL- and FAS-mediated apoptosis.
- Antiapoptotic proteins, such as BCL2 and MCL1 can be highly expressed in cholangiocarcinoma cells; MCL1 overexpression may be a consequence of miR-29 downregulation; furthermore some data suggest NOS-, Notch-, and COX2-mediated antiapoptotic effects.
- Constitutive activation of RAS-RAF-MAPK signaling has been reported at varying frequency. KRAS mutations (20–50%; codon 12>13) were reported to correlate with perineural infiltration and poor prognosis. BRAF mutations are also described in CC.
- Alterations in tumor suppressor genes encompass p53 (20–80%) and p21 mutations, together with MDM2 upregulation leading to functional in activation of p53 in the vast majority of cholangiocarcinomas; furthermore, APC and DPC4 deletions and mutations or hypermethylation (up to 80%) in the promoter region of p16, leading to its downregulation, occur in cholangiocarcinoma.
- CDNK2A inactivation occurs in PSC-related cholangiocarcinoma; enhanced CDNK2A promoter methylation and suppression in hepatolithiasis-associated cholangiocarcinoma.
- Microsatellite instability appears to be infrequent.

Diagnosis

- Diagnosis is based on standard morphology aided by immunohistology.
- Diagnosis of resection specimen includes typing, staging (TNM criteria including vascular and perineural infiltration and resection margin status), grading, and analysis of the nontumorous liver tissue.
- Since in biopsy differentiation from other tumors of the pancreatobiliary system is not definitively possible by histology alone and differentiation from metastatic lesions of other tumors may require additional immunohistological analyses, clinical and imaging information regarding potential extrahepatic tumor manifestations is mandatory.

Prognosis and Predictive Factors

- Intrahepatic and extrahepatic metastases, macroscopic vascular invasion, advanced TNM stage, and noncurative resection are negative prognostic factors.
- Macroscopic type is of prognostic relevance after operation (5-year survival for mass-forming type: 40%; for intraductal growing type: 70%; for periductal infiltrating type: <5%).
- Grading is not an unequivocal prognostic factor; (partial) squamous or sarcomatoid

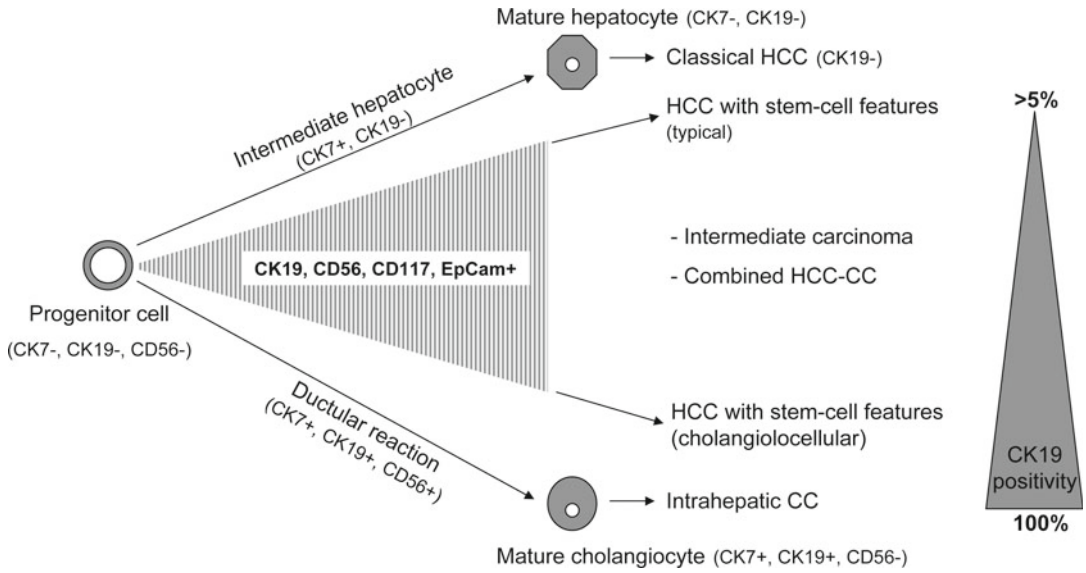


Fig. 3.5 Schematic representation of the possible histogenesis of hepatocellular carcinoma, mixed hepatocellular–cholangiocarcinoma, and cholangiocarcinoma (material adapted from Komuta et al., 2008). *CC* cholangiocarcinoma; *CK* cytokeratin; *EpCam* epithelial cell adhesion molecule; *HCC* hepatocellular carcinoma

differentiation has been correlated with poorer prognosis; mucin typing (MUC5AC vs. MUC2) has been correlated with prognosis but independency has not been demonstrated so far.
- There is no established predictive marker for targeted therapy so far; KRAS mutation status may have potential to predict response to EGFR-targeted therapy.

Combined Hepatocellular–Cholangiocarcinoma

Definition

- Malignant primary liver carcinoma with unequivocal and intimately mixed (↔collision tumor) components of hepatocellular and cholangiocellular differentiation.

Epidemiology

- Combined hepatocellular–cholangiocarcinoma (HCC–CC) represents about 1–5% of all primary liver tumors.
- Whether age, sex, geographic distribution, and etiological risk factors are similar to classical HCC or CC varies significantly between different studies.

Histopathology

- Typically intimately mixed areas of classical HCC and CC are seen.
- Immunohistochemistry can support the dual differentiation (e.g., HCC: Hepar1, CD10, pCEA, (AFP) and CC: CK7, CK19 positive, but HCC areas with progenitor cell features may also be positive).
- A mixed phenotype can be found at the interface of both regions, which has to be separated from HCC–CC with stem cell features.
- HCC–CC with stem cell features (Fig. 3.5) may show nests of mature hepatocytes with peripheral clusters of small cells positive for CK7, CK19, CD56, CD117, and/or epithelial cell adhesion molecule (EpCam); intermediate small ovoid cells with scant cytoplasm (positive for Hepar1 or AFP and CK7, CK19, or CD117) surrounded by a desmoplastic

stroma that may contain ill-defined glands; or cholangiocellular differentiation with small (CK19, CD56, CD117, and EpCam positive) cells with mild atypia growing in a so-called antler-like pattern that consists of cord-like anastomosing tubuli arranged in a fibrous stroma. The latter pattern is considered to reflect the cholangioles of the canal of Hering.

Differential Diagnosis

- The differential diagnosis includes HCC, CC, and a collision tumor of HCC and CC depending on the tissue (biopsy) available for diagnosis.

Molecular Pathology

- Genomic alterations in HCC–CC include loss of heterozygosity (LOH) at 4q, 8p, 16q, and 17p, which are at least partially identical between HCC and CC areas in most cases.
- HCC–CC may show genomic alterations that are more common in classical CC than in HCC (e.g., LOH of 3p and 14q).
- In contrast to HCC, β-catenin mutations have not been described in HCC–CC, whereas TP53 mutations (25–30%) and RB1 deletions may be present.

Diagnosis

- Definite noninvasive diagnosis by imaging is impossible due to the similarities with either classical HCC or CC, but HCC typical imaging combined with elevation of both AFP and carbohydrate antigen 19–9 (CA19-9) may be indicative.
- Radiological imaging aids in the detection of liver cirrhosis, the extent of intrahepatic disease, vascular involvement, and extrahepatic spread.
- Diagnosis may be incidentally made on liver biopsy specimen, but most diagnoses evolve from histological evaluation of surgical specimen.

Prognosis and Predictive Factors

- The prognosis of HCC–CC is worse than HCC and similar to CC with repeatedly reported 5-year survival rates of less than 25%.
- HCC–CC tends to behave like HCC with respect to vascular invasion and like CC with respect to lymph node metastasis
- In noncirrhotic patients partial hepatectomy with hilar lymph node dissection is the best treatment option, whereas in cirrhotic patients the functional reserve of the remaining liver has to be considered before surgery.
- Although the present data are based on experience of single cases, liver transplantation seems no choice due to the short survival time compared to classical HCC.
- Local ablative therapy (e.g., radiofrequency ablation) may be a treatment option for local recurrences.
- Predictive factors have to be evaluated through long-term followup of larger cohorts.

Hepatoblastoma

Definition

- Primary malignant blastomatous liver tumor with hepatic precursor cell differentiation that may show various combinations of several, variably mature epithelial and mesenchymal lineages.

Epidemiology

- Hepatoblastoma (HB) is rare (incidence 1/1,000,000), but represents the most frequent liver tumor in children and occurs slightly more often in males.
- 80–90% of HBs are detected before the age of 5 years, the median age at diagnosis is 1 year.
- Most cases arise sporadic, but the incidence is up to 2,000-fold increased in kindreds with

familial adenomatous polyposis (FAP) and Beckwith–Wiedemann syndrome (BWS; imprinting defect with overexpression of IGF2).

Etiology

- HB is associated with premature birth and low birth weight.
- HB can be associated with congenital diseases and syndromes (e.g., BWS, FAP monosomy 7, trisomy 9, 18 and 21, Acardia syndrome, Goldenhar syndrome, neurofibromatosis type 1, and Prader–Willi syndrome).

Clinical

- Symptoms are typically an enlarged abdomen that may be accompanied by anorexia or weight loss.
- Other unspecific symptoms like nausea, abdominal discomfort or pain may be present, whereas jaundice is rare (<5%).
- HB is associated with paraneoplastic hematological syndromes (e.g., anemia, thrombocytosis).
- Pulmonary metastases are present in 10–20% of patients at diagnosis. Other sites of metastases are skeleton, brain, ovaries, and eyes.
- Marked AFP elevation is found in 90% of cases, the remaining (AFP-negative) cases show a more aggressive course and are associated with a high risk histology (see below).
- In most cases (up to 85%) a single mass is detected in imaging studies.
- Up to 50% of HBs show areas of calcification or ossification.
- Preoperative chemotherapy is able to reduce tumor size in >80% of cases.

Histopathology

- HBs display a variety of morphological differentiation and growth pattern that may be present to a variable extent.
- HBs are classified into pure epithelial type, a mixed epithelial and mesenchymal (MEM) type (with/without teratoid features), and hepatoblastoma, not otherwise specified.
- Wholly epithelial type HB can be subtyped into fetal, mixed fetal and embryonal, macrotrabecular, and small cell undifferentiated (SCUD).
- Fetal subtype HB account for nearly 1/3 of all HBs and the neoplastic cells resemble hepatocytes of the developing liver. Due to variable glycogen and lipid content the cytoplasm of these hepatoblasts shows a typical light and dark pattern at scanning magnification. The nuclei are small and round with fine chromatin distribution. Immunohistologically fetal HBs show a variable AFP and a membranous β-catenin expression.
- Mixed fetal and embryonal HB accounts for 1/5 of cases and areas with embryonal differentiation resemble hepatoblasts of gestational weeks 6–8. The hepatoblasts lack visible glycogen or lipids and have a scant, dark granular cytoplasm and a large nucleus with coarse chromatin. β-catenin is expressed at the cell membrane and in less differentiated areas nuclear staining is seen.
- Macrotrabecular HB is rare and shows wide trabeculae (>6 cells) as the predominant growth pattern. The trabeculae may be composed of embryonal, fetal, and/or hepatocyte-like cells. Macrotrabecular HB (MT) can be divided in MT1 (exclusively hepatocyte-like cells, more aggressive course) and MT2 (fetal/embryonal cells).
- SCUD HBs are composed entirely of noncohesive sheets of so-called small blue cells without discernible cytoplasm. SCUD HBs are very rare and represent the least differentiated subtype that is not associated with AFP elevation. The SCUD phenotype, even if expressed focally, is an adverse prognostic factor (Fig. 3.6).
- MEM type HBs account for 45% of cases and consist of neoplastic epithelial and neoplastic mesenchymal components. About 80% of MEM type HBs do not show teratoid features, whereas the remaining cases display complex heterologous tissues of all three germ layers (e.g., endodermal, neuroectodermal, complex

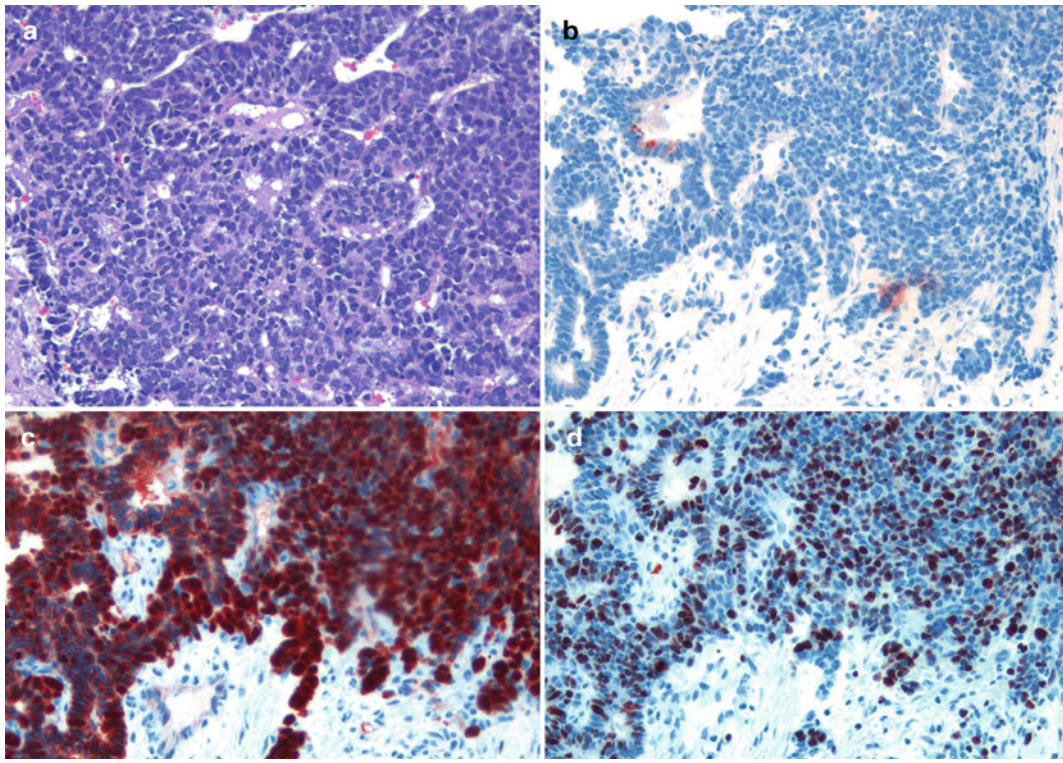


Fig. 3.6 Small cell undifferentiated hepatoblastoma. (**a**) Small cell blue tumor arranged in solid sheets. (**b**) AFP expression is only seen in individual tumor cells. (**c**) Strong nuclear and cytoplasmic β-catenin expression. (**d**) Ki67 stains demonstrate high proliferative activity (original magnification 200-fold)

mesenchymal). Osteoblast-like cells adjacent to osteoid may be epithelial-derived as indicated by CK8 and AFP expression and blending with epithelial components.

- Independent of the subtype neoadjuvant chemotherapy may result in fibrosis, hemorrhage, and necrosis, whereas squamous metaplasia may be seen in residual viable tumor areas. Other treatment-induced changes include ballooning, fatty change, nuclear pleomorphism, and foreign body reaction.
- Special variants include HB with cholangiocellular components, transitional liver cell tumor (TLCT, intermediate between hepatoblast and hepatocyte, giant cells, very high serum AFP), and the calcifying nested stromal epithelial tumor.

Differential Diagnosis

- HB must be differentiated from infantile hemangioendothelioma, mesenchymal hamartoma, or undifferentiated embryonal sarcoma that occur in the same age group.
- Rhabdoid features may occur in SCUD HB and then require differentiation from a primary rhabdoid tumor of the liver, which shows no immunohistological expression of SMARCB1/INI1.

Molecular Pathology

- Although no consistent pattern of chromosomal anomalies has been detected, low number alterations that include gains of 1q, 2, 4q,

Table 3.2 Molecular findings in hepatoblastoma (Material adapted from Zimmermann and Saxena, 2010)

Microsatellite instability	17/21 cases (81%)
TGFβ signaling	Upregulation
PPARα	Upregulation
Adipocytokine signaling	Upregulation
Extracellular matrix-receptor interaction	Upregulation
Apoptosis	Downregulation
WNT signaling	Upregulation
β-catenin	Mutation, deletions in exon 3 and 4
AXIN1	Mutations
APC	Mutations (in cases associated with familial adenomatosis polyposis)
Cell cycle dysregulation	
PLK1	Upregulation
CDKN2A	Promoter methylation
CDKN2B	Loss of expression
DLK1	Upregulation
Dysregulation of IGF-signaling	
PLAG1	Overexpression
11p15 gene cluster	Imprinting errors (BWS)

7, 8q, 10, 12q, 17q, 20, Xp, and Xq as well as losses of 1p, 2q, 4q, and 11p have been commonly reported.

- Gains of 2q, 8q, and 20q have been associated with a poor outcome.
- LOH of the maternally imprinted allele at 11p15 is nearly pathognomonic for patients with BWS. This region encodes p57/kip2, IGF2, and H19.
- Alterations in the APC/ β-catenin pathway play an important role in the pathogenesis of HB as demonstrated by the association with FAP, the presence of APC mutations in sporadic HBs, and the detection of nuclear β-catenin accumulation due to deletions in exons 3 and 4 in about 70–80% of cases.
- Besides the most commonly affected β-catenin and IGF-signaling pathways altered gene expression is found for components of several other pathways (Table 3.2).
- The molecular changes correlate in part with the histological differentiation (Table 3.3), whereas others, like p53 mutation and mismatch repair defects, do not.

Table 3.3 Genotype–phenotype correlation in histological subtypes of hepatoblastoma (Material adapted from Zimmerman and Saxena, 2010)

Histological subtype	Molecular alterations
Small cell undifferentiated hepatoblastoma	Overexpression of MAPK pathways
	Loss of CDKN1B
	FOXG1 overexpression
	Downregulation of Notch signaling (HES1)
	Downregulation of GS
Fetal hepatoblastoma	Large deletion in exons 3 and 4 of β-catenin
Mesenchymal hepatoblastoma	Upregulation of pathways responsible for extracellular matrix-receptor interaction

Table 3.4 Staging of hepatoblastoma according to the Children's Oncology Group

Stage	Criterion
I	Tumor completely resected without microscopic residual disease
II	Presence of microscopic residual disease
	Pre- or intraoperative tumor rupture
III	Unresectable tumor
	Resectable tumor with grossly visible residual disease
	Nodal metastasis
IV	Distant metastasis

Diagnosis

- HB diagnosis is reached via imaging studies in combination with serum AFP level and biopsy.

Prognosis and Predictive Factors

- The Children's Oncology Group staging system (Table 3.4) is used to assess completeness of resection, the most important prognostic factor.
- The PRETEXT (pretreatment extent of disease) system is used for staging before initiating therapy and correlates with overall and event-free survival.

- High PRETEXT stage, low serum AFP, vascular invasion, and certain histological subtypes (SCUD, rhabdoid features, TLCT) predict aggressive behavior, whereas low stage and purely fetal morphology are favorable prognostic factors.
- HB is usually chemosensitive and thus preoperative chemotherapy allows downstaging and enables secondary resection, but this approach has been questioned since about 40% of HBs are primarily resectable and those with pure fetal histology and low mitotic rate do not require additional chemotherapy.
- Liver transplantation is the only treatment option for unresectable HBs and results in 10-year survival rates of 66–78%.
- The only contraindication for transplantation of HBs is the persistence of extrahepatic disease unresponsive to chemotherapy.
- Polo-like kinase 1 (PLK1) expression has negative prognostic value independent of β-catenin mutation, age, stage, and histology.
- Hypermethylation of the RASSF1A promoter is associated with less response to preoperative chemotherapy.

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Molecular Pathology of Gallbladder Cancer

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Contents

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DOI 10.1007/978-1-4614-4900-3_4,

Epidemiology of Gallbladder Cancer

- Gallbladder cancer (GBC) is an infrequent neoplasm in most Western countries but is much commoner in some other parts of the world. It is not included in the statistics of World Health Organization and International Agency for Research on Cancer databases
- High incidence rates of GBC have been detected in indigenous Chilean Mapuches (35/100,000), in women in Delhi, India (21.5/100,000), women in La Paz, Bolivia (15.5/100,000), women in South Karachi, Pakistan (13.8/100,000) and Quito, Ecuador (12.9/100,000).
- GBC affects women two to six times more than men, and its' incidence increases progressively with age in both sexes.
- GBC is not frequent in North America, except for Pima Indians of New Mexico (11.3/100,000) and female immigrants from Latin America, confirming a role for both genetic and environmental factors.
- Susceptibility in native North and South American women, who also present an increased risk of cholelithiasis, may be explained by common ancestral origins, suggesting a genetic component may be responsible for the greater risk in Chile.
- The geographical zone and ethnic and cultural variations in the incidence of GBC suggest that there is a considerable genetic and environmental influence on the development of the disease, which includes diet and lifestyle.
- In Chile, it is the main cause of cancer death in women, with a mortality rate of 7 deaths per 100,000 in men and 15.6 deaths per 100,000 in women, and this pattern has remained constant over the last 18 years.

Risk Factors of Gallbladder Cancer

Gallstones

- The most significant risk factor for GBC is cholelithiasis present in over 95% of chronic cholecystitis, and in 65–90% of patients with GBC. The link between cholelithiasis and GBC has been described since 1861.
- One potential connection between genetic predisposition to stones and incidence of GBC has been suggested by studies on populations where there is a predominance of gallstones.
  - In a study that included three ethnic groups (Mapuches, Chilean Hispanics and Maoris on Easter Island), it was concluded that lithogenic genes are very frequent among Chilean indigenous peoples and the Hispanic populations with the highest rates of GBC mortality.
  - These studies strongly support the thesis that genetic factors play an important role in some specific populations, contributing to lithogenic bile production.
  - Specific polymorphisms of apoproteins and plasma cholesterol ester transfer proteins seem to correlate with cholesterol cholelithiasis.
- Although it is certain that a high percentage of cholelithiasis cases are associated with GBCs, close to 10–25% of patients with this disease have no cholelithiasis reported, and only a small proportion (1–3%) of patients with gallstones develop cancer.
- Various studies have shown that of all the gallbladders operated for cholelithiasis, between 3 and 4% present with GBC at different stages in the specimen or its presumed precursors. For this reason, it has been suggested that cholecystectomy is the fundamental intervention for reducing GBC in high risk areas.

Hyalinizing Cholecystitis ("Porcelain Gallbladder")

- The term "porcelain gallbladder" (PG) has been employed for extensively calcified gallbladder, which has traditionally been regarded as a high risk for cancer and long reported to be associated with carcinoma in 12.5–62% of the patients. However recent large scale radiologic studies have all but eliminated this dogma finding carcinoma in only a very small percentage of cases with calcifications (44 of 25,900 and 15 of 10,741 cholecystectomies, respectively).
- It has now become clear that it is a distinctive type of chronic cholecystitis termed hyalinizing

cholecystitis characterized by extensive hyalinization and effacement of the gallbladder wall by a hyaline fibrosis with minimal or no calcifications ("incomplete porcelain gallbladder") that is a very high risk for carcinoma. In contrast, extensively calcific versions of this process are seldom, if at all, associated with carcinoma.

Gallbladder Polyps

- The presence of polyps is another predisposing factor for GBC. Recent evidence suggests that polyps larger than 10 mm in diameter have higher potential to become malignant.
- If it is diagnosed in asymptomatic patients, even in the absence of gallstones, removal of the gallbladder is recommended.
- Small polyps (less than 10 mm in diameter) must be removed if they are producing symptoms or are associated with gallstones.

Anomalous Pancreatobiliary Ductal Junction

- An anomalous pancreatobiliary ductal junction (APBDJ) is associated with the development of GBC. This anomalous ductal junction is associated with duodenal or pancreatic content reflux into the gallbladder, and this causes bile stasis leading to precancerous changes in the gallbladder mucosa.
- Patients who develop GBC in association with APBDJ are generally young, have a low incidence of gallstones, and are more frequently Asians rather than Westerners.
- This condition is more frequent in the Japanese population and is seen in close to 17% of patients with carcinoma, compared to less than 3% of patients with other hepatobiliary disorders.

Carcinogens

- Studies have shown that methylcholanthrene, o-aminoazotoluene, and nitrosamines can produce GBC in animal experiments.
- Occupational exposure to chemical carcinogens in individuals who work in the rubber industry suggests these compounds may play a role in gallbladder carcinogenesis. In northern India, the use of mustard oil loaded with carcinogenic impurities has been proposed as an etiological factor for GBC.
- A high concentration of the products of free radical oxidation and secondary bile acids has been reported in patients with GBC compared to a control group of patients with cholelithiasis.
- In other analyses conducted on workers who handle cellulose acetate or who work in industries exposed to radon, a higher risk of suffering from GBC was also found.

Chronic Gallbladder Infection

- Although the participation of chronic bacterial infections like *Salmonella* and *Helicobacter* have been reported in gallbladder carcinogenesis, there are contradictory evidences.
- This association has been assumed particularly in countries like India, where typhoid fever is endemic.
- With regards to *Helicobacter*, findings are not consistent and do not support a significant role with respect to the presence of this bacterium in gallbladder carcinogenesis.
- Persistent/recurrent infections are also implicated in the GBCs that occur in patients with hyper-IGM syndrome who develop gallbladder carcinoma in very young ages, as early as teenage years.

Genetic Susceptibility and Polymorphism

- Polymorphisms of several genes have been identified in cholelithiasis includingABCB4, ABCB11, CYP7A1, and ApoB. The genetic variant located in the first exon of ABCG8 gene (ABCG8–D19H) has been found to have a threefold to sevenfold risk of cholelithiasis when heterologous and homologous, respectively.
- ABCG8 is expressed in the canalicular membrane of hepatocytes and small bowel epi-

thelium acting in collaboration with ABCG5 in facilitating the cholesterol secretion. The risk variant would produce an increase in the cholesterol secretion favoring the formation of cholesterol stone.
- This polymorphism is also associated with GBC in Indian, Asian, and Danish populations. It has been suggested that this alteration could be responsible of at least 25% risk to develop GBC.
- Others genetic variants of Ex10 + 837TC (rs5275) en PTGS2 which code the COX2 synthesis have also been implicated.
- Also variants of VEGF have been associated with billary tract cancers and variants of IL8RB, IL8, IL8RB, RNASEL, and variants of NOS2 for cholelithiasis (and thus presumably, indirectly with GBC).

Other Factors

- Epidemiological studies have shown that there is a strong association between GBC and obesity. Gallbladder adenomyomatosis, chronic inflammation of the intestine and polyposis coli have also been linked to GBC.

Carcinogenic Pathways

- In most epithelial tumors, particularly glandular tumors (adenocarcinomas), two models of malignant progression have been shown to be in play: The *metaplasia–dysplasia–carcinoma pathway* and the *adenoma–carcinoma* sequence.
- The first is based on alterations to the normal gallbladder epithelium, whereby metaplasia first appears as an adaptive process secondary to chronic irritation or inflammation. Subsequently, dysplasia develops in the metaplastic epithelium, and gradually progresses into a carcinoma in situ and finally, to invasive cancer. We refer to this as the pathway of non-tumoral intraepithelial neoplasia.
- The second pathway, the adenoma–carcinoma sequence, represents the malignant transformation process from an initially benign glandular proliferation that forms a mass (polyp or papillary lesion) that is variably termed as adenoma or intracystic papillary neoplasm or, as proposed recently, as intracholecystic papillary tubular neoplasms.
- The evidence at the genetic molecular level also demonstrates that the two pathways correspond to two different biological events (see below).

Metaplasia–Dysplasia–Carcinoma Sequence

- In gallbladder carcinogenesis model, the initial lesions on the epithelium of the mucosa are attributable to inflammation.
- The formation of stones may be the primary initiating process.
- Two types of metaplasia can be observed in the gallbladder mucosa: the pyloric and intestinal type. While the former is more ubiquitous the latter appears to be more closely associated with cancer.
- Epithelial dysplasia has been found adjacent to GBCs. Over 80% of infiltrative GBCs show foci of epithelial dysplasia, including its most advanced form, carcinoma in situ.
- In addition to common coexistence of these lesions, molecular evidence also points towards this transformation.
- According to our observations, the period required to progress from dysplasia to advanced GBC is believed to be ~15 years, because a continuum in the progression of the lesions is observed over such period.

Adenoma–Carcinoma Sequence (Intracholecystic Papillary Tubular Neoplasms)

- In the WHO 2010 classification, these lesions are regarded under two groups, "adenomas" and "intracystic papillary neoplasms," We consider those >1 cm under one unified category of intracholecystic papillary tubular neoplasms, similar to what is being done in pancreatic intraductal papillary mucinous neoplasms, or biliary intraductal papillary neoplasms (Figs. 4.1 and 4.2).

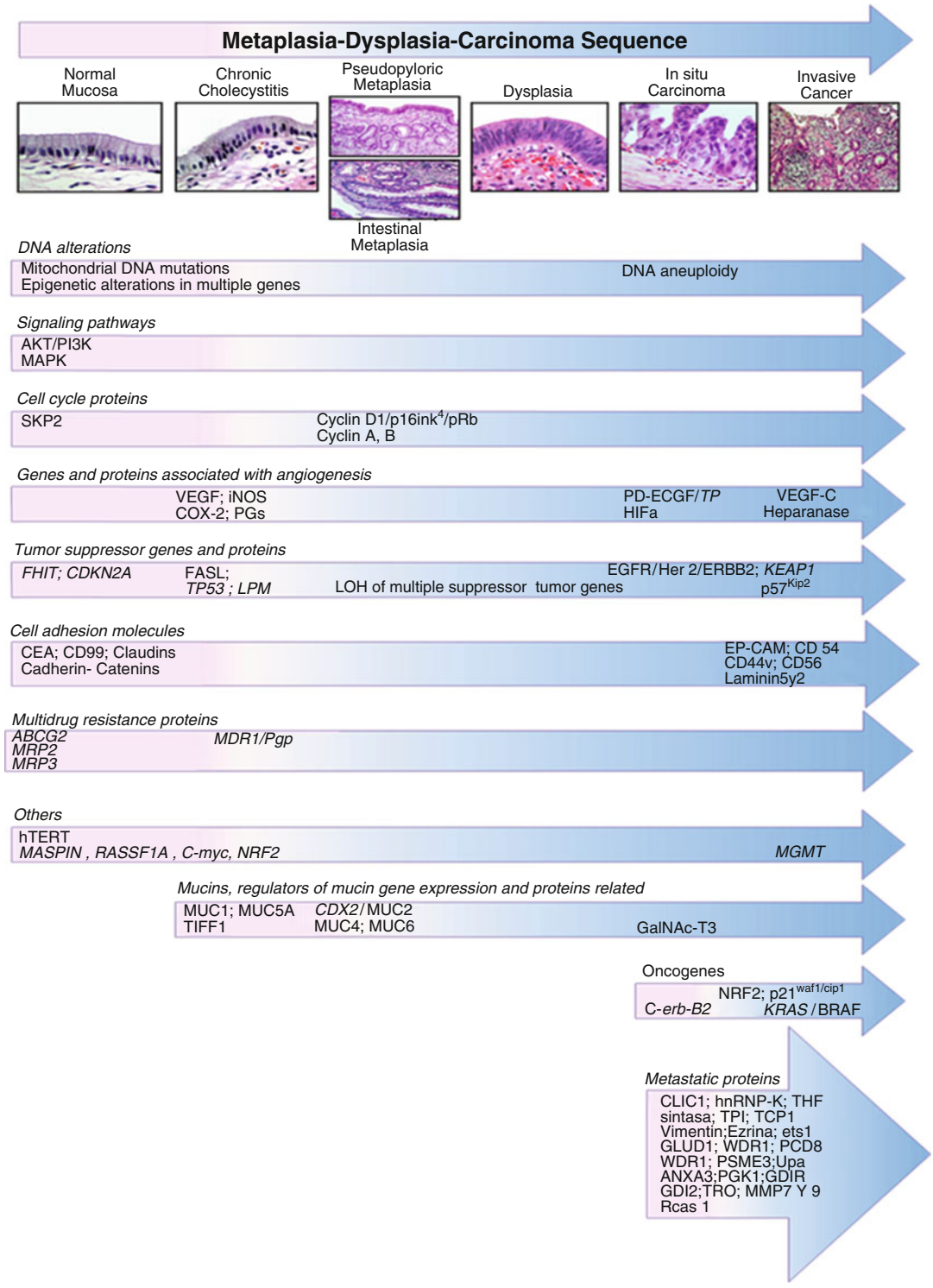

Fig. 4.1 Metaplasia–dysplasia–carcinoma sequence and its main molecular alterations

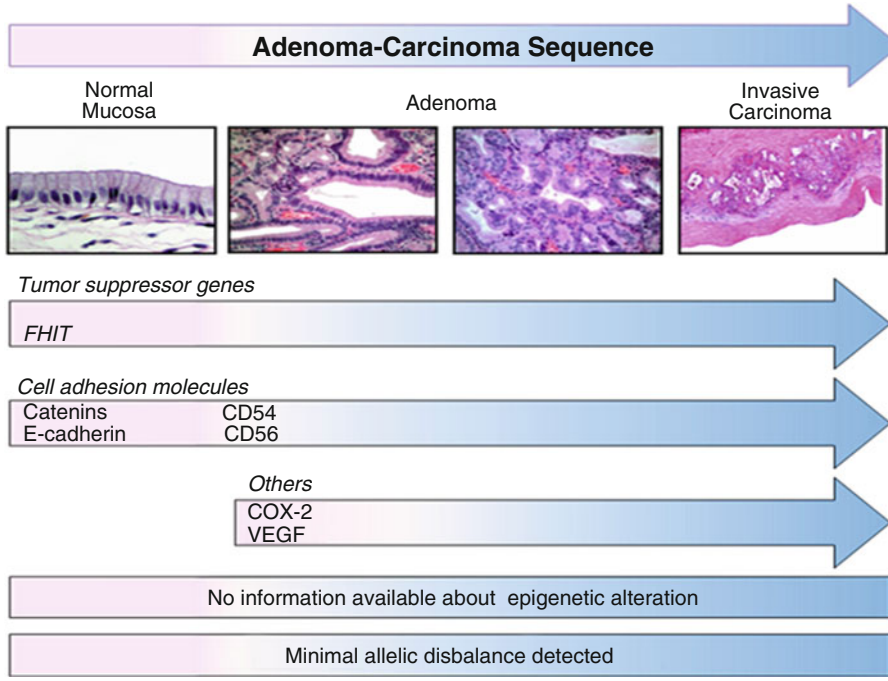

Fig. 4.2 Adenoma–carcinoma sequence and its main molecular alterations

- The adenoma–carcinoma carcinogenic sequence was initially suggested by Kozuka, who reviewed the histology of 1,605 GBCs and found 11 benign "adenomas," 7 with signs of malignancy and 79 with invasive carcinomas.
- In our experience, these "adenomas" and "papillary neoplasms" occur in 0.4 of cholecystectomies.
- About 6–7% of the invasive cancers arise in association with these lesions.
- While the smaller ones and those with pyloric cell phenotype (MUC6 expressing) have a much less likelihood of cancerous transformation, those >1 cm are associated with invasive carcinoma even if they have pyloric differentiation, in 18%.
- The more papillary examples, and especially those with foveolar (MUC5AC expressing) or biliary (MUC1 expressing) phenotype and >1 cm have an association with invasive cancer in more than half of the cases.
- Intestinal (MUC2 and CDX2 positive) and oncocytic types are less common and less well characterized.
- Invasive carcinomas arising from this pathway may have a more indolent behavior, although the data on this is fairly limited.

Oncogenes

KRAS

- A wide range of figures have been reported with respect to activating mutation of the *KRAS* oncogene in GBC.
- While some have failed to identify any mutations in this gene. Others, detected them in between 40% and 50% of GBCs.
- The overall rate of *KRAS* mutation is significantly less than in pancreatic and distal biliary tract carcinomas.
- Those patients with anomalous union of pancreatobiliary ducts have a significantly higher frequency of mutation.
- The increasing incidence of *KRAS* mutation with exposure to pancreatic juice has led to the hypothesis that this mutation may be induced by the pancreatic secretions.

- Mutation of *KRAS* is not detected in carcinomas associated with an "adenoma" (intracholecystic papillary tubular neoplasms), supporting the idea that there are two different genetic pathways related to the two morphological pathways, flat versus tumoral.

BRAF

- Mutations in exon 15 of *BRAF* were observed in 33% of GBCs. The activation at several levels of the *RAS/RAF/MEK/ERK* pathway may constitute an event secondary to Ras activation or it may be due to the activation of points not yet studied in gallbladder carcinogenesis.
- Disruption of MAP kinase *RAS/RAF/MEK/ERK* signaling pathway occurs in around 65% of GBCs, and could therefore be considered one of the most common defects in the pathogenesis of this disease.

Epidermal Growth Factor Receptor

- Overexpression of epidermal growth factor receptor (*EGFR, HER1*) has been reported in 12–100% of GBCs and has been linked to shorter survival.
- The genomic instability due to amplification of *MYC* may be the cause of the amplification of *EGFR* and/or *ERBB2* (*HER2*).
- Overexpression of ERBB2 has been reported between 63 and 69% of gallbladder adenocarcinomas
- *HER2* amplification was identified in more than 20% of GB and extrahepatic bile duct carcinomas. There was strong correlation between *HER2* immunohistochemistry and fluorescence in situ hybridization positivity.

SKP2

- *SKP2* is believed to be a protooncogene causally involved in the pathogenesis of lymphomas. It is considered a potential target for phosphatase and tensin homologue (*PTEN*)-deficient cancers
- *SKP2* overexpression was associated with gene amplification on chromosome 5p11–13 in 53% of the GBC cases.
- Overexpression of *SKP2* immunohistochemically was found to be an independent prognostic factor in GBC, in addition to its correlation with signs of aggressiveness including vascular invasion, advanced T stage, histologic grade, and proliferation index.

Tumor Supressor Genes

TP53

- Even though the mutations in this gene are seen infrequently early on, it is relatively common at the later stages of this disease.
- Mutations of the *TP53* gene, and associated accumulation of p53, have been found in between 27 and 70% of GBCs.
- Molecular studies have revealed that mutations between exons 5–8 are directly related to the deregulation of gene *TP53*, which was observed in patients from two separate geographic areas with high prevalence of GBC, Chile and Japan, with some differences in the type of mutations.

p16/CDKN2/INK4

- The inactivation of this tumor suppressor gene is a common event in human cancers.
- We observed *p16* potential inactivation in 41% of cases, either by LOH (11%) or by methylation (24%).
- There is a loss of immunoexpression from normal epithelial tissue adjacent to GBC (50–90% of cells), decreasing in dysplasia or adenoma (over 50%), and with the least expression observed in carcinoma (10–50%).
- Inactivation of the *p16* gene is associated with a poor prognosis, and also appears to be associated with mutation of the *KRAS* gene.

Fragile Histidine Triad

- The reduction in the immunohistochemical expression of fragile histidine triad (*FHIT*) along with the loss of the alleles is almost universal in GBC, and is detected early in the sequential development of this neoplasia.
- This suggests that the *FHIT* gene is a possible tumor suppressor involved in the pathogenesis GBC.

SERPINB5 (Maspin)

- Maspin is a tumor suppressor gene in the Serpin family.
- *SERPINB5* gene encodes maspin, which is upregulated in GBC when compared with normal tissue.
- Maspin expression is highest in early stages of gallbladder carcinogenesis with a decrease in expression levels in disease progresses.

Promyelocytic Leukemia

- Patients with normal promyelocytic leukemia (*PML*) and p53 expression have been shown to have more favorable outcome, compared with abnormal expression of either of the two proteins.
- *PML*, along with p53, is thus proposed as a prognostic marker.

Microsatellite Instability

DNA Repair System and Microsatellite Instability

- Although in some studies, loss of hMLH1 and hMSH2 (mismatch repair system) has been reported to be high; in our experience, high levels of microsatellite instability (MSI-H) is present in only 10% of GBC, which is slightly less frequent than the incidence in colon and gastric cancers.
- MSI-H phenotype was also found in 33% of intestinal metaplasias and 83% of dysplastic areas associated with MSI-H tumors. In over 90% of cases, the pattern found in these preneoplastic lesions matched the one found in the normal tissue adjacent to the tumor, suggesting that microsatellite instability may be significant in the initial stages of gallbladder carcinogenesis.
- Separately, altered immunohistochemical expression of O6-Methylguanine-DNA methyltransferase (MGMT) was detected in approximately 60% of specimens of GBC and correlated with hepatic invasion and poor prognosis.
- A combined status of altered MGMT and mismatch repair was shown to be a significant prognostic factor in GBC reflecting the accumulation of genetic mutations.

Adhesion Molecules and Mucins

e-Cadherin–Catenin Complex

- The changes to the cadherin–catenin complex is known to lead to alterations of cell–cell adhesion, that facilitates the spreading of neoplastic cells independently in the tissue.
- The cytoplasmic and nuclear expression of beta-catenin in carcinomas is associated with a better prognosis.
- In our experience, loss of E-cadherin, beta-catenin, and alpha-catenins occurs in 20–30% of advanced (subserosally invasive) GBC.
- See Fig. 4.3.

Claudins

- Claudins are a family of proteins with a key role in the tight junctions, and regulate the flow of molecules between the cells.
- Decrease in immunoexpression of some claudins, in particular, claudin 10, but to lesser degree also of claudin-2, -3, and -4 was found in carcinomas compared to the normal samples.

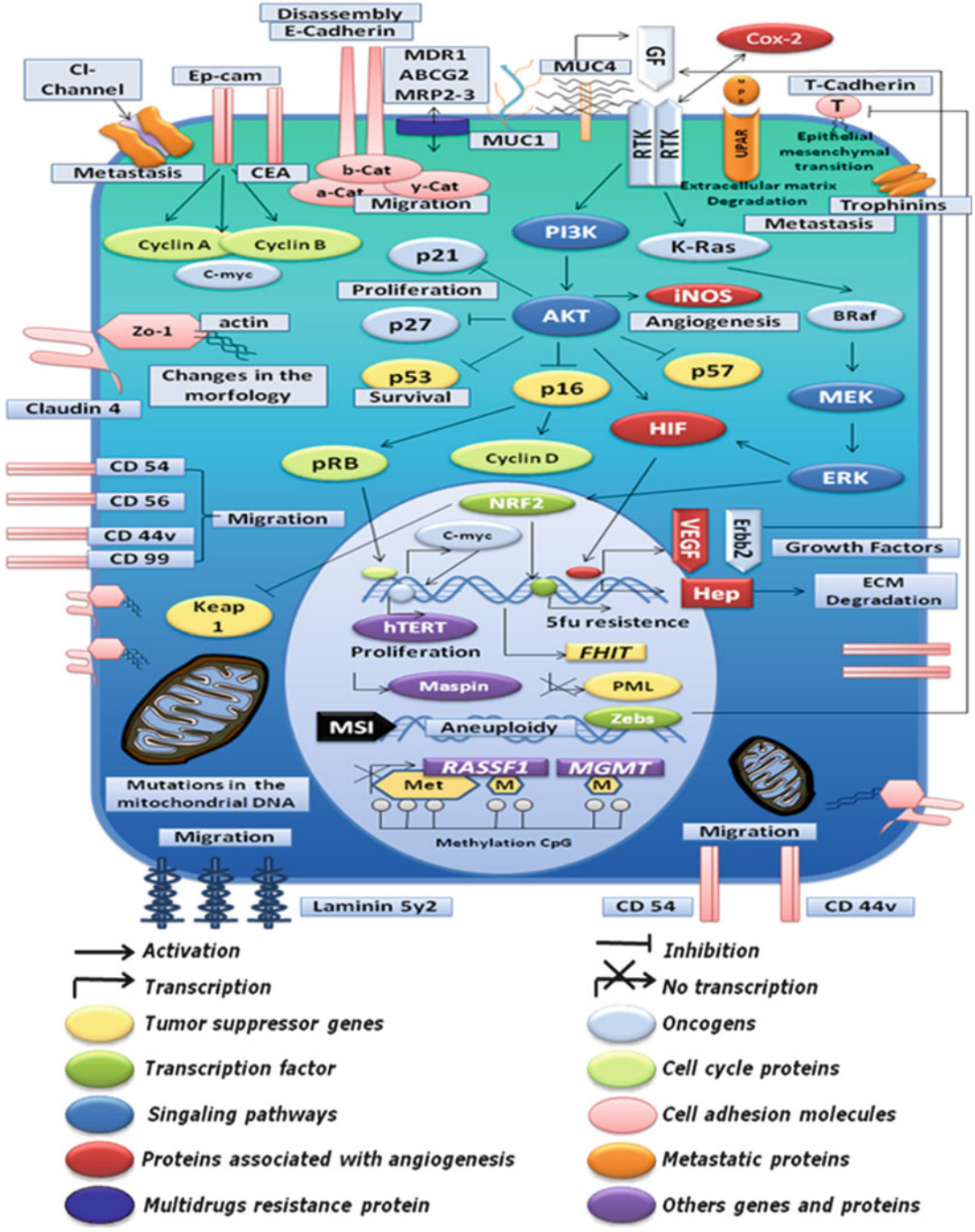

Fig. 4.3 Summary of key molecular aspects involved in GBC (Adapted from Dakubo GD. Field cancerization: basic science and clinical applications. Toronto: Nova Science; 2011)

- Caudin-4, on the other hand, was found to be expressed uniformly in GBC.

EPCAM

- EPCAM is a panepithelial differentiation antigen that is expressed on almost all carcinomas.
- EPCAM appears to be an independent prognostic marker in GBC.

MUC Glycorpoteins, 1, 2, 4, 5a, and 6

- As in the pancreas, MUC1 expression was significantly higher in cancerous tissue than in normal and was associated with GBC progression and lymphatic invasion.
- MUC2 is rarely expressed in GBC but is detected in the goblet cells and intestinal type differentiation areas in nondysplastic as well as dysplastic tissue. It is also detected in some mucinous carcinomas.
- The levels of MUC4 immunoexpression and messenger RNA were increased in GBC specimens. Immunoprecipitation experiments showed an interaction between MUC4 and ERBB2 which are partially colocalized in the apical cell surface.
- In preneoplastic lesions of the gallbladder, inflammation was dependent upon signaling pathways induced by EGFR inducing overproduction of MUC5AC, which accumulates in cholecystitis produced by cholesterol stones.
- As expected MUC6 expression is frequent in pseudo pyloric metaplasia just as in some forms of dysplasia and a subset of carcinomas.
- In intracholecystic papillary tubular neoplasms (adenomas, and "intracystic" papillary neoplasms), the MUC profile corresponds to the cell lineages, with the biliary differentiation areas and high grade dysplastic foci typically showing MUC1, the gastric types showing either MUC5AC or MUC6, and those rare intestinal subtypes showing MUC2.

Other Surface Glycoproteins, CD54, CD56, CD44, CD99, and CEA

- Among the surface glycoproteins involved in cell–cell interactions, adhesion and migration, CD54 expression was detected in 14% of adenomas and in 39% of carcinomas, associated with advanced stages.
- The expression of CD44 was found in 20% of normal tissues studied, in 33% of metaplasias, in 37% of low grade dysplasias, 43% of adenomas, and 24% of carcinomas. CD44v6 variant was found only in late stages of carcinomas in 26% and in 43 of adenomas.
- CEA was demonstrated to increase progressively from 3% in normal to 11% in metaplasia, 33% in low grade dysplasia and 70% in carcinoma.
- The immunohistochemical expression of CD99 showed modest decreases in neoplastic transformation with 95% in a normal gallbladder, 88% in metaplasia, 86% in adenoma, and 67% in carcinoma.

Heparanase

- Heparanase, also known as HPSE, is an enzyme that acts both at the cell surface and within the extracellular matrix to degrade polymeric heparan sulfate molecules into shorter chain oligosaccharides.
- Expression of heparanase was directly associated with tumor size, level of tumor invasion, and poor survival in patients with GBC.

ZEB1 and T-cadherin

- ZEB1 has been implicated as one of the key molecules responsible for epithelial–mesenchymal transition (EMT) and the invasiveness of cancers.
- It has been speculated that the transcriptional regulator ZEB1 physically binds to the T-cadherin promoter, repressing the promoter activity in E-box through a form depending on the sequence, and thus suppresses T-cadherin

expression, and ultimately increasing the invasiveness of GBC cells.

Laminin 5

- Laminin 5 is a component of the extracellular matrix that exert myriad effects on tissues throughout the body.
- The immunostaining of laminin-5y2 has been shown to be a strong predictor of stromal and lymphovascular invasion and subserosal neural infiltration in GBC as well as the frequency of lymph node metastasis.

UDP-*N*-Acetyl-a-*D*-Galactosamine

- UDP-N-acetyl-a-D-galactosamine is the terminal carbohydrate forming the antigen of blood groups, and is necessary for intercellular communication.
- In stage pT2 GBC, a correlation between the immunohistochemical expression of GalNAc-T3 and the depth of invasion into the subserosa was detected.
- It was suggested that as an independent prognostic factor in pT2 carcinomas, the expression of this GalNAc- may help early identification of those patients who need more aggressive treatments.

Epigenetic Alterations in GBC

- Epigenetics is regarded as those heritable changes in gene expression that are not the result of alterations in the nucleotide sequence.
- The main components of the epigenetic code that act as transcription repressors are: DNA methylation, histone modification (phosphorylation, acetylation, and methylation), and altered regulation mechanisms using miRNA.
- The inactivation of tumor suppressor genes by methylation is a frequent epigenetic mechanism in the carcinogenic process and seems to be an early, progressive, and cumulative event in gallbladder carcinogenesis. Its presence has been used as a marker for prognosis and treatment selection.
- Epigenetic alterations also increase from chronic cholecystitis without metaplasia to chronic cholecystitis with metaplasia, and may thus have a role in metaplasia–dysplasia–carcinoma sequence.
- In GBC, ten genes have showed a relatively high frequency of aberrant methylation: *SHP1* (80%), *3-OST-2* (72%), *CDH13* (44%), *P15INK4B* (44%), *CDH1* (38%), *RUNX3* (32%), *APC* (30%), *RIZ1* (26%), *P16INK4A* (24%), and *HPP1* (20%).
- Significantly high methylation frequencies in GBC compared to chronic cholecystitis were detected for eight genes (*3-OST-2*, *CDH13*, *CDH1*, *RUNX3*, *APC*, *RIZ1*, *P16INK4A,* and *HPP1*). The average methylation index of the cases studied was relatively high for GBC (0.196 ± 0.013), compared to chronic cholecystitis (0.065 ± 0.008; $P < 0.001$).
- In a series of 109 advanced cancers examined in our laboratory, a significant association with survival was found in the methylation of *p73* ($P < 0.006$), *MGMT* ($P < 0.006$), and *DCL1* ($P < 0.044$). Subserosal tumors with methylation index equal to or greater than 0.4 were associated with significantly worse survival. A multivariate analysis found methylation of *MGMT* to be a prognostic factor independent of survival ($P < 0.01$). Other studies have shown a high methylation frequency in *SEMA3B* (92%) and *FHIT* (66%); intermediate in *BLU* (26%) and *DUTT1* (22%); and a very low frequency in *RASSF1A* (8%) and *hMLH1* (4%) on chromosome 3p, a tumor suppressor gene candidate locus.
- We have also found that *DAPK1*, *DLC1*, *TIMP3*, and *RARB2* show a progressive increase in their methylation state from chronic cholecystitis without metaplasia to advanced carcinoma that invades the serosal layer.
- In addition, an increase in methylation of *p15*, *APC*, *DLC1*, and *CDH13* was related to poor survival. This finding illustrates the important role that epigenetic process may play in

gallbladder carcinogenesis, and the use of epigenetic changes as prognostic factors as well as in the potential selection of alternative treatments for GBC.

Inflammation

Prostaglandin and Cyclooxygenase

- COX2 is believed to play an important role in gallbladder carcinogenesis. Its overexpression seems to be an early event, occurring in preneoplastic lesions, and is associated with the dysfunction of p53.
- High expression of COX2 in GBC correlates significantly with Nevin stage and lymph node metastasis.
- Prostaglandin PGE2 produced by COX2-expressing carcinoma cells and stromal cells could also be playing an important role in tumor growth and progression through 3 potentials mechanisms: stimulation of cancer cell proliferation by induction and phosphorylation of the AKT PI3-kinase pathways; expression of insulin-like growth factor 1 reception via PI3/AKT; and/or modification of the MAP kinase cell proliferation pathway.

Inducible Nitric Oxide Synthase

- It has been suggested that nitrous oxide (NO) has a dual effect on the development of gallbladder tumors.
- One aspect of this is that the excessive NO induced by inducible nitric oxide synthase (iNOS) may have a significant tumor-inducing effect in chronic cholecystitis and cholecystitis with adenomyoma: iNOS expression has been detected in 88% in cholecystitis, 100% in cholecystitis with adenomyoma and a 71% in adenocarcinoma.
- On the other hand, low iNOS expression in gallbladder adenocarcinomas has been associated with early metastasis and poor prognosis.

FAS Receptor and FAS Ligand in Immune Escape

- FAS receptor (FASR), an important cell surface receptor protein of the TNF receptor family known also as CD95, induces apoptosis by binding FAS ligand (FASL). The FASL protein expression in malignant gallbladder tissue is significantly higher than in normal, and the amount of apoptotic lymphocytes associated with carcinomas are significantly higher as well.
- It is speculated that the overregulation of FASL allows the tumor cells to escape the body's immune monitoring by inducing infiltrating lymphocyte apoptosis in GBC tissues.
- It is also believed that the upregulation of FASL expression plays an important role in the invasiveness, progression to higher grade and metastasis in GBC.

KEAP1 and NRF2

- KEAP1 has been shown to interact with NRF2, a master regulator of the antioxidant response, which is important for the amelioration of oxidative stress.
- KEAP1 mutation occurs more frequently in GBC than in cancer of the bile ducts. These mutations result in the accumulation and activation of NRF2, an important transcriptional factor for many genes.
- NRF2 provides cytoprotection against oxidative agents and increases cell growth, and can partially provide resistance to 5-FU in cancer cells.
- Because NRF2 activation leads to a coordinated antioxidant and antiinflammatory response, and KEAP1 suppresses NRF2 activation, KEAP1 has become a very attractive drug target.

CDX2

- It has been shown that transcription factor CDX2 regulates MUC2 expression by binding to *MUC2* promoter.
- Wu et al. observed *CDX2* and *MUC2* expression in intestinal metaplasia in the gallbladder. In

addition to intestinal metaplasia, CDX2 is expressed in a small subset of intracholecystic papillary tubular neoplasms with intestinal differentiation.
- It is believed that the transcriptional control of *CDX2* may be altered by abnormal stimulation of inflammation.

Trefoil Factor Family 1 Protein

- Trefoil factor family 1 (TFF1) protein is not present in normal gallbladder mucosa, but is upregulated in the inflamed gallbladder epithelium and expressed in large amounts in both primary and metastatic GBC.
- On the other hand, TFF1 expression in primary tumors has been found to decrease with progression of disease (higher tumor stage and higher grade). Furthermore, patients with TFF1-positive tumors showed a more favorable outcome compared to TFF1-negative tumors.

Molecular Carcinogenesis

ERK1/2 and PI3K Signaling Pathway

- The activation of the p-ERK1/2 and PI3K/AKT signaling pathways seems to be involved in the initiation and progression of gallbladder adenocarcinoma.
- A strong immunohistochemical expression was detected for p-ERK1/2 and PI3K in 59 and 51% of GBCs, respectively, compared with very low staining in benign lesions and peritumoral tissues.
- The expression of p-ERK1/2 and PI3K also correlated with poor differentiation, larger tumor size, and the presence of local invasion and lymph node metastasis.

c-FLIP and TRIAL

- c-FLIP was shown to be overexpressed in 74% of GBCs studied and was not expressed in normal or adenomatous tissues.
- Targeted small interfering RNA (siRNA) substantially deregulated the levels of c-FLIP mRNA expression and produced significant apoptosis in GBC cells (GBC-SD and SGC-996) in a TRAIL-dependent fashion.
- It appears that the expression of c-FLIP is upregulated in GBC and the deregulation of c-FLIP sensitizes TRAIL-induced apoptosis, providing a different insight and potentially, a powerful strategy for the treatment of advanced GBC by blocking c-FLIP.

Cyclin E

- Cyclin E is a member of the cyclin family that allows the cell division progress into S phase.
- Overexpression of cyclin E was detected more in women, and in the GBCs with signs of aggressiveness including vascular invasion, tumor necrosis, high histological grade, and high proliferation index.
- In addition, the overexpression of cyclin E was also predictive of patient survival.

Cyclin D1/p16/Rb Pathway

- Disruption of the cyclin D1/p16^{INK4}-pRb pathway is believed to play an important role in the progression of GBC.
- Loss or reduction in p16 and pRb expression has a strong correlation with grade, T stage and metastasis as well as the prognosis.
- Over expression of cyclin D1 is an early event in gallbladder carcinogenesis.

Other Genes or Alterations

Human Telomerase Reverse Transcriptase

- The shortening of telomeres seems to be specific to cells undergoing neoplastic transformation and progression, not identified as commonly in regenerative or reactive processes. It appears to be an early and consistent

finding in the development of biliary tract carcinomas, found in metaplastic (63%) and dysplastic (90%) gallbladder epithelium.
- Along the same lines, the nuclear expression of human telomerase reverse transcriptase (hTERT) in GBC increases progressively with the degree of abnormality in the gallbladder epithelium, form 3% in the normal, to 4% in regenerative, to 25% in low grade dysplasia, 82% in high grade dysplasia and finally 93% in adenocarcinomas.

DNA Ploidy

- Aneuploidy is detected in up to half of GBCs and correlate with higher mitotic Index. This has been found to be higher, up to 80% in those with subserosal invasion.
- No correlation was observed between DNA ploidy patterns and histological type of cancer or other clinical parameters. However, most poorly differentiated adenocarcinomas are aneuploid.

Mitochondrial DNA Mutations

- Mutations in the mtDNA displacement loop (regulatory region) are relatively frequent and early events in the sequential pathogenesis of GBC, being detected in seemingly normal epithelia and chronic cholecystitis.
- The D310 mutations were found in 38% of GBC, 57% of dysplasia and in 46% of uninvolved gallbladder epithelium adjacent to GBC, showing a clonal relationship to the corresponding tumor.
- In patients without GBC, these alterations were detected in 21% of dysplasia and in 25% of injured epithelium in chronic cholecystitis. But only 7% of uninjured normal mucosa showed abnormality of D310.

Vascular Endothelial Growth Factor

- Vascular endothelial growth factor (VEGF) expression is significantly higher in GBC than in normal tissue (80 vs. 63%).
- VEGF expression is directly related to the level of wall infiltration in GBC and is believed to play an important role not only in tumor progression but also metastasis.

Platelet-Derived Endothelial Cell Growth Factor and Thymidine Phosphorylase

- Platelet-derived endothelial cell growth factor and thymidine phosphorylase (PDGF/TP) are important molecules in angiogenesis. Part of the angiogenic effect of Mucose is believed to be through its chemotactic effect on endothelial cells as shown in vitro studies.
- Immunohistochemical expression of PDGF in GBC was found to be associated with advanced disease.
- Mucose in GBC was also associated with angiogenesis and this expression appeared to be an independent prognostic factor.

Hypoxia-Inducible Factors

- Hypoxia-induced factors (HIF), HIF1α and HIF2α, were found to be independently related to VEGF overexpression and high microvascular density in GBC.
- It is of interest that HIF2a was also related to high TP expression.

Vascular Endothelial Growth Factor-C

- Vascular endothelial growth factor-C (VEGF-C) is a lymphangiogenic polypeptide that has been shown to be involved in several solid human cancers.
- VEGF-C may play an important role in tumor progression in GBC through lymphangiogenesis and lymph node metastasis.
- In one study, 63% GBCs studied revealed high VEGF-C protein expression by immunohistochemistry, which correlated with the involvement of lymphatic vessels, lymph node metastasis, and poor prognosis after surgery.

Chloride Intracellular Channel Protein 1 and Urokinase-type Plasminogen Activator Receptor

- Chloride channels are a diverse group of proteins that regulate fundamental cellular processes including stabilization of cell membrane, transepithelial transport, maintenance of intracellular pH, and regulation of cell volume. Chloride intracellular channel protein 1 (CLIC1) expression has been shown to be significantly upregulated in highly metastatic GBC-SD18H cell line compared to the less metastasis-prone GBC-SD18L cell line, and furthermore, it causes increased cell motility and invasiveness of the latter. Additionally, the knockdown of CLIC1 deregulated cell migration and invasiveness of the GBC-SD18H cells.
- The levels of urokinase-type plasminogen activator receptor (UPAR) expression in GBC tissues correlated significantly with metastasis, but not with the degree of differentiation and size of the tumor.
- It has been suggested that UPAR expression may serve as a marker of invasive phenotype of GBC and may be used for prognostication, and even as a target for treatment.

Trophinins

- Trophinins have been found overexpressed in GBC cells.
- The upregulation of trophinins increased cell invasion in vitro. The increase was associated with an increase in proteins found in metastasis, such as alpha3 integrin, MMP7, MMP9, and ETS1.

Conclusion

- In the past decade, many of the puzzles of the cancer of the gallbladder have begun to be gradually revealed. However, most of the information generated is still in its infancy and ought to be verified. The main challenge remains is to determine the true significance of each of the molecular/genetic alteration identified and how they fit the rest of the puzzle, and how they can be translated into reliable prognostic and predictive markers or targets for therapy.

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Molecular Pathology of Lung Cancers

5

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Contents

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Lung Tumor Overview

- The lung is one of the most commonly affected sites of cancer in the body, with involvement by a variety of different types of neoplasms
  - Primary lung carcinomas are subdivided into nonsmall cell lung carcinoma (NSCLC) and small cell lung carcinoma (SCLC) categories
    - NSCLC category includes:
    - Adenocarcinomas (~45%)
      - These most commonly have mixed histologic features, and are categorized according to the dominant pattern (Fig. 5.1)
        - Lepidic (formerly bronchioloalveolar) adenocarcinomas contain extension of cancer cells, often with "hobnail" cytology, along alveolar septae, with minimal invasion of the lung parenchyma
        - Papillary/micropapillary adenocarcinomas contain frond-like growths of cancer cells into alveolar spaces, with (papillary) or without (micropapillary) central fibrovascular cores
        - Acinar adenocarcinomas are the "classic" pattern, with rounded or jagged arrays of cancer cells with a central lumen
        - Solid adenocarcinomas contain tumor cells in solid sheets or detached ribbons, often with areas of "signet ring" cytology

L. Cheng and J.N. Eble (eds.), *Molecular Surgical Pathology*,
DOI 10.1007/978-1-4614-4900-3_5,

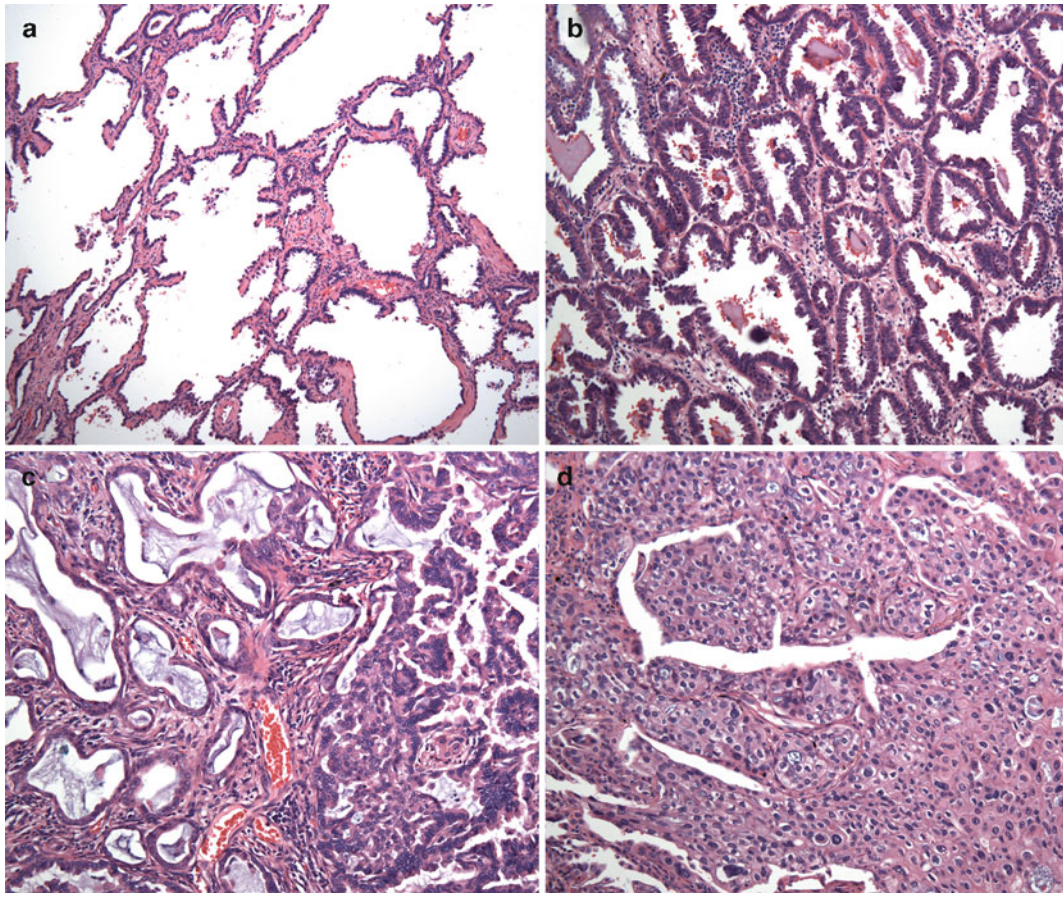

Fig. 5.1 Representative images of lung adenocarcinoma including (**a**) lepidic; (**b**) acinar; (**c**) combined acinar and papillary; and (**d**) solid patterns

    - Large cell carcinomas (~10%)
    - Squamous cell carcinomas (~20%)
  - NSCLC, not otherwise specified (10–30%)
    - This category includes tumors with limited representation on biopsy that cannot be clearly defined based on morphologic and immunohistochemical grounds
  - Well-differentiated neuroendocrine tumors (<5%)
    - Typical carcinoid tumor
    - Atypical carcinoid tumor
  - SCLCs (~15%)
- Metastases to the lung are extremely common, as the predominant organ to receive venous and lymphatic drainage from the rest of the body. Solid cancers of all types can metastasize to the lung
  - Not uncommonly, cancers arising in other sites may present initially with lung metastases. Accordingly, evaluation of a new cancer in the lung should exclude the possibility of a metastasis from another organ
- Other tumor types including sarcomas and lymphomas may arise in the lung, but will not be discussed further here

- Clinical features
  - Primary lung cancer is the most common cancer in the USA
    - Estimated 220,000 new cases in 2011
  - ~90% of lung cancer cases are associated with tobacco exposure

    - Lung cancer was uncommon prior to the mass commercialization of tobacco products in the late nineteenth century
  - Primary lung cancer is the most lethal cancer in the USA
    - Disease-specific mortality rate is ~85%, second only to pancreatic cancer
    - More deaths to lung cancer than the next four most lethal cancers (breast, prostate, colon, pancreas) combined
  - Survival depends heavily on stage
    - Early stage (I–II) disease (ipsilateral lung involvement only, peribronchial, or intraparenchymal nodal metastases): survival is 30–50% at 5 years
    - Advanced stage (III–IV) disease (contralateral lung involvement, mediastinal, subcarinal, contralateral nodal, and/or distant metastases): survival is <10% at 5 years
  - Clinical presentation can be nonspecific
    - Cough, pleuritic chest pain, dyspnea
    - Some patients present with symptoms from distant metastases
  - Diagnosis is suspected by signs, symptoms, and radiology
    - Areas of consolidation on chest X-ray
    - Mass lesions or "ground glass opacities" by CT scan
  - Diagnosis is confirmed by microscopic examination of a lesion
    - Patients often present in advanced stage with unresectable disease
    - Small biopsies and cytopathology specimens are common
  - Treatment
    - Small cell carcinomas: chemotherapy/radiation therapy
    - Nonsmall cell carcinomas
      - Early stage: surgical resection
      - Advanced stage: chemotherapy/radiation therapy and/or molecular targeted therapy
- Molecular genetic pathology
  - Significant advances have been made in the past decade concerning the molecular pathology and clinical applications thereof in adenocarcinoma of the lung, which will be the primary focus of this section. Data are emerging regarding the genetic alterations in squamous cell carcinomas and small cell carcinomas, which have not garnered widespread clinical application as of September 2012. These will be discussed subsequently.

Epidermal Growth Factor Receptor-Mutated Lung Adenocarcinoma

- *EGFR* (epidermal growth factor receptor) gene at 7p12
- Transmembrane receptor protein with cytoplasmic tyrosine kinase involved in transduction of growth factor signaling
  - Member of the ERBB family of receptor tyrosine kinases; also known as *HER1* or *ERBB1*
- 20% of adenocarcinomas have activating somatic mutations in the cytoplasmic tyrosine kinase domain of *EGFR* (exons 18–24), leading to constitutive activation of downstream pathways in the absence of growth factor receptor binding
  - Exon 19 deletions (65%)
    - Variably-sized small in-frame deletions that include at least a four-residue LREA motif in codons 746–749
  - p.Leu858Arg (25%)
    - Missense point mutation (L858R) in exon 21
  - Less common mutations (10%) include:
    - A variable point mutation involving glycine at codon 719
    - Small variable duplication/insertions involving 767–774 in exon 20
    - p.Leu861Gln point mutation (exon 21)
    - p.Thr790Met point mutation
- *EGFR*-mutant lung cancers are more common in women, nonsmokers, and patients of Asian ancestry, although these clinical characteristics are insufficient to use for selecting patients for testing or for therapy
- *EGFR* mutations, *KRAS* mutations, and *ALK* rearrangements are mutually exclusive, except in rare case reports

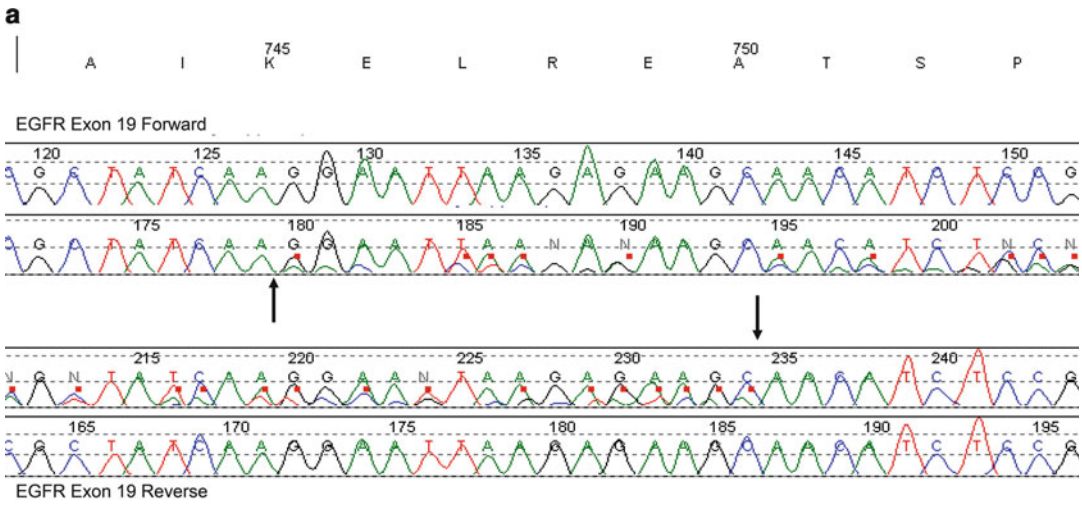

Fig. 5.2 (**a**) Sanger sequencing tracing of EGFR exon 19, showing c.2236_2250del (p.Glu746_Ala750del), a common 15 bp deletion involving the LREA motif. The boundaries of the deletion are defined by the point on the tracing where two overlapping sequences become detectable in the forward and reverse strands (*black arrows*). (**b**) Low levels of mutant are readily detected with the addition of peptide nucleic acids (PNA). Sanger sequencing tracings of EGFR exon 21 showing c.1537T>G (p.Leu858Arg) in (*A*) a sample containing 50% mutant allele; (*B1*) 25% mutant without PNA; (*B2*) 25% mutant with PNA; (*C1*) 0.5% mutant without PNA; (*C2*) 0.5% mutant with PNA

- The mutated *EGFR* allele is frequently amplified
- Molecular diagnostics
  - Indication for testing: therapeutic selection
    - Tumors with the common *EGFR* mutations show better response rates and progression-free survival when treated with *EGFR* tyrosine kinase inhibitors (TKIs) gefitinib or erlotinib than when treated with standard platinum-based chemotherapy
      - Objective response rate (tumor shrinkage by >25%) is ~70–90% in patients with *EGFR* mutations treated with an EGFR TKI, but only 20–30% with chemotherapy
      - Median progression-free survival doubles for patients with *EGFR*-mutant tumors treated with an EGFR TKI, as compared to chemotherapy
      - *EGFR* mutations do NOT predict response to therapeutic antibodies directed against the extracellular ligand-binding domain of the EGFR protein, such as cetuximab
    - Mutations in exon 20 are associated with resistance to EGFR TKIs
      - Exon 20 duplications/insertions are associated with primary treatment resistance
      - Thr790Met point mutation is found in approximately 50% of cases with acquired (secondary) resistance
        - Rare germline Thr790Met mutations have been seen in families with inherited predisposition to lung cancer
  - Additional technical considerations
    - PCR Sanger dideoxyterminator sequencing is the reference method (Fig. 5.2a)
      - Sensitivity is limited to samples with ≥50% malignant cell content
        - The utility of Sanger sequencing is limited in many samples, including small biopsies and cytology samples with benign stromal contamination

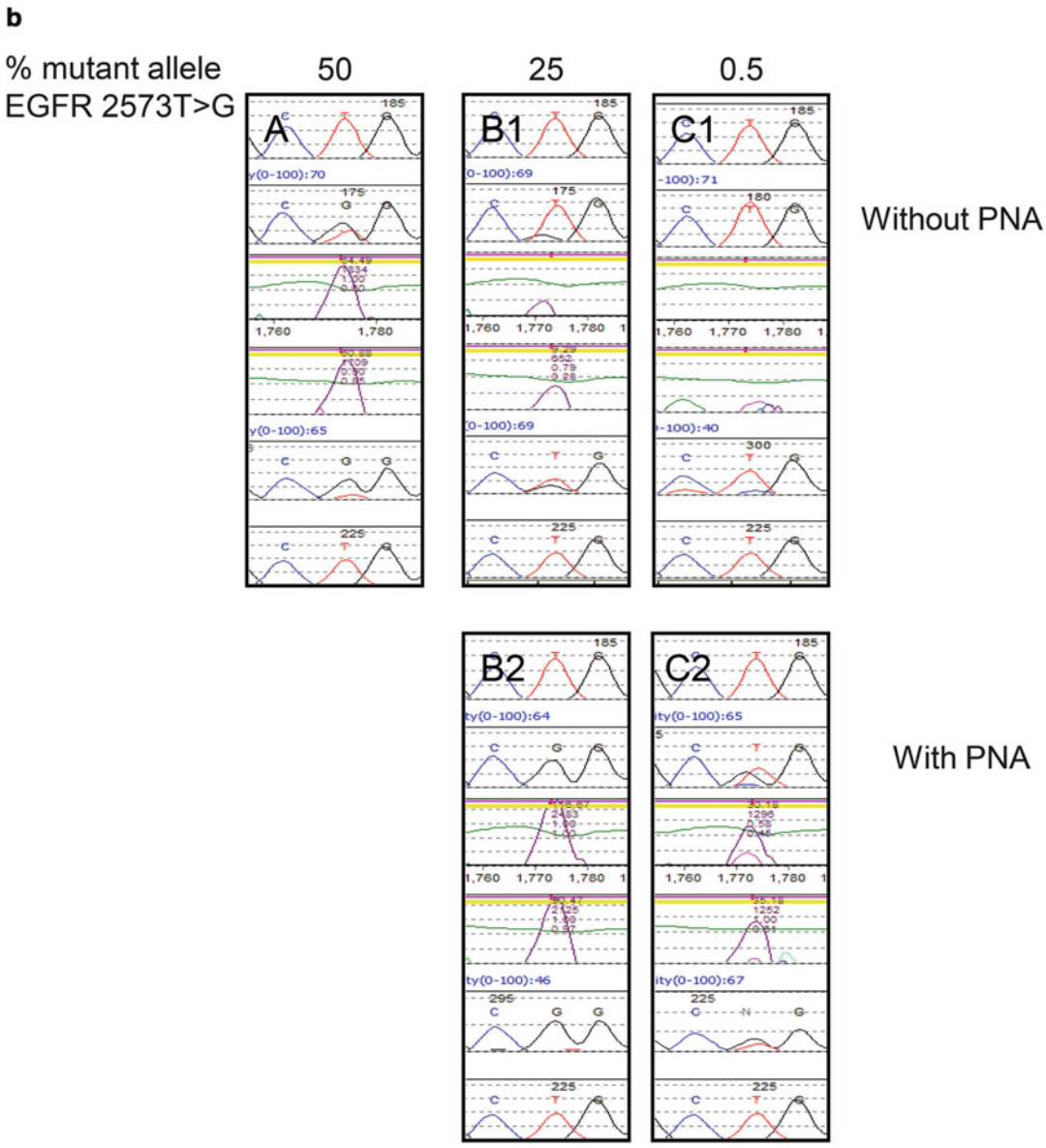

Fig. 5.2 (continued)

- Enhanced sensitivity using:
  - Mutation enrichment strategies
    - Microdissection prior to analysis
      - Gross dissection is preferred to laser capture microdissection, which can be associated with false positive errors due to amplification of a small number of template molecules
    - Selective silencing of wild-type sequences
      - Restriction digestion of wild-type alleles
      - Blocking amplification with peptide nucleic acid (PNA) or locked nucleic acid (LNA) probes (Fig. 5.2b)

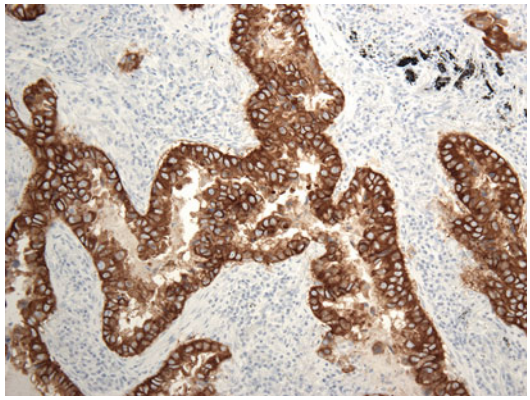

Fig. 5.3 Mutation-specific immunohistochemistry for the Leu858Arg-mutated EGFR protein

- Allele-specific PCR with/without scorpion probes
- Real-time PCR
- PCR with electrophoresis for exon 19 deletions and exon 20 insertions
- Mutation screening with heteroduplex analysis, DHPLC, or high resolution melting

- FISH or CISH to assess *EGFR* copy number is an inappropriate surrogate for mutation analysis. Mutant alleles are often polysomic or amplified, but the association is not absolute, and *EGFR* copy number gain does not correlate with treatment response or progression-free survival
- EGFR immunohistochemistry (Fig. 5.3)
  - Leu858Arg mutant-specific antibody is sensitive and specific for this point mutation
  - Exon 19 deletion mutant-specific antibodies are insufficiently sensitive due to the variability of the amino acid changes at this site and are therefore unreliable as a surrogate for mutation analysis
  - Antibodies to total EGFR or phosphorylated EGFR are NOT appropriate surrogates for *EGFR* mutation analysis in lung cancer

Anaplastic Lymphoma Kinase-Rearranged Lung Adenocarcinoma

- *ALK* (anaplastic lymphoma kinase) gene at 2p23
- Transmembrane receptor tyrosine kinase, originally described in anaplastic large cell lymphoma
  - Member of the insulin receptor superfamily, but its function is poorly understood
  - Also known as CD246
- Approximately 5% of adenocarcinomas of the lung have activation of the ALK kinase by chromosomal rearrangements
  - Most cases contain an inversion: inv(2)(p21p23)
    - Amino terminus of *EML4* fused to the entire cytoplasmic portion of *ALK*
  - Other *ALK* rearrangements have been reported
    - *TFG–ALK, KIF5B–ALK*
    - t(2;5), associated with *NPM–ALK* fusion, the characteristic finding in anaplastic large cell lymphoma, has not been reported in lung cancer
  - Patients with *ALK*-rearranged lung cancers respond better to treatment with a targeted inhibitor of the ALK tyrosine kinase, crizotinib, than to conventional chemotherapy
    - Response rate to crizotinib is ~60% in patients with *ALK* rearrangements
  - *ALK* rearrangements are mutually exclusive with *EGFR* mutations and *KRAS* mutations, except for rare case reports
  - *ALK* rearrangements are associated with younger age, and are more common in males and never or light smokers; ethnic associations are less clear. However, clinical variables are inadequate for selection of patients for testing or treatment
- Molecular diagnostics
  - Test indications: treatment selection
  - Additional technical considerations
    - FISH is the reference method (Fig. 5.4a)
      - Split-apart probes to *ALK* enable the detection of *EML4–ALK* rearrangements as well as other less common *ALK* rearrangements

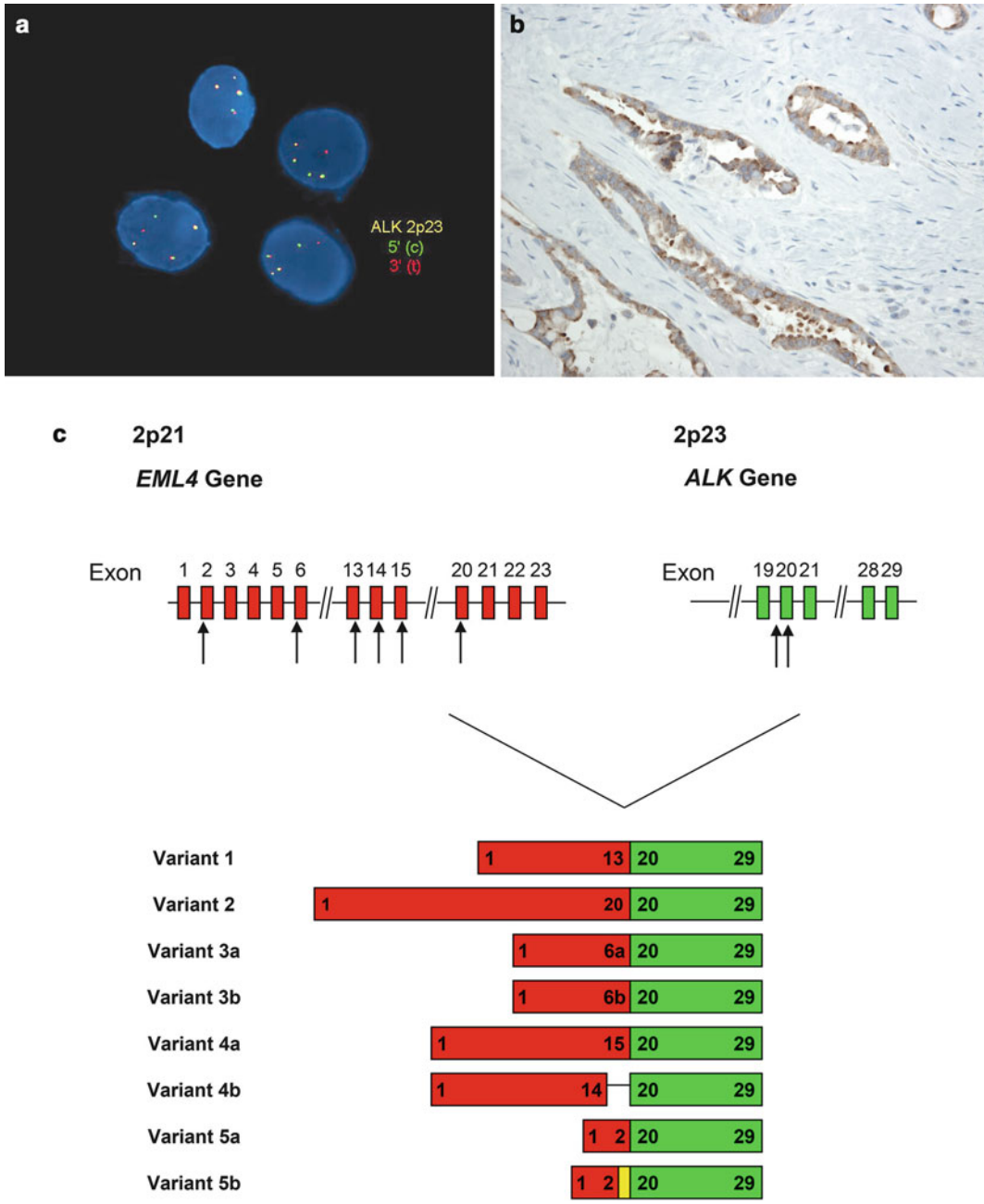

Fig. 5.4 (**a**) *ALK* FISH using *ALK* breakapart probes. The separation of the *green* and *red* signals indicates the presence of a balanced translocation involving the *ALK* locus and is typical of the *EML4-ALK* fusion. Additional copies of the fused (*yellow*) signal indicate the presence of polysomy at this locus, a common finding both in *ALK*-translocated and *ALK* wild-type tumors. (**b**) Immunohistochemistry for ALK using clone 5A4 in an *ALK*-translocated tumor. The ALK protein is expressed in the cytoplasmic compartment. (**c**) Schematic of *EML4-ALK* fusion products, variants 1–5. The breakpoints in *EML4* are highly variable. In most cases, the *ALK* breakpoint occurs in exon 20, however the breakpoint in variant 5 occurs in intron 19. Variant 4b contains an 11 bp linker between *EML4* and *ALK*

- A commercial break-apart assay (Abbott Vysis) is approved by the US FDA for selecting patients to treat with crizotinib
  - Probes can narrowly be split when hybridized to normal (nonrearranged) interphase nuclei, therefore careful interpretation is required
- IHC (Fig. 5.4b)
  - Standard ALK antibodies used for anaplastic lymphoma are insufficiently sensitive to distinguish *ALK*-rearranged lung cancer from nonrearranged lung cancer without additional signal amplification strategies
  - Newer monoclonal antibodies (Cell Signaling clone D5F3, Novocastra clone 5A4) have been shown to correlate well with FISH in small studies
- RT-PCR (Fig. 5.4c)
  - At least 13 molecular variants of *EML4–ALK* have been reported involving fusion of *EML4* exons 2, 6, 13, 14, 15, 17, 18, or 20 to *ALK* exon 20 or intron 19
  - RT-PCR is less practical than FISH for routine testing due to the number of molecular and chromosomal fusion variants and the challenges of analyzing RNA from formalin-fixed tissues

– Resistance to crizotonib is ascribed to secondary mutations in the ALK tyrosine kinase domain, some of which are analogous to common resistance mutations in *EGFR* and *BCR–ABL*
  - L1196M confers high-level resistance and is analogous to T315I in *BCR–ABL* and T790M in *EGFR*
  - Other mutations that affect the crizotonib or ATP-binding sites include 1151Tins, L1152R, C1156Y, F1174L, L1198P, and D1203N
  - Routine testing for these is not yet commonplace, and effective treatments in the setting of secondary resistance have not yet emerged

Kirsten Rat Sarcoma 2 Viral Oncogene Homolog-Mutated Lung Adenocarcinoma

- *KRAS* (Kirsten rat sarcoma 2 viral oncogene homolog) gene at 12p12
- A membrane-associated G-protein mutated in many cancer types
  – One of the earliest discovered oncogenes
- Approximately 30% of lung adenocarcinomas contain *KRAS* mutations
  – Variable substitutions in codons 12 and 13 (>90%) and 61 (uncommon)
  – *KRAS* mutations, *EGFR* mutations, and *ALK* rearrangements are mutually exclusive, except in rare case reports
  – *KRAS* mutations are more common in patients with a history of tobacco smoking
- Molecular diagnostics
  – Test indications: treatment selection
    - *KRAS* mutations are not associated with any targeted treatment response
    - *KRAS* mutations are mutually exclusive of *EGFR* mutations and *ALK* rearrangements; therefore, the presence of a *KRAS* mutation in a tumor can be used to exclude a patient from more expensive and time-consuming testing for *EGFR* mutations and *ALK* rearrangements
      - "Double positive" cases are very rare
  – Additional technical considerations
    - PCR with sequencing is the standard technique (Fig. 5.5)
      - Essentially all relevant mutations are missense substitutions in codon 12, 13, or 61
      - Several different missense substitutions can occur within these codons, so a targeted sequencing strategy is more appealing than hybridization, real-time PCR, or allele-specific PCR
    - *KRAS* has very high homology to other *RAS* family genes, including *NRAS* and *HRAS*, which must be taken into account when designing a *KRAS* assay

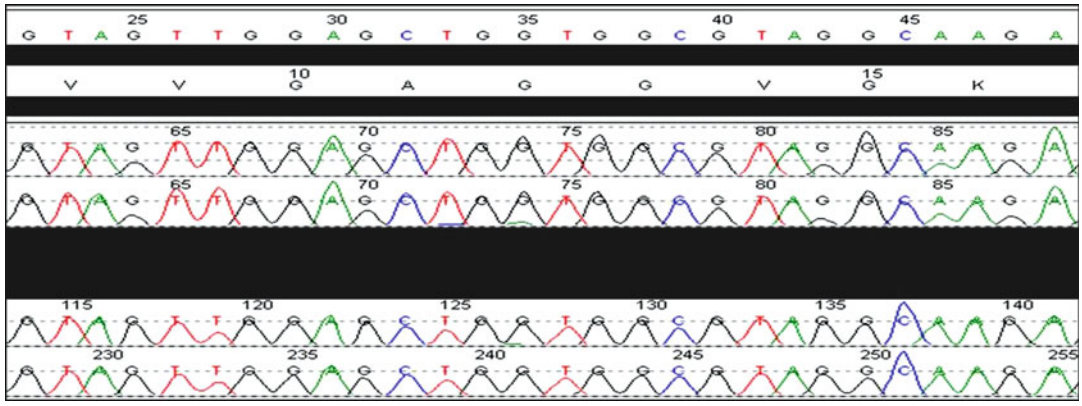

Fig. 5.5 Sanger sequencing tracings of *KRAS* exon 2 showing a low level of c.35G>A (p.Gly12Asp)

Other Mutations in Lung Adenocarcinoma

Less Common Oncogenic Mutations

- Several other genes may play a role in the molecular pathology of lung adenocarcinoma, but are less commonly analyzed in clinical molecular pathology laboratories, as of September 2012
- *BRAF* mutations
  - Seen in ~3% of lung adenocarcinomas, mutually exclusive with *KRAS, EGFR*, and *ALK*
  - Include the p.Val600Glu (V600E) mutation seen in many other tumor types (i.e., melanoma, colorectal carcinoma, papillary thyroid carcinoma), as well as several other mutations that appear to be restricted to lung cancer (especially point mutations in codons 466 and 469)
- *ERBB2* insertion mutations in exon 20
  - Seen in ~2% of lung adenocarcinomas
  - Amplification-mediated *ERBB2* activation typically seen in breast cancer is not common in lung cancer
  - *ERBB2* mutations are mutually exclusive of mutations in *KRAS, EGFR, BRAF*, and *ALK*
- *PIK3CA* mutations
  - Frequently concurrent with mutations in other genes including *EGFR, KRAS, BRAF*, and *ERBB2*
  - Have been described as secondary resistance mutations in TKI-treated *EGFR*-mutant tumors

Tumor Suppressor Gene Mutations

- *LKB1/STK11* mutations are common, found in up to 35% of lung adenocarcinomas, but not in squamous cell carcinomas
- *LKB1/STK11* is commonly inactivated by nonsense mutations together with the loss of heterozygosity
- In vitro studies suggest that concurrent *LKB1* and *KRAS* mutations may predict response to MEK and mTOR inhibitors in lung adenocarcinomas

Other Gene Fusions and Amplifications

- *ROS1* fusions
  - Detected by FISH in ~1% of lung carcinomas
  - *ROS1* is phylogenetically related to *ALK*
  - Lung adenocarcinomas harboring *ROS1* fusions appear to respond to crizotinib therapy, albeit less robustly than tumors with *ALK* fusions

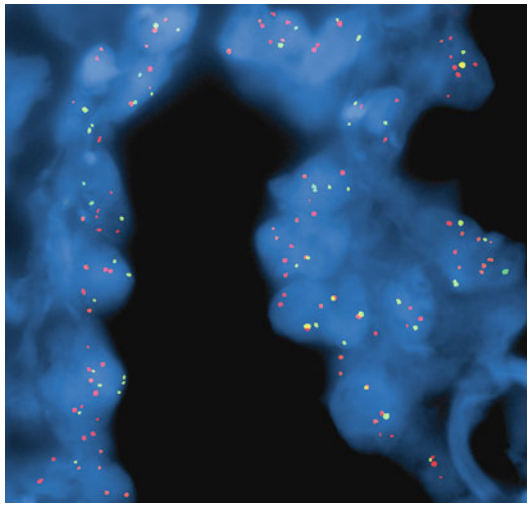

Fig. 5.6 MET FISH using MET (*red*) and centromeric 7 control (*green*) probes. This case shows polysomy at both the MET and Cep7 loci

- *MET* amplification/polysomy
  - As detected by FISH, is seen in ~25% of patients with *EGFR* mutations who develop secondary resistance to EGFR TKIs (Fig. 5.6)
  - In vitro data suggest that MET signaling inhibition can overcome resistance to EGFR TKIs, however there is little evidence for efficacy of MET inhibition in clinical practice

Important Mutations in Other Lung Carcinomas

Squamous Cell Carcinomas

- Typically associated with tobacco exposure
- Approximately 20% have *FGFR1* amplification
  - Response to targeted inhibitors has been shown in preclinical studies
- Approximately 5% have an *FGFR2* mutation
  - Six different mutations have been reported, none more frequent than others
- Approximately 4% have *DDR2* mutation
  - DDR2 is a collagen-binding receptor tyrosine kinase
  - Mutations occur throughout the gene
  - *DDR2* mutated cancers demonstrate response to dasatinib in preclinical studies
- Approximately 2% have *PIK3CA* mutation
  - Missense substitutions in codons 542, 545, and 1047, as are seen in lung adenocarcinomas and in cancers of other organs

Small Cell Carcinoma

- Almost exclusively associated with tobacco exposure
- Initially responds to radiation and chemotherapy but quickly relapses and is rapidly fatal
- Most common mutations are in tumor suppressor genes: *TP53, RB1*, and *PTEN*
- Recent studies of recurrent disease following TKI therapy have shown that *EGFR*-mutant adenocarcinomas can recur as small cell carcinomas and retain the original *EGFR*-activating mutation

Suggested Readings

http://seer.cancer.gov/statfacts/html/lungb.html

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Molecular Pathology of Breast Cancer

6

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Introduction

- Breast cancer is the most common cancer affecting women, with an estimated 250,000 new cases in 2011 in the US alone and 1.5 million worldwide. It is one of the first major diseases where basic laboratory research has had a large impact on the routine clinical management of patients, ranging from detection, to diagnosis, to therapy. Molecular approaches to pathology, in particular, have had an enormous influence, especially in the areas of diagnosis and therapeutic decision-making. The topic of molecular pathology in breast cancer is very large and evolving far too rapidly to cover completely in a chapter of this nature. This chapter will primarily focus on reviewing aspects that are already in routine clinical use, some of the more promising applications on the near horizon, and scientific questions that are currently at the forefront of translational research. From an etiological point of view, the molecular pathology of breast cancer is the result of molecular abnormalities occurring in important normal processes, including the gross, microscopic, and molecular anatomy of the breast, breast development, and adult physiology—which is where we begin

L. Cheng and J.N. Eble (eds.), *Molecular Surgical Pathology*,
DOI 10.1007/978-1-4614-4900-3_6, © Springer Science+Business Media New York 2013

Normal Characteristics of the Female Human Breast

Gross, Microscopic, and Molecular Anatomy

- Grossly, the size of the adult female breast varies enormously. On average, it is about 10–12 cm in diameter, 5–8 cm in thickness, and weighs about 700 g. Weight may almost double during pregnancy and lactation. Pathologists typically divide the breast into four quadrants (Q): upper outer (UOQ), upper inner (UIQ), lower outer (LOQ), and lower inner (LIQ). Other important regions are the areola/nipple complex and the lymph nodes in axillary tail extending from the UOQ. Lymphatic (and vascular) drainage is important as the main pathway for breast cancer cells to metastasize. Most regions of the breast, especially the UOQ and LOQ, drain to the axillary nodes, although the LIQ and UIQ also drain to a chain of internal mammary nodes beneath the sternum and extending upwards
- Internally, the breast is composed of 15–20 segments or lobes, somewhat analogous to segments of an orange, but less well defined. Each lobe contains thousands of lobules, which are small grape-like clusters of glands lined by epithelial cells specialized to produce milk. Small ducts that join to form larger ducts that eventually exit through the nipple, transmitting milk to nourish our young, interconnect the lobules. All known precursors of breast cancer, also referred to as premalignant lesions, develop and progress from abnormal cells within the ductal system, primarily in the lobules and smallest ducts connected to them, referred to as the terminal duct lobular unit (TDLU)
- The entire normal ductal and lobular system is delineated from the mesenchymal stroma ("connective tissue") by a continuous basement membrane (BM) which is an important barrier which must be breached for cancer cells to invade and metastasize
- The lumens of the ducts and lobules are generally lined by two distinct layers of cells; an outer layer directly on top of the BM referred to a myoepithelial cells (MECs), and an inner layer directly on top of the MECs referred to a luminal epithelial cells (LECs)—although LECs also have many subtle points of attachment with the BM interspersed with the MECs
- Nearly, all LECs typically express large amounts of keratin proteins, particularly CK8, CK18, and CK19. MECs express abundant CK5 and CK6, but are generally negative for keratins found in LECs, and they do not express ER or PR. MECs also typically express several other molecules distinct from LECs, including smooth muscle actin (SMA), calponin, S100, p63, CD10, and stratifin (SFN), which appear to be important in certain specialized normal functions such as contraction of duct lumens to expel milk, and to maintain normal cell polarity within ducts, which can actively suppress the invasion of cancer cells
- These keratins play an important role in a new molecular classification of breast cancers—the so-called intrinsic molecular subtypes, which is discussed in more detail later. Briefly, the most common subtype is referred to as "luminal" breast cancers, primarily because they have many similarities at the gene expression level with normal LECs, including these keratins. Another important subtype, referred to as "basal" breast cancers, expresses keratins normally associated with MECs, which historically have been referred to as "basal" cells because of their location in ducts and lobules. There is a common misconception that luminal and basal breast cancers evolve from genetically altered LECs and MECs, respectively, partly because of molecular similarities including keratins—which is probably not true, although the "stem" cell origin of all breast cancers is far from clear and a topic of much debate and research
- A proportion of LECs (20–30%) also express nuclear estrogen receptors (ER) and progesterone receptors (PR). ER and PR are important mediators of growth and differentiation stimulated by the hormones estrogen and progesterone. The majority of cancer cells also

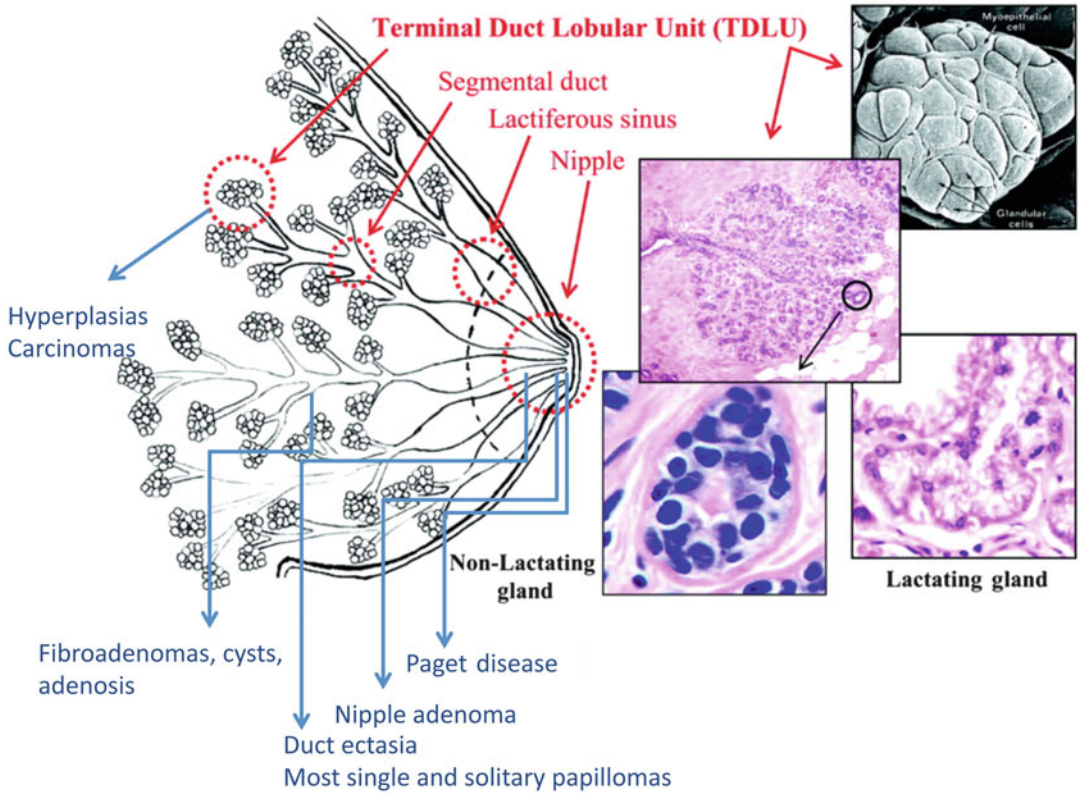


Fig. 6.1 Anatomy of the adult mature human breast. Correlation between compartments and different distinct pathologic processes arising in the breast

express these receptors, which may promote tumor growth

- Recent studies have shown that histologically normal appearing breast epithelial cells are not always normal at the molecular level, and some of these morphologically silent biological abnormalities may predispose the cells to premalignant or malignant transformation. For example, chromosomal gains and losses have been observed in normal breast epithelium. Although the overall frequency of imbalances is quite low, it is significantly higher in normal cells adjacent to cancer cells than normal cells at a distance. Some of these genetic defects may be shared with the adjacent cancer, although the majority are not and appear to be random. Other studies have shown that breast tissue, especially in women at high risk for breast cancer, may contain patches of histologically normal appearing cells in which activity of the p16 tumor suppressor gene is suppressed. Compared to adjacent cells with normal p16 function, these cells show increased proliferation and elevated expression of cyclooxygenase 2 (COX2), and the latter appears to be associated with the development of many types of cancers. There are likely to be many other acquired and inherited molecular abnormalities in otherwise normal appearing cells (Figs. 6.1 and 6.2)

Breast Development

- The molecular mechanisms responsible for human breast development are poorly understood because it is extremely difficult to study directly. Most of what we know is inferred

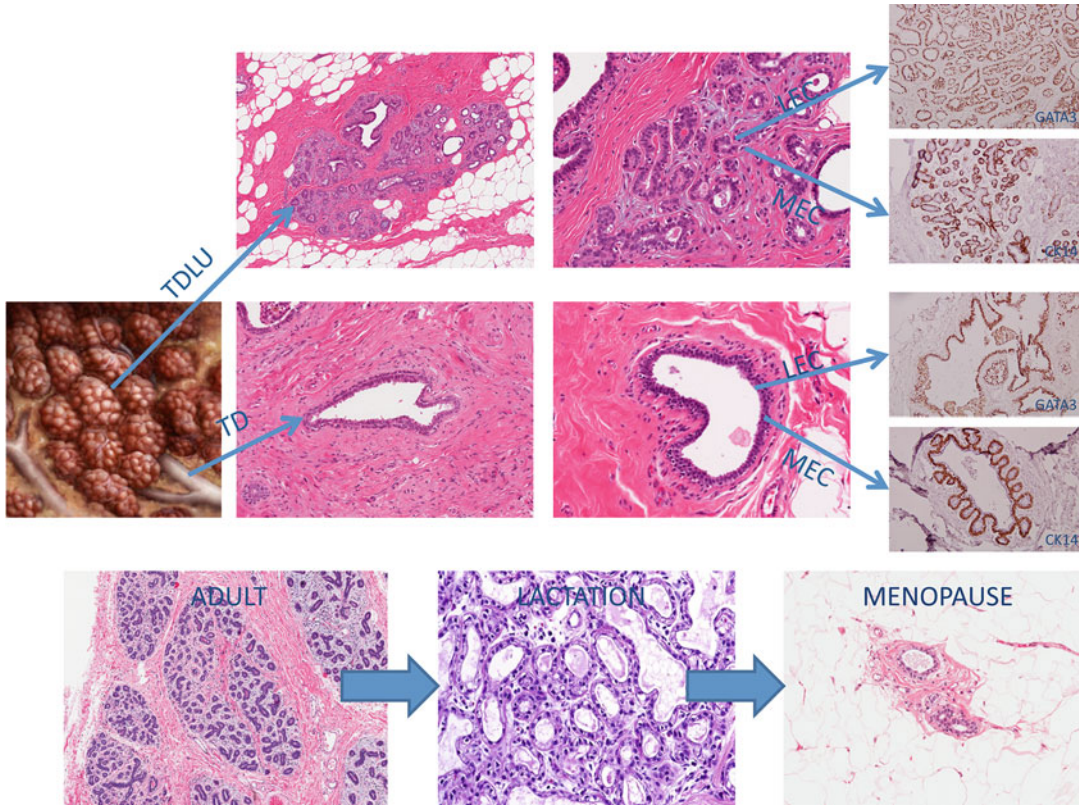


Fig. 6.2 Breast histology. Differences between luminal epithelial cells (LEC) and myoepithelial cell layer (MEC) compartments. GATA 3 is a representative marker of LEC in both TDLU and TD. CK14 is a distinctive marker of MEC. *Lower*: Histological changes associated with lactation and menopause. During lactation, the acini are closely packed, with reduced amount of stroma; secretory material in the lumens is seen. With menopause, there is a marked reduction of acini and ducts, with replacement by fat

from animal studies, particularly involving genetically engineered mice, where the effect of altering specific genes on breast development can be directly observed. However, there are probably many important parallels in breast development among all mammals, and studies using mice and other models almost certainly reveal molecular mechanisms shared with humans. Many normal developmental mechanisms play a central role in the development and progression of breast cancers. For example, cells in the earliest potential precursors of breast cancer, referred to as hyperplasias, demonstrate suppression of molecular pathways involved in adult differentiation, and reactivation of embryonic pathways, which is also true of later stages such as the progression of ductal carcinoma in situ (DCIS) to invasive breast cancer (IBC)

- Mammary glands are derived from ectodermal buds or ingrowths along mammary lines in the embryo. Between 14 and 18 weeks of gestation, distinct mesenchymal and ductal compartments start to develop. By 28 weeks, there are two clearly defined cell compartments (LECs and MECs). The ductal and lobular system continues to develop and mature throughout the second half of gestation, as well as the areola and nipple. Many genes are known to play critical roles in regulating development. For example, BCL2, which suppresses apoptosis, increases dramatically beginning at about 18 weeks, and plays a important role in duct formation by inducing

Table 6.1 Genes involved in breast development

Gene	Disease	Pathway	Clinical features
TBX3	Ulnar mammary syndrome	Linked to FGF pathway	Abnormalities in limbs and apocrine glands TBX3 overexpression linked to breast carcinomas
PTHR1	Blomstrand chondrodysplasia	Mutation in receptor or parathormone (PTH) mediates cross-talk between epithelium and mesenchyme in early mammary bud	Dwarfism PTHrP is commonly secreted in breast cancers
Ectodysplasin	Hypohidrotic ectodermal dysplasias	Development of ectodermal appendages	X-linked ectodermal dysplasia receptor (which binds ectodysplasin) promoter methylation is linked to breast cancer
Ska (*neuroregulin 3*)	None known	Affects patterning of mammary glands, along the body axes. NRG-3 is a ligand to EGFR family	Upregulated in breast cancer, particularly those with HER2 overexpression
WNT		LEF1, the transcriptional mediator of WNT signaling at placode stage	LEF1−/− embryos placodes 2 and 3 do not form; the other placodes develop into small buds and degenerate. Corresponding ducts and nipples are missing in newborn

Clinical consequences of mutations involving those genes and associated clinical syndromes

cells in the center of solid cords of primitive epithelial cells to die, forming patent lumens. Ductal budding and branching depends on prolactin which sensitizes cells to the growth stimulating effects of insulin. Aldosterone promotes differentiation of buds into ducts and lobules, forming primitive TDLUs. ER is expressed in LECs by third trimester and PR, 2–3 months after birth. Genetic alterations of these regulatory molecules can play important roles in the development and progression of breast cancer, in general, by promoting "embryonic" growth in an inappropriate setting. Other important genes are discussed later in the context of what happens to breast development when they are altered in transgenic and knockout mice (Tables 6.1 and 6.2)

- There are no structural or known molecular differences between male and female breasts during the postnatal period. At birth, nipple ducts finally open onto the surface. Closely after birth, prolactin, estrogen, and progesterone decrease dramatically, resulting in involution of newborn breast tissue. During this time, apocrine and cystic changes become prominent, which are also common in postmenopausal breasts. Between 2 years of age and puberty, the breasts are very small, and the main constituents are scattered small ducts embedded within a dense collagenous stroma. Pubertal changes are characterized by greatly increased growth of stroma, MECs, and LECs, which are prominently caused by increased levels of estrogen, although full differentiation requires other hormones and growth factors as well, including insulin, cortisol, thyroxin, prolactin, and growth hormone. ER is necessary for duct elongation, and ER knockout mice only develop rudimentary ducts without terminal end buds or alveolar buds. Interestingly, these glands are highly resistant to cancer development. PR is necessary for duct elongation and alveolar development, which are lacking in PR knockout mice. After menarche, prominent cyclical developmental changes occur with the menstrual cycle. Early on,

Table 6.2 Animal transgenic models in breast carcinogenesis

Gene (KO or overexpression)	Pathway	Clinical features
BRCA1 KO	BRCA1 and p53	Increased mammary tumor development in BRCA1 KO that was p53 heterozygous (p53+/−) suggested that BRCA1 loss may induce tumor development due to genetic instability causing LOH LOH in p53 was seen in majority of BRCA1 KO mice
Erα OE	ER	Mammary carcinomas with similarities to human breast cancer and ER+ phenotype
Aromatase OE	Aromatase	Male mice developed gynecomastia, and homozygous mice were infertile and developed Leydig testicular cancers Females developed ductal hyperplasias and dysplasia. However, no mammary tumors were seen Mice exhibited increased ERα and ERβ levels, as well as PR, cyclin D1, and cyclin E levels (cyclin D1 overexpression correlates with ER+ phenotype in human cancers) DMBA treated mice with AO developed mammary tumors, whereas WT only showed hyperplasia. Letrozole effectively inhibited dysplastic growth in MMTV-aromatase mice
TGFα/HER2	TGFα and HER2	Double transgenic mice developed significantly less breast tumors than parental lines. Double transgenic mice with HER2 aromatase overexpression show less hyperplasias
ERα KO	ER	Mammary glands resembled prepubertal wild-type mice. ERα KO mammary epithelium underwent ductal morphogenesis when transplanted to wild-type mice. Transgenic mice with MMTV-aromatase/ERα KO did not develop hyperplastic growth and exhibited morphology similar to ERα KO mice. ERα mediated growth of the mammary duct network is a prerequisite for aromatase induced changes within the transgenic mammary gland
WNT	WNT	Mice developed ductal hyperplasias early in life and mammary adenocarcinomas in most animals by 1 year of age. MMTV-wnt/ERα KO−/− exhibited stunted growth similar to parental ERα KO mice
PELP-1	Coactivator of ER, PR, AR. Mediates G1-S transition. Aromatase pathway	MMTV-PELP1 developed mammary tumors in over 40% of cases. Tumors show ER and aromatase expression. Human breast cancers commonly show PELP1 overexpression and are associated with poor response to tamoxifen
AIB1 KO/OE	Binds to steroid receptors and transcription factors	AIB-1 levels have been correlated with poor prognosis in breast cancer. Coinduction of AIB1 and HER2 was associated with decreased DFS and tamoxifen resistance. AIB1 KO resulted in decreased oncogenesis with decreased HRAS, HER2, and IGF1 expression. MMTV-AIB1 resulted in tumor development in 48% of mice. The carcinogenic potential was abrogated in double transgenic mice with MMTV-AIB1 ERα KO (ER is important in the AIB1 pathway). Induction of IGF1 signaling in the mammary gland is typical of the AIB1 transgenic model. Treatment with the mTOR inhibitor RAD001 resulted in block of hyperplasia and atypia in the AIB1 transgenic model
CSF1	CSF1	The macrophage colony-stimulating factor, CSF1, is commonly elevated in breast cancer. CSF1 op/op is deficient in lactation and develops osteopetrosis. Cross-linked species of MMTV-PYMT CSF op/op showed less progression of disease and lung metastases compared to the parental strain of MMTV-PYMT

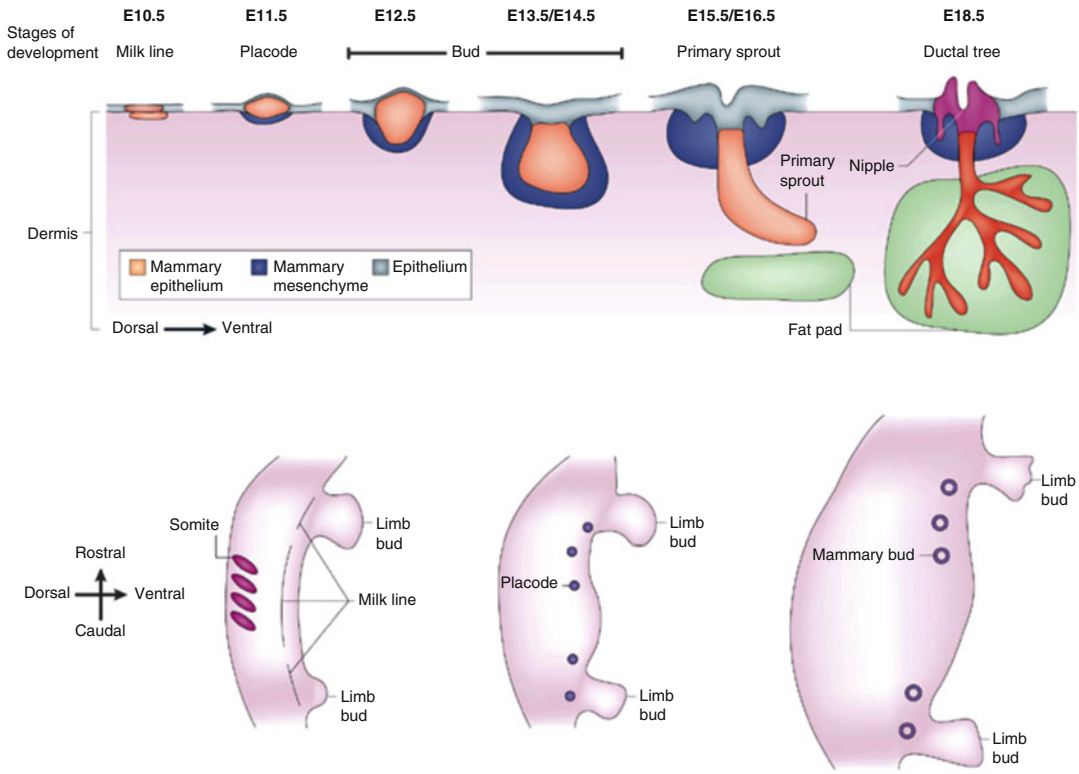
TDLUs develop more alveoli with each successive cycle. From menarche on, the mammary gland is fully anatomically and functionally developed to support pregnancy and lactation

- During pregnancy, proliferation of essentially all types of cells, especially LECs, dramatically increases, mediated by increasing levels of estrogen, progesterone, ER, and PR. After delivery circulating ER and PR decrease to low levels, in preparation for lactation. Once lactation begins, cell proliferation ceases as the cells terminally differentiate to produce milk. When lactation ceases, secretory LECs undergo apoptosis, alveoli collapse, and the mammary gland involutes back to the nonpregnant condition, although the ductal system postpregnancy retains a somewhat more complex ductal framework than prior to pregnancy. In the adult female breast, there is a relatively large reserve of normal stem cells which support the dynamic changes in growth and differentiation associated with menstrual cycling, pregnancy, and lactation. Presumably, various genetic alterations of normal stem cells may give rise to precancerous or cancer-stem cells, which eventually grow uncontrollably. However, there are probably other sources of cancer-stem cells, including dedifferentiation of mature LECs due to specific mutations
- After menopause, both lobules and ducts are decreased in number. Intralobular stroma is replaced by collagen and the breast stroma undergoes replacement by fat (Fig. 6.3)

Molecular Biomarkers in Routine Clinical Practice

Estrogen Receptor and Progesterone Receptor: Molecular and Clinical Aspects

- The measurement of ER and PR has become a standard of practice in the evaluation of patients with primary breast cancer. The measurements can be performed accurately on formalin-fixed paraffin-embedded (FFPE) tissue by using immunohistochemistry (IHC) and the results have good correlation with those of biochemical testing
- The ER is the paradigm tumor marker for management of patients with cancer. It dates back to at least 1896 when G Batson reported regression of advanced breast cancer in women who underwent oophorectomy
- ER controls essential developmental and physiological processes. It interacts with the receptor as estradiol, regulates growth and differentiation, and helps maintain homeostasis. Studies have shown that dysregulation of ER and PR during development are important in carcinogenesis
- The effects and actions of estradiol are mediated through interaction with two nuclear receptor proteins, ERα and ERβ, located in chromosomes 6q and 14q, respectively, which are encoded by two separate genes *ESR1* and *ESR2*, respectively. Both, ERα and ERβ show substantial homology in the DNA binding domain. Role of ERβ in breast cancer has not yet been determined. Hereafter, ERα will simply be referred to as ER
- The "classical" function of ER involves binding of 17βestradiol to ER located in the cell nucleus. This induces receptor dimerization, which binds to estrogen response elements (EREs) on many other genes, which are then indirectly regulated by estrogen and ERα
- ERE activated genes perform many important functions, including inhibition of apoptosis and stimulation of the cell cycle. There is cross talk with other mitogenic pathways (ras, raf, cyclin D1)
- Activation of estrogen target genes is accomplished through direct hormonal binding with the ER. This recruits protein regulators known as coactivators and repressors. Coregulators are responsible for chromatin remodeling to facilitate binding of RNA-polymerase. Histone acetylation, through acetyl transferases, correlates with a more actively transcribed state of chromatin regulation, whereas methylation favors more tightly coiled chromatin, which is less accessible to transcription and less gene expression



	Milk line	Placode	Mammary bud epithelium	Mammary bud mesenchyme
Secreted molecules and receptors	FGF10/FGFR2	WNTs	PTHLH	PTHR1
	WNT10b,6	FGFs	IGF1	BMP4/BMPR1A
	NRG3/ERBB2,4	BMP4	BMP4	
Transcription factors	TBX2,3	TBX2,3	LEF1	MSX2
	GLI3	LEF1	MSX1,2	
			TBX3	
			GLI3	

Fig. 6.3 Morphological stages in the embryonic development of the mammary gland in the mice: Around embryonic day 10 (E10) of mouse development, the milk line (*orange*) is defined by a slight thickening and stratification of the ectoderm (*gray*) as depicted here in this series of cross sections through the trunk. On E11.5, the milk line breaks up into individual placodes (*orange*) and the underlying mammary mesenchyme (*blue*) starts to condense. Over the following days, the placodes sink deeper into the dermis and the mammary mesenchyme becomes organized in concentric layers around the mammary bud (*orange*). Starting on E15.5, the mammary epithelium (*orange*) starts to proliferate at the tip and the primary sprouts pushes through the mammary mesenchyme towards the fat pad (*green*). On E18.5, the elongating duct has grown into the fat pad and has branched into a small ductal system. The cells of the mammary mesenchyme have formed the nipple, which is made of specialized epidermal cells (*purple*). *Lower*: The schematic diagram shows the position of the milk line, placodes, and mammary buds along the lateral body wall of early mouse embryos. Secreted molecules, receptors, and transcription factors that are important at the different stages are listed in the table below. At the mammary bud stage, proteins that are expressed in the epithelium and in the mesenchyme are listed separately. *BMP* bone morphogenic protein; *ERBB* erythroblastic leukemia viral oncogene homologue; *FGF* fibroblast growth factor; *FGFR1* fibroblast growth factor receptor; *GLI* Gli-Kruppel family member; *IGF* insulin growth factor; *IGFR* insulin growth factor receptor; *LEF* lymphoid enhancer-binding protein; *MSX* muscle segmentation homeobox; *NRG* neuroregulin; *PTHLH* parathyroid hormone-like hormone; *PTHR1* parathyroid hormone receptor; *TBX* T-box; *WNT* wingless-related MMTV integration site. (Reprinted with permission of Nature publishing group)

- ER status is highly predictive of clinical benefit from endocrine therapy in both adjuvant and metastatic disease settings. ER-positive tumors are more likely to respond to hormonal therapy, and have a better prognosis, when compared to ER− tumors
  - Harvey et al. showed in a cohort of 1,982 patients, using ligand binding assays (LBA) >3 fmol/mg and, retrospectively IHC (Allred Score >2 or 1–10% weakly positive cells), showed IHC to be a stronger predictor of disease-free survival (DFS) in patients receiving endocrine therapy when compared to LBA
  - Elledge et al., in a cohort of 205 patients, showed significant correlation of IHC ER and clinical response in patients with advanced metastatic disease (ER negative 25%, intermediate 46%, and high 66%)
- Accurate measurements of ER are of considerable importance, because it represents one of the strongest predictive factors of responsiveness to endocrine management. In some cases, endocrine therapy alone is an option, without additional cytotoxic therapy. About 70–80% of breast cancers are ER-positive and 20–30% are ER-negative. Only 70% of ER-positive tumors show clinical response to estrogen manipulation, but measuring ER expression alone is insufficient to distinguish responders from nonresponders. A significant fraction of patients with ER-positive disease eventually develop resistance to endocrine therapy
- Clinical progression of the ER-positive breast cancer typically correlates with hormone resistance. Loss of response and decreased ER expression are associated with a more aggressive clinical course. Epigenetic alterations of the ER promoter, including methylation of *ESR1* gene, are thought to be important events in the development of ER-negative breast cancers
- In the last decade, prospective randomized clinical trials have shown the superiority of aromatase inhibitors over tamoxifen in postmenopausal receptor-positive women
- Tamoxifen is a partial agonist (both antagonistic and agonistic effects) of the ER receptor, and induces dimerization and nuclear translocation and is designated as a selective ER modulator (SERM)
- Fulvestrant directly binds to ER monomers, inhibits dimerization, and suppresses activation, thereby functioning as a pure antiestrogen. Its benefits have been demonstrated in the metastatic setting, and ongoing trials are underway in the adjuvant setting
- Anastrozole, letrozole, and exemestane are aromatase inhibitors (AI) which block the conversion of adrenally produced precursor compounds to estrogenic molecules. Recent trials also showed the benefits of estrogen deprivation persist for many years even after completion of the initial hormonal therapy in reducing both unilateral and contralateral breast cancers
- Recently, the Women's health Initiative Estrogen-Alone trial, analyzed, prospectively, the use of equine-conjugated estrogen (CEE) among patients with prior hysterectomy. The trial was stopped earlier and showed a decreased risk of breast cancer in the treatment group
- Progesterone has an essential role in regulating breast maturation. A clear role in carcinogenesis has been shown in animal models, particularly in respect to induction, maintenance, and progression of the neoplastic phenotype. An increased risk of breast cancer is documented in long-term users of progestin-only containing hormone-replacement therapy (HRT) regimens
- ER is important for regulating PR expression. Colocalization studies show that PR expressing cells also express ER. In fact, PR expression is regarded as a marker of an intact ER axis. However, discrepancies exist: the relative risk of disease recurrence is higher in patients with ER+/PR– cancers, compared to ER+/PR+ tumors
- About 60% of breast cancers express PR. This expression is regarded as a marker of intact ER function. PR receptor is also nuclear. Progesterone effects are mediated through the intracellular proteins PRA and PRB. Both are coded from the same gene using two distinct translation initiation sites

- Expression of PR in breast cancer is also associated with higher responsiveness to endocrine therapy. The majority of HER2-positive cancers are PR-negative, suggesting that nuclear ERα may be nonfunctional in these cases. However, membrane ER appears to remain functional and promotes tumor cell proliferation in cooperation with overexpressed *HER2*. In this setting, tamoxifen (as a partial agonist) may theoretically help induce cell proliferation. In this setting, AI will remain beneficial. A role for highly quantitative assessment of PR might be helpful in more precisely predicting response in patients with ER-positive/HER2-positive tumors
- Most testing for ER and PR today is done using IHC. However, errors have been problematical when using IHC. For example, the United Kingdom National External Quality Assessment Service (UK NEQAS) evaluated the frequency of hormone-receptor-positive cancers in more than 7,000 patients, highlighting significant variation in ER and PR positivity rates. Similar results were obtained by the Royal College of Pathologists of Australasia ($n=8{,}000$ patients). Approximately one-third of 1,023 ER tests performed on patients, in Canada, between 1997 and 2005 were scored falsely negative, which was revealed by retesting in an expert central laboratory in Ontario. More than 100 of these patients have since died and a class action lawsuit ensued claiming negligence in ER testing and failure to provide Tamoxifen to these patients. Investigation into the matter identified many causes of false negative IHC results, including: poor sample fixation, improper staining procedures, and improper interpretation:
  - The International Breast Cancer Study Group (IBCSG) conducted a series of studies comparing chemo and endocrine treatment to endocrine treatment alone in years before the establishment of IHC testing: Most studies of ER testing used LBA or ELISA. They compared with results obtained after the primary tumor blocks were collected and reanalyzed in a single central lab using IHC. Discordant ER results between institutional and central results were 16% (ER+) and 24% (ER−) for specimens from premenopausal women, and 9% (ER+) and 24% (ER−) from postmenopausal women. Overall concordance rate was 82 and 88% for pre- and postmenopausal women, respectively
  - In the ECOG 2197 trial, 11% of local ER− tests were scored positive on central testing, with an overall concordance rate of 90%
  - In the ALTTO trial (5,000 patients from countries worldwide), so far, 4.3% of tumors that tested ER+ in local laboratories were found to be negative (false-positive) on central review. More than 20% of tumors exhibited at least some expression of ER (false-negative) on central review

Guidelines for Estrogen Receptor and Progesterone Receptor Testing by Immunohistochemistry

- In an effort to improve the quality of testing for ER and PR by IHC, the American Society of Oncologists (ASCO) and College of American Pathologists (CAP) jointly developed and recently published guidelines for pathologists to follow (Fig. 6.4 and 6.5). Compliance with the guidelines is now mandatory for laboratories in the US to receive CAP accreditation
- Immunohistochemistry on FFPE tissue replaced LBAs for testing in the late 1980s. Harvey et al. compared the predictive abilities of LBA and IHC using the 6F11 antibody in a large cohort of patients with newly diagnosed breast cancer. This cohort received a variety of types of adjuvant therapy that ranged from none to endocrine alone, chemotherapy alone, or a combination of the above. Receptor status was scored as the sum of the proportion and average intensity scores of positive staining tumor cells (Allred Score on a scale ranging from 0 to 8). On the basis of clinical outcome in patients with adjuvant endocrine therapy, patients with Allred Score >3 (corresponding

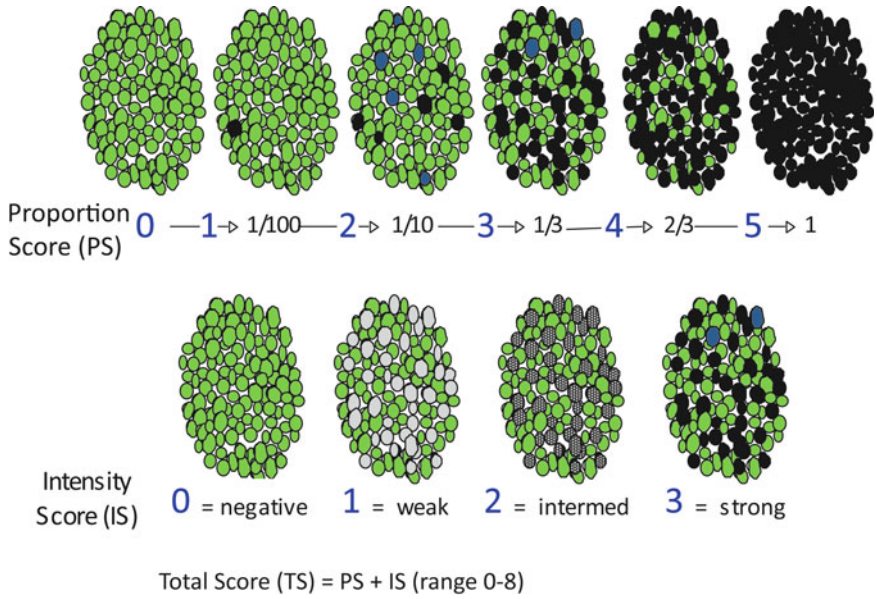


Fig. 6.4 Algorithm for scoring biomarkers (ER, PR) according to recent ASCO guidelines. Allred Score. A combination of number of cells (Proportion Score) and intensity of staining (Intensity Score) is used. (Adapted from Allred et al.)

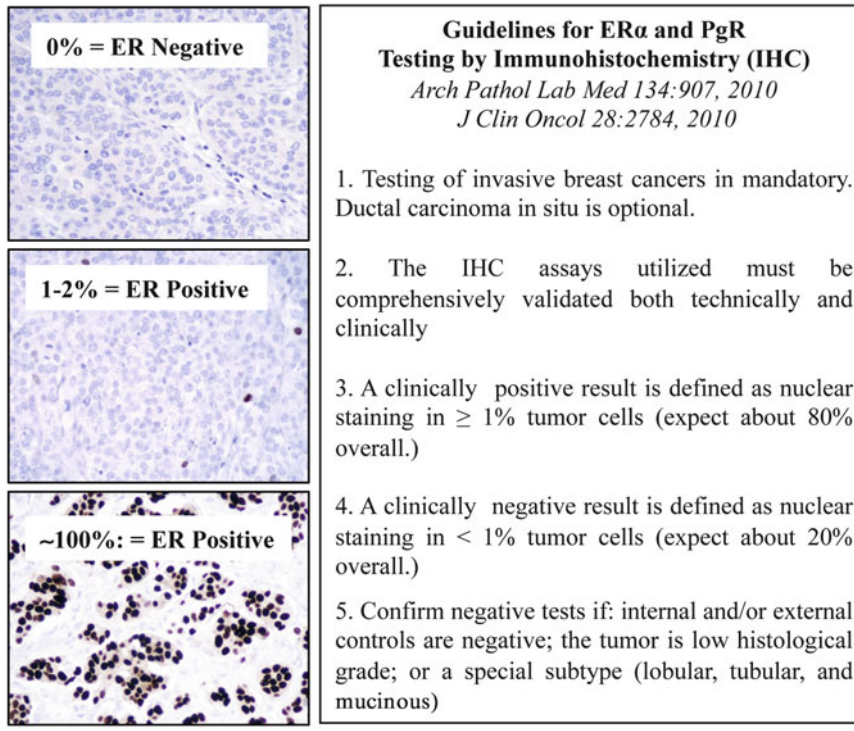


Fig. 6.5 Schematic example of ER interpretation

to as few as 1–10% positive cells) had a substantially and statistically significant better prognosis than patients with scores less than 3 (<1% positive cells). The predictive ability of IHC was superior to LBA previously performed in the same tumors

- There is no gold standard available for IHC assays of ER and PR. A relevant standard would be any assay whose specific preanalytic and analytic components conformed to assays whose results have been validated against clinical benefit from endocrine therapy (clinical validation). Several assays meet this criteria such as the methods described in the publication by Harvey et al. and Mohsin et al., the FDA 510(k)-cleared ER/PR pharmDx assay kit (Dako, Glostrup, Denmark)
- ER status can also be determined at the RNA level. The Oncotype DX® Assay measures RNA expression of 21 genes to determine a recurrence score (RS). ER and PR are among the most prevalent genes in the signature. Comparison between measures of the ER/PR protein by IHC and of mRNA by RT-PCR showed a discordance rate of 9% and 12%, respectively. There are no published correlations of the individual measures of ER and PR mRNA from the 21-gene signature with the clinical outcome
- A laboratory that performs ER testing should validate its proposed or existing assay against one of the clinically validated assays and demonstrate acceptable concordance. To be considered acceptable, the results of the assay must be initially 90% concordant with those of the clinically validated assay for the ER-positive and PR-positive categories, and 95% concordant for the ER-negative or PR-negative categories
- The cutoff from distinguishing a "positive" from "negative" cases should be ≥1% ER+ positive tumor cells. Patients whose breast tumors show at least 1% ER+ cells are candidates for endocrine therapy and those with less are not. Percentage of stained tumor cells provides valuable predictive and prognostic information to inform treatment strategies
- Eight studies described the relationship between hormone-receptor levels and patient outcomes. Overall survival, DFS, recurrence/relapse-free survival, 5-year survival, time to treatment failure, response to endocrine therapy, and time to recurrence were positively related to ER levels
- PR status provides additional predictive value independent of ER values, especially among premenopausal women. Its predictive value has been demonstrated in retrospective studies using 1% as cutoff point. Among patients who received adjuvant endocrine therapy, the best cutoff for both DFS ($P=0.0021$) and OS ($P=0.0014$) was a total PR Allred Score >2, which corresponds to greater than 1% of carcinoma cells exhibiting weakly positive staining. In patients with metastatic breast cancer who received first-line endocrine therapy on relapse, a correlation with PR status and response to endocrine therapy was found at a 1% staining threshold ($P=0.044$) or response to tamoxifen at 10% ($P=0.021$). Patients with carcinomas >1% PR staining had a better survival after relapse ($P=0.0008$)
- Reporting results for ER, PR, and HER2: The percentage and proportion of tumor cells staining positively should be recorded and reported. All tumor areas of the tissue section on the slide should be evaluated. This can be achieved manually by counting cells or through image analysis
- The intensity of the staining should be recorded and reported as weak, moderate, or strong. This measurement should represent an estimate of the average staining of the intensity of the positively stained tumor cells on the entire section relative to the intensity of the positive controls run on the same batch. A cutoff of a minimum of 1% of the tumor cells positive for ER/PR for a specimen is considered to be positive. The term equivocal must not be used
- Less than 1% of the tumor cells positive for ER/PR for a specimen is considered to be negative. Such patients do not receive meaningful benefit from endocrine therapy

- Any specimen lacking intrinsic elements (normal breast epithelium) that is negative on ER and/or PR assay should be repeated using another tumor block or another specimen, and reported as not interpretable rather than as negative
- "Not interpretable" receptor results refer to samples that did not conform to preanalytic specifications of the guidelines, were processed using procedures that did not conform to guideline specifications of the lab operating procedures, or the assay used to analyze the specimen was not validated and controlled as specific in the guideline. Examples of circumstances leading to not interpretable results include testing of needle biopsies or cytology samples fixed in alcohol, use of fixatives other than 10% NBF, biopsies fixed for intervals shorter than 6 h or longer than 72 h, samples where fixation was delayed more than 1 h, samples with prior decalcification, and samples without internal or external controls
- Negative ER and PR interpretations in tumors that characteristically have an ER+ phenotype (e.g., lobular, tubular, and mucinous carcinomas) should be confirmed by retesting
- ER and PR should be documented in all newly diagnosed breast cancers. Recurrences should also always be tested to exclude prior false negatives, and to document changes in biologic behavior. In the routine practice, DCIS is also commonly tested for ER and PR based on the NSABP-24 clinical trials. The trial compared placebo versus tamoxifen after lumpectomy and radiation. There was a significant reduction (40–50%) in subsequent breast cancer (ipsilateral and contralateral) restricted to patients with DCIS ER+ at 10 years followup

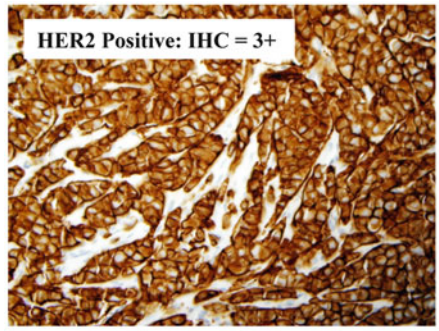
Human Epidermal Growth Factor Receptor 2 Gene: Molecular and Clinical Aspects

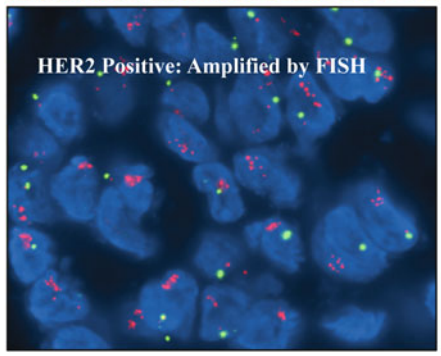
- The human epidermal growth factor receptor 2 gene, more commonly referred to as *HER2*, is amplified in 15–25% of human breast cancers. *HER2* amplification and overexpression are highly correlated, which are significantly associated with aggressive disease (i.e., poor prognostic factors), and are the molecular targets for specific therapies, such as trastuzumab
- *HER2* is a protooncogene located on chromosome 17. It encodes a tyrosine–kinase receptor residing in the surface membrane of breast epithelial cells. It forms complexes with similar proteins (erbB1, erbB3, and erbB4) and acts as receptors for several ligands, such as EGF, heregulin, and amphiregulin. It regulates many normal cell functions, including proliferation, survival, and apoptosis
- The overall relationship between HER2 and clinical outcome is complex and varies with the clinical setting. A weak but significant association between poor outcome and a positive HER2 (overexpression or amplification) in patients receiving no additional therapy after initial surgery is seen. But this only represents a small fraction of patients today. The majority of patients typically receive some form of adjuvant treatment. Some studies have shown that HER2+ breast cancers are resistant to certain types of cytotoxic chemotherapy (e.g., the combination of cyclophosphamide, methotrexate, and 5-fluorouracil) but sensitive to others (e.g., anthracyclines and taxanes). In general, it is accepted that HER2+ cancers appear to be associated with relative, but no absolute, resistance to endocrine therapies in general. However, this issue remains very controversial. The most promising and useful findings are based on recent studies showing that HER2+ cancers respond favorably to new antibody-based therapies, targeting specifically the HER2 protein, such as trastuzumab. Although this therapy was originally demonstrated effective in patients with metastatic disease, more recent clinical trials have shown significant benefits in the adjuvant setting for patients with less advanced disease. The NSABP-B31 clinical trial, which randomized patients with HER2+ cancer to adjuvant chemotherapy +/− trastuzumab, showed a 52% improvement in disease-free survival with the monoclonal antibody

- A long and persistent controversy in the evaluation of the HER2 status by protein expression through IHC, or gene amplification by FISH exists. However, many studies have shown that, when properly performed, a very strong correlation between the two methods exists, and they are equivalent and complimentary in the clinical practice
  - Owens et al. observed a similar frequency of HER2 amplified cases by IHC (20%) among 116,736 specimens and FISH (22%) among 6,556 specimens
  - Most clinical trials using trastuzumab enroll patients with IHC positive, or reflex FISH positive, or ISH alone
- In general, approximately 70% of breast cancers show little or no protein overexpression, a normal gene copy number, and do not respond to trastuzumab. Roughly 15% show low to intermediate levels of protein expression, and the gene is amplified in nearly a third of those cases. There is still uncertainty of how well these patients respond to the drug. The remaining 15% of cases show very strong membrane staining, indicating high levels of protein expression and the gene is nearly always amplified. This is the population who shows best response to trastuzumab

Guidelines for HER2 Testing in Breast Cancer

- ASCO and the CAP jointly developed and published guidelines to improve the quality of HER2 testing (Fig. 6.6)
- A positive HER2 test is defined as a result of 3+ surface protein expression (formed as uniform intense membrane staining of >30% of invasive tumor cells) or FISH result of amplified *HER2* gene copy number (average of >6 copies/nucleus for test systems without internal control probe) or *HER2/CEP17* ratio of more than 2.2, where CEP17 is a centromeric probe for chromosome 17 on which the HER2 gene resides
- Originally, FISH testing results were reported as either positive or negative, but an intermediate range (referred as equivocal range) has since been described and its clinical significance remains unclear. Much of the confusion using this term comes from the need to define the need for trastuzumab treatment. There is also significant variation in the intermediate (equivocal) ranges for both the IHC and FISH assays. The equivocal range for IHC consists of samples scored 2+, which includes up to 15% of samples. An equivocal result (2+) is complete membrane staining that is either nonuniform or weak in intensity but with obvious circumferential distribution in at least 10% of cells. Some, but not all of these samples may have *HER2* gene amplification and require additional testing to define the true HER2 status. The equivocal range for FISH assays is defined as *HER2/CEP17* ratios from 1.8 to 2.2 or average gene copy numbers between 4.0 and 6.0 for systems without an internal control probe. About 3% of patients have ratios of 2.0–2.2 and were previously included in treatment arms with trastuzumab. Polysomy 17 is a vague term, seen in up to 8% of tumors. If polysomy 17 is defined as three or more copies of CEP17, most are not associated with protein or mRNA overexpression
- Discordant results (IHC3+/FISH– or IHC<3+/FISH+) have been documented in approximately 4% of cases. The significance of this is unclear. Equivocal results of a single test require additional action, which should be specified in the report. Equivocal results by IHC should follow confirmatory FISH analysis. Counting additional cells or repeating the test confirms equivocal FISH results. If the results remain equivocal, confirmatory IHC is recommended
- A negative HER2 test is defined as either an IHC result of 0 or 1+ for cellular membrane protein expression (no staining or weak, incomplete membrane staining in any proportion of tumor cells), or a FISH result showing *HER2/CEP17* ratio of less than 1.8 or an average of fewer than four copies of *HER2* gene per nucleus for systems without an internal control probe





Guidelines for HER2 Testing
Arch Pathol Lab Med 131:18, 2007
J Clin Oncol 25:118, 2007

1. Testing of invasive breast cancers is mandatory.

2. Either immunohistochemistry (IHC) or fluorescent in situ hybridization (FISH) assays may be utilized, providing that they have been comprehensively validated technically and clinically.

3. Positive by IHC is defined as strong circumferential membrane staining in >30% tumor cells (referred to as 3+; expect about 15% of tumors overall). Absent (0) or weak (1+) intensity staining are considered as negative results (about 70% overall). Staining in between is considered equivocal (2+) and the tumor must be retested for confirmation (usually by FISH).

4. Positive by FISH is defined as a gene-to-chromosome ratio > 2.2, or gene copy number > 6 (about 15% overall). A ratio of < 1.8 is negative (about 70% overall). A ratio in between is equivocal (about 15% overall) and the tumor must be retested and confirmed (by IHC or counting more cells by FISH).

Fig. 6.6 Algorithm for scoring HER2 according to recent ASCO guidelines

- The ASCO/CAP guidelines establish that, in order to classify a test as positive or negative, the laboratory must have performed concordance testing with a validated FISH assay and confirmed that only 5% or less of samples classified as either + or − disagree with the validated assay on an ongoing basis. Equivocal cases are not expected to be 95% concordant, but rather subjected to a confirmatory test

Recent Advances in the Molecular Pathology of Breast Cancer of Clinical Significance

Multigene Prognostic Indices

- Oncotype DX® is a prognostic test measuring the RNA expression of 21 genes, which provides a recurrence score (RS; range 0–100) using FFPE tumor samples. The genes include proliferation markers (*Ki67, survivin, cyclin D1*), invasion-related (*MMP11, cathepsin*), *HER2*, ER, PR, and others (*GSTM1, CD68, BCL2*), as well as five housekeeping genes used to normalize expression overall. The RS quantifies the likelihood of disease recurrence based on studies in women with early stage hormone estrogen receptor (ER) positive only breast cancer, and assesses the likely benefit from certain types of chemotherapy. Scores are reported as: low (<18), intermediate (18–31), or high (>31) relative to risk of recurrence. Typically, patients in the high risk receive chemotherapy and those in the low risk do not. Studies have demonstrated that treatment is modified in 31% of patients who are tested by Oncotype DX®, including omission of presumed unnecessary chemotherapy in 22%. Based on these findings, it is estimated that the cost of gene expression against the relative costs of ER, PR, and HER2 are likely to result in an overall cost saving, as well as reduced toxicity and quality of life

improvements for patients. Recently, the test has also shown similar prognostic and predictive significance in women with receptor-positive node-positive received adjuvant treatment with the aromatase inhibitor anastrozole, and in cancer patients receiving neoadjuvant hormonal therapy and chemotherapy. There is an important ongoing phase III clinical trial, referred to as the TAILORx study, designed to help optimize the use of adjuvant endocrine and chemotherapy in patients with receptor-positive breast cancer. Based on their recurrence score, women will be assigned to three different treatment groups: women with a recurrence score higher than 25 will receive chemotherapy plus hormonal therapy (the standard of care); women with a recurrence score lower than 11 will receive hormonal therapy alone; and women with a recurrence score of 11–25 will be randomly assigned to receive adjuvant hormonal therapy, with or without chemotherapy. The study is primarily designed to evaluate the effect of chemotherapy on those with a recurrence score of 11–25. Because the degree of benefit of chemotherapy for women with recurrence scores between 11 and 25 is uncertain, strong preliminary evidence suggests that may only require endocrine therapy, which would be an important benefit

- The Mammaprint®: 70-gene prognostic index was validated as clinically useful in studies of younger women with node-negative breast cancer by classifying them into low risk and high risk for disease recurrence. It requires frozen tumor samples. Genes involved in the regulation of cell cycle, invasion, and angiogenesis heavily weight it. Genes of interest do not include known prognostic markers such as ER, PR, and HER2. High risk patients are most likely to benefit from cytotoxic chemotherapy. In contrast, the low risk group typically responds very well to endocrine therapy without chemotherapy. The prospective validation of the MammaPrint® signature's prognostic value is currently ongoing through the Microarray in Node-Negative Disease May Avoid Chemotherapy (MINDACT) trial. This trial opened in February 2007 as has enrolled over 6,000 patients from five European countries. It assesses all patients by the standard clinicopathologic prognostic factors included in adjuvant setting and by the 70-gene signature assay. If both traditional and molecular assays predict a high risk status, the patient receives adjuvant cytotoxic chemotherapy and also hormonal therapy if ER positive. If both assays indicate a low risk, no chemotherapy is given and ER-positive patients are given adjuvant hormonal therapy only. When there is discordance between the traditional clinicopathologic prognostic factor prediction of risk and the 70-gene signature prediction of risk, the patients are randomized to receive treatment based on either the genomic or the clinical prediction results. The primary goal of the study is to confirm that breast cancer patients with a "low risk" molecular prognosis by MammaPrint® and "high risk" clinical prognosis can be safely spared chemotherapy without affecting distant metastases-free survival (DMFS)
- PAM50 assay: was developed to efficiently determine intrinsic molecular subtypes based on evaluating 50 carefully selected genes using next generation sequencing and FFPE tissue samples. It is currently performed in a commercial reference laboratory, but an instrument dedicated to perform this will be available to pathology laboratories in the future. The PAM50 test provides a risk of relapse score (ROR) initially based on studies of patients with node-negative breast cancer who did not receive adjuvant systemic therapy. The ability of ROR to predict prognosis has recently been confirmed as useful in an independent set of 786 patients with ER+ treated only with tamoxifen. In these studies, ROR was a better predictor than standard clinicopathologic variables, including Ki67, PR, and histological grade. Most recently, PAM50 outperformed OncotypeDX® for predicting response to endocrine therapy in a large prospective clinical trial of receptor-positive node-negative patients

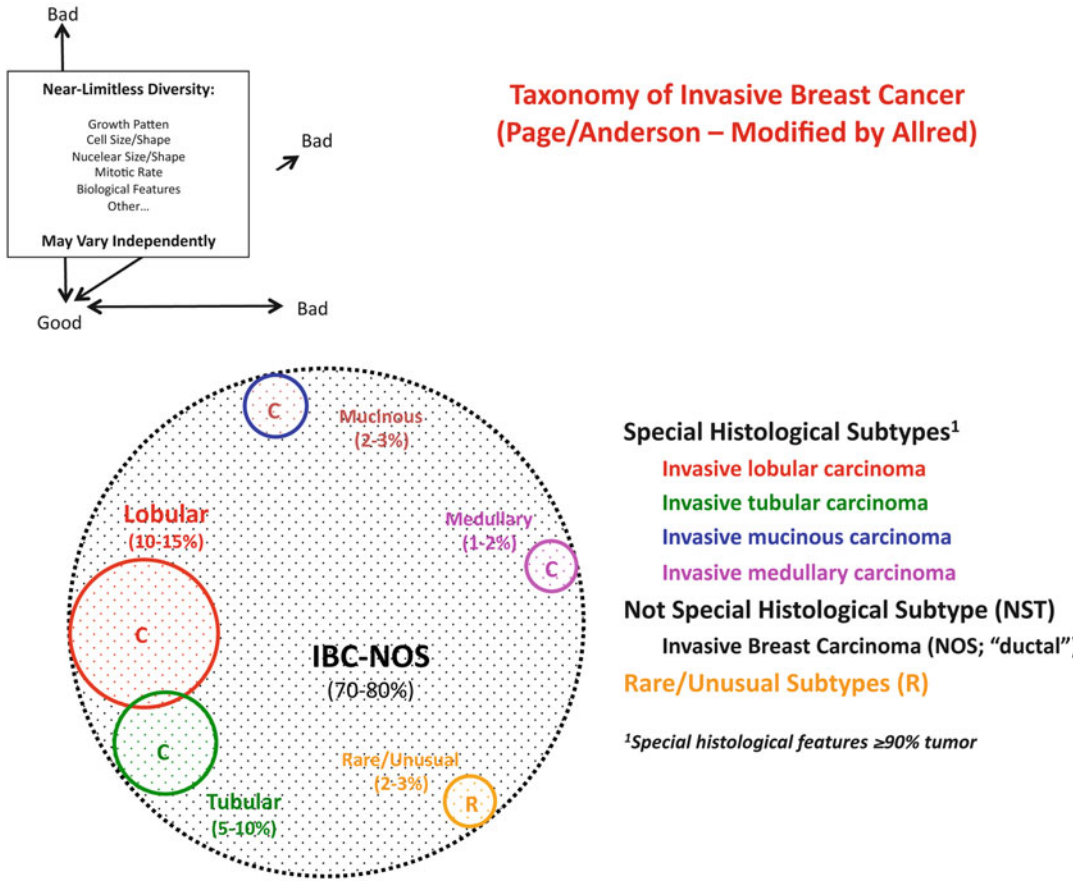


Fig. 6.7 Taxonomy of breast cancer. WHO classification of common histologic subtypes

- MapQuant Dx® genomic grade: is a predictor test derived by identifying 97 differentially expressed genes from grade 1 and 3 breast cancers using a training set of 64 ER+ tumors. Most genes are cell cycle regulators and proliferation. Genomic grade index (GGI) was strongly associated with risk of recurrence among patients with grade 2 tumors. It requires fresh tissue, similar to Mammaprint
- Breast cancer index (BCI): provides assessment of likelihood of distant recurrence in patients with ER+, node-negative breast cancer treated with endocrine therapy (primarily tamoxifen). BCI was developed from a combination of two indices: HOXB13:IL17BR and a proliferation related five-gene molecular grade index. Technically, it involves using a qRT-PCR assay with FFPE tissue samples
- The clinical use of Mammaprint®, Oncotype DX®, BCI, PAM50 assays have all been proven most useful in studies of patients with receptor-positive node-negative breast cancer, which are highly enriched with luminal A molecular subtypes, which may explain why the prognostic ability of these different gene expression-based assays is similar, as most of them are differentiating luminal A from all other subtypes (Figs. 6.7 and 6.8)

Intrinsic Molecular Subtypes of Breast Cancer

- Understanding the more recent advances in the molecular biology of breast carcinogenesis, imply acknowledging the major contribution of

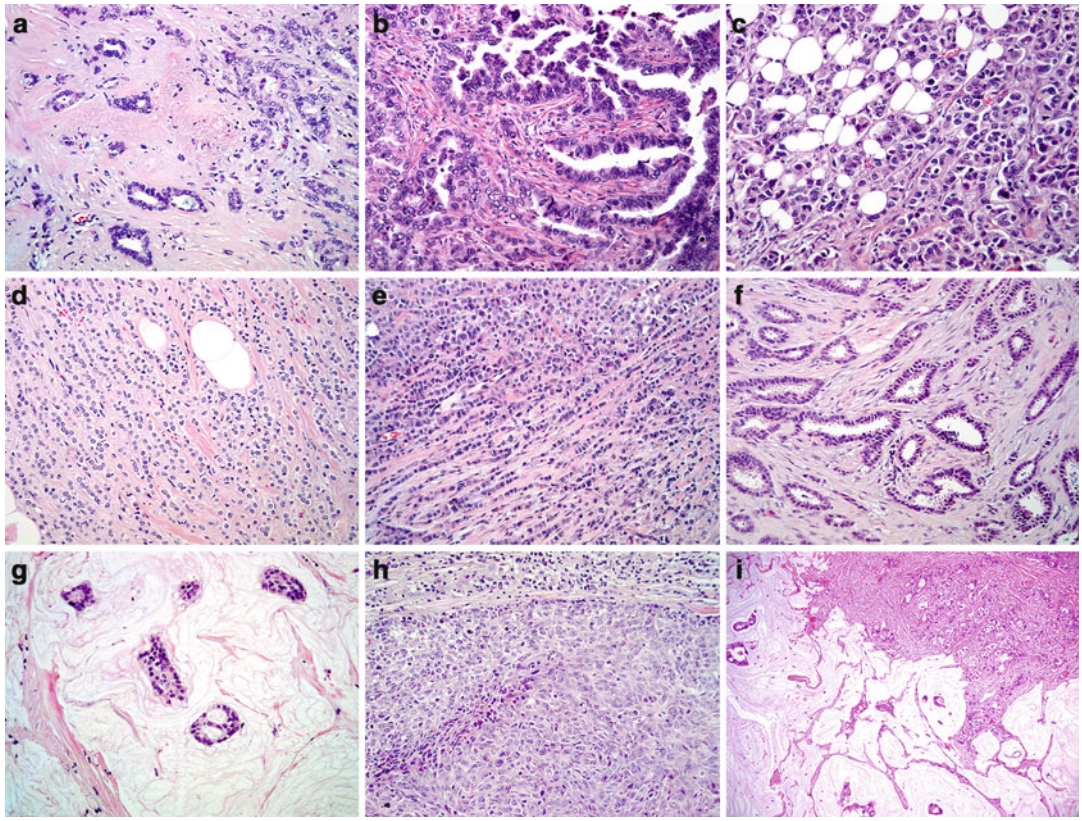


Fig. 6.8 Breast cancer pathology, common histologic subtypes. Grading invasive carcinomas depend on the degree of tubular formation, nuclear features, and mitotic index. Invasive ductal carcinoma of no special type. 8a-Grade 1; 8b-Grade 2; 8c-Grade 3. 8d-8i, common histologic types. 8d and 8e: invasive lobular carcinoma; 8f: invasive tubular carcinoma; 8g: invasive mucinous carcinoma; 8h: invasive medullary carcinoma; 8i: invasive ductal carcinoma with mucinous features

Perou et al. in the description of the molecular intrinsic subtypes of breast carcinomas. This work represents the first molecular classification of tumors, not considering the histology but a description of gene expression profiles of different breast tumors

- Four molecular subtypes were originally described: luminal, normal breast-like, HER2, and basal like. Subsequently luminals were further subdivided into Luminal A and Luminal B
- • Luminal tumors are reminiscent of "normal luminal epithelial cells," including CK8/18+. Lum A are ER+ and enriched with genes associated with active ER pathway, low levels of proliferation related genes, low histological grade, and generally good prognosis. The Lum B tumors are typically higher grade, with high proliferation indexes, and worse outcome, and a significant proportion are HER2+. Recent data show no good separation between Lum A and Lum B based on proliferation
- The normal breast-like subtype has gene expression profiles similar to fibroadenomas and normal breast enriched in adipose tissue genes. They are relatively poorly characterized and their prognostic significance is unclear. Recent studies suggest that the normal breast-like group may be an artifact caused by contamination of samples with normal tissue
- The HER2+ subtype shows amplification or 3+ reactivity by IHC, and expresses many other genes associated with the HER2 pathway.

However, a good number of *HER2* amplified, ER+ cancers fall into Lum B category

- The basal subtype expresses genes found in normal basal/MECs of breast, such as *CK5, CK14, p-cadherin, caveolins 1–2, CD44*, and *EGFR*. A minority has *EGFR* amplification. However, unlike MECs, they also express certain proteins characteristic of LECs, such as CK8, CK18, and KIT. Basal-like carcinomas are usually high histological grade tumors with high proliferation, necrosis, pushing borders, and lymphocytic infiltrate. Histological subtypes commonly seen in this category include medullary or metaplastic carcinomas. The basal-like subtype more commonly happen in younger individuals, often of African–American or Hispanic decent. The tumors usually show high initial response to cytotoxic chemotherapy, although the majority relapses and overall prognosis is very poor. These features are similar to those seen in tumors of patients with *BRCA1* mutation and the BRCA1 pathway is dysfunctional in basal-like cancers
- Three new ER-negative molecular subtypes have recently been described: One, referred to as "Molecular apocrine," is similar to HER2 subtype but shows activation of androgen receptor signaling; another, referred to "Interferon subtype," are characterized STAT1; and the third are referred to as the "claudin-low" group, which typically demonstrate a cancer-stem cell like phenotype
- Recently, several studies have questioned whether intrinsic subtyping is reproducible or stable, and whether it has any useful clinical significance
- The relationship of intrinsic molecular subtypes to special histological subtypes of breast cancer: Some studies, mainly using microarray-based technology, have shown that at the transcriptional level, tubular, mucinous, and lobular subtypes are more homogeneous than invasive ductal carcinomas of no special type (IDC/NST). Tubular, mucinous, and neuroendocrine carcinomas are typically included in the luminal phenotype. Adenocystic, medullary, and metaplastic are basal-like in agreement with previous studies
- The use of IHC has recently been advocated as a surrogate to microarray analysis to define the intrinsic molecular subtypes (Fig. 6.11): Expression by IHC of ER, PR, and luminal CKs (CK8 and CK18), lack of HER2 overexpression, and low Ki67 are typical of Lum A. Expression of ER, PR, and luminal CKs, and HER2 overexpression are seen in Lum B. Absence of ER and PR, and HER2, and expression of basal CKs (CK5/6) define basal-like tumors
- In the neoadjuvant settings, pathologic complete response (pCR) has been used to determine response to chemotherapy. pCR is only seen in 20–30% of patients (with use of standard anthracycline and taxane-based chemotherapy): Different rates have been shown across the different molecular subtypes: rates are 7% for Lum A, 17% for Lum B, 36% for HER2, and 43% for basal-like. This is one of the few scenarios where the use of molecular subtypes is advocated to translate into clinical practice. It is important to understand that molecular subtypes do not add much additional information of prognostic significance compared to the current standards of histologic subtypes and pathologic grading
- Even though the molecular classification has been one of the greatest advances in breast cancer in the last two decades, differences in molecular aspects of common histologic subtypes have been also recognized. Here are some examples: Medullary carcinomas show a prominent T helper cell immune response. Adenoid cystic carcinomas of the breast show a characteristic translocation *t*(6;9), which creates a *MYB–NFIB* fusion transcript. Secretory carcinomas also have an associated translocation, *t*(12;15) with the conformation of a *ETV6–NTRK3* fusion transcript. Micropapillary carcinomas have a high rate of lymph node metastasis and are typically included in the luminal B subtype, but a distinct set of gene clusters on their own, including high rate *FGFR1* amplification. Metaplastic breast cancers are a mixture of adenocarcinoma with metaplastic elements, homologous (squamous and spindle metaplasia) or heterologous (chondroid, osteoid, skeletal muscle). They

are typically associated with *PI3K/AKT* mutations—over 90% are HER2 and ER negative, and typically show a basal-like immunophenotype. A dysfunctional BRCA1 pathway is seen with over 60% of metaplastic carcinomas, which is caused by methylation silencing of the *BRCA1* gene promoter. In addition, a mouse model with *BRCA1* inactivation and wild-type allele of *TP53* show classical morphologic features of metaplastic carcinomas, including HER2 and basal markers (CK14 and EGFR), as well as activation of WNT pathway (Figs. 6.9, 6.10, and 6.11)

Important Somatic Mutations in Breast Cancer

- *TP53* is mutated in up to 30% of sporadic breast cancers, as well as many other types of cancers. The gene is located on chr 17 and encodes a nuclear transcription factor normally involved in cellular pathways activated in response to stress by inhibiting the proliferation, and inducing apoptosis, of cell damaged in a variety of ways. P53 acts as a transcriptional activator of genes involved in inhibition of the cell cycle, blood vessel formation, stimulation of apoptosis, and promotion of DNA repair. Currently, 2,500 different inactivating *TP53* mutations have been described in breast cancer. About 75% are single nucleotide substitutions leading to substitution of a single amino acid, and the remaining 25% are insertions, deletions, and nonsense mutations. Mutations in one allele are associated with inactivation of the other one by loss of heterozygosity (LOH) in most affected breast cancers. Mutation of the gene often correlates with increased nuclear p53 expression by IHC, which can be used as an easy surrogate assay in certain situations. Somatic mutations of *TP53* occur in IBCs and DCIS. In both settings, they are associated with increased tumor size and grade, as well as axillary metastasis and the rate of *TP53* mutations is very high in *BRCA1/BRAC2* carriers. The presence of *TP53* mutations is associated with poor prognosis: shorter DFS and OS in both node-negative and node-positive cancers. However, one study has shown an advantage in survival in node-negative breast cancer with mutated *TP53* treated with XRT compared to node-negative with WT *TP53*
- *ESR1* mutations: ERα has been reported as mutated and amplified in low percentage of breast cancers. Those with an ER A86V mutation are associated with lower activity of the receptor. The ER K303R mutation makes the receptor hypersensitive to activation by estrogen, which may promote tumor progression. An ER 437 stop codon mutation has been identified in metastatic breast cancers, and may be important in promoting metastatic spread, although the mutation is very rare
- Gene copy number alterations (referred to as allelic imbalance): AI is very common in breast cancers, occurring in as many as 50%. Gene amplification is a pathologic change commonly associated with increased mRNA transcription and protein expression of affected genes. Gene deletions are associated with loss of expression and function. Amplification of several regions in the breast cancer genome contains genes coding for oncogenes. For example, the chromosome 17q12 amplicon contains the *HER2* gene, the 8p24 amplicon the *MYC* gene, the 11q13 amplicon the *CCND1* gene, and the 6p11 the *ESR1* gene
  - Amplification of *HER2* is common in breast cancer and was discussed in detail above
  - Amplification of *ESR1* on chromosome 6p occurs in 5–20% of breast cancer, it is associated with increased ER expression, and it appears to increase responsiveness to tamoxifen therapy—so determining this feature may help optimize the use of endocrine therapy
  - 8q24 *MYC* on chromosome 8q24 is frequently amplified. MYC regulates cell growth and proliferation, and amplification is associated with higher histological grade, high proliferation rate, early recurrence, and death. Coamplification of *MYC* and *HER2* is very common, and trastuzumab is

Gene expression patterns of 85 experimental samples representing 78 carcinomas, three benign tumors, and four normal tissues, analyzed by hierarchical clustering using the 476 cDNA intrinsic clone set.

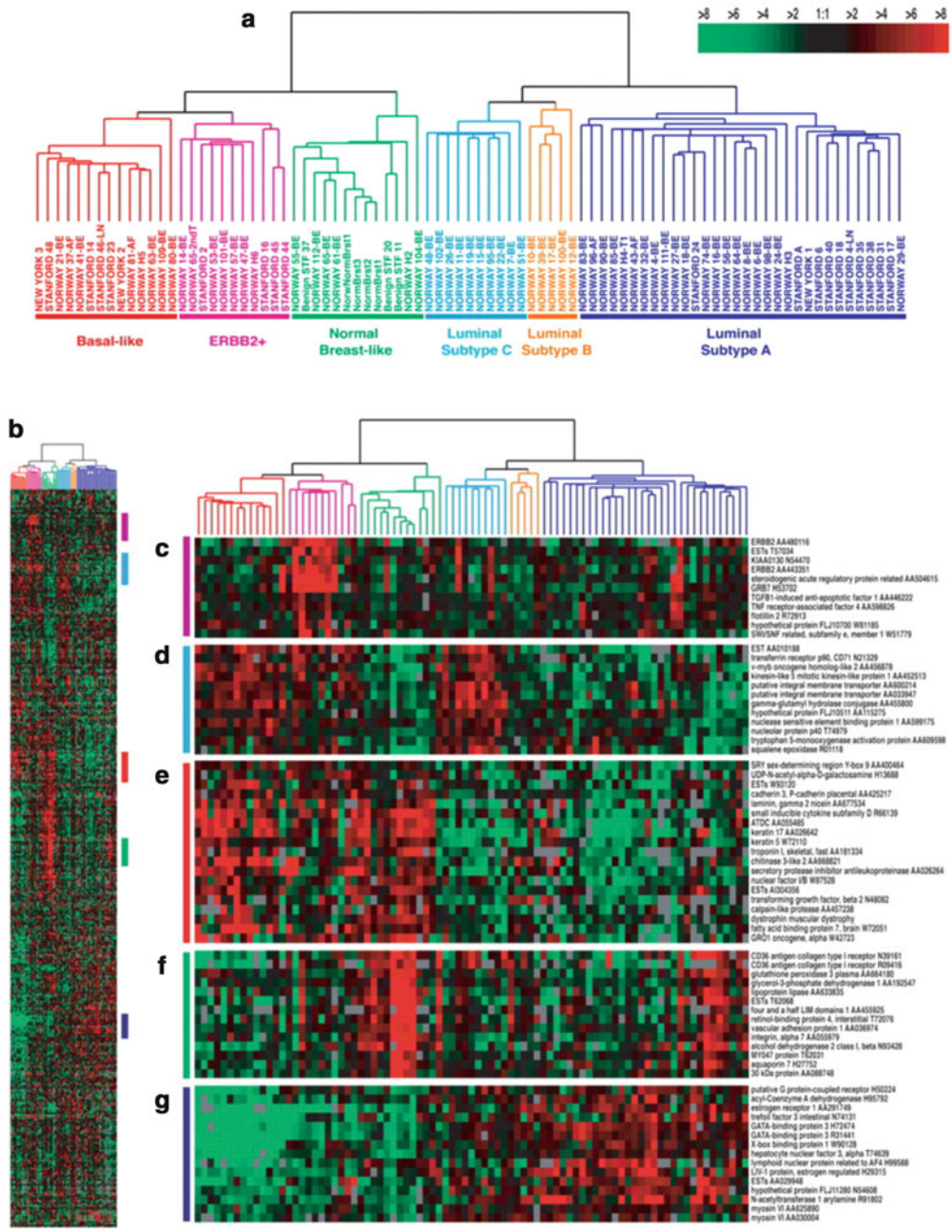


Fig. 6.9 Breast cancer molecular intrinsic subtypes. Gene expression patterns of 85 experimental samples representing 78 carcinomas, 3 benign tumors, and 4 normal tissues, analyzed by hierarchical clustering using the 476 cDNA intrinsic clone set. (**a**) The tumor specimens were divided into five (or six) subtypes based on differences in gene expression. The cluster dendrogram showing the five (or six) subtypes of tumors are colored as: luminal subtype A, *dark blue*; luminal subtype B, *yellow*; luminal subtype C, *light blue*; normal breast-like, *green*; basal-like, *red*; and ERBB2+, *pink*. (**b**) The full cluster diagram scaled down. The *colored bars* on the right represent the *inserts* presented in **c–g**. (**c**) ERBB2 amplicon cluster. (**d**) Novel unknown cluster. (**e**) Basal epithelial cell-enriched cluster. (**f**) Normal breast-like cluster. (**g**) Luminal epithelial gene cluster containing ER. (Copyright 2001 National Academy of Sciences, USA, with permission)

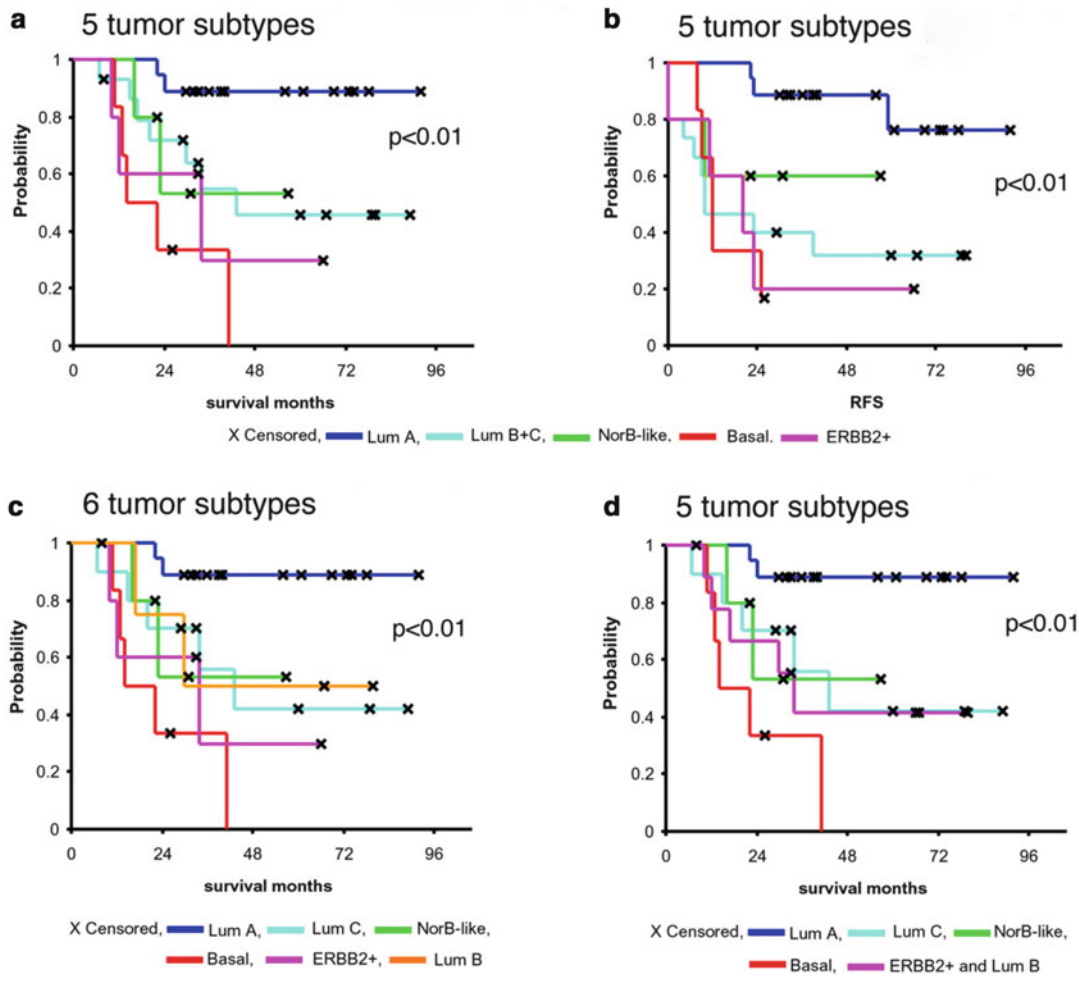


Fig. 6.10 Overall and relapse-free survival analysis of 49 breast cancer patients, uniformly treated in a prospective study, based on different gene expression classification. Overall and relapse-free survival analysis of the 49 breast cancer patients, uniformly treated in a prospective study, based on different gene expression classification. (**a**) Overall survival and (**b**) relapse-free survival for the five expression-based tumor subtypes based on the classification presented in Fig. 6.9 (luminals B and C were considered one group). (**c**) Overall survival estimated for the six-subtype classification with the three different luminal subtypes presented in Fig. 6.1. (**d**) Overall survival based on the five-subtype classification. (Copyright 2001 National Academy of Sciences, USA, with permission)

associated with improved outcome when coamplification exists compared to tumors with amplified *HER2* alone

– *CCND1* on chromosome 11q13 encodes a cell cycle regulatory protein that plays an important role in normal mammary gland development. The amplification is seen in up to 20% of breast cancers, which is significantly higher in lobular and with ER+/PR+ tumors. Coamplification of *MYC* and *CCND*1 occurs and is associated with aggressive phenotype. Coamplification with *FGFR1* has also been reported and is associated with worse outcome

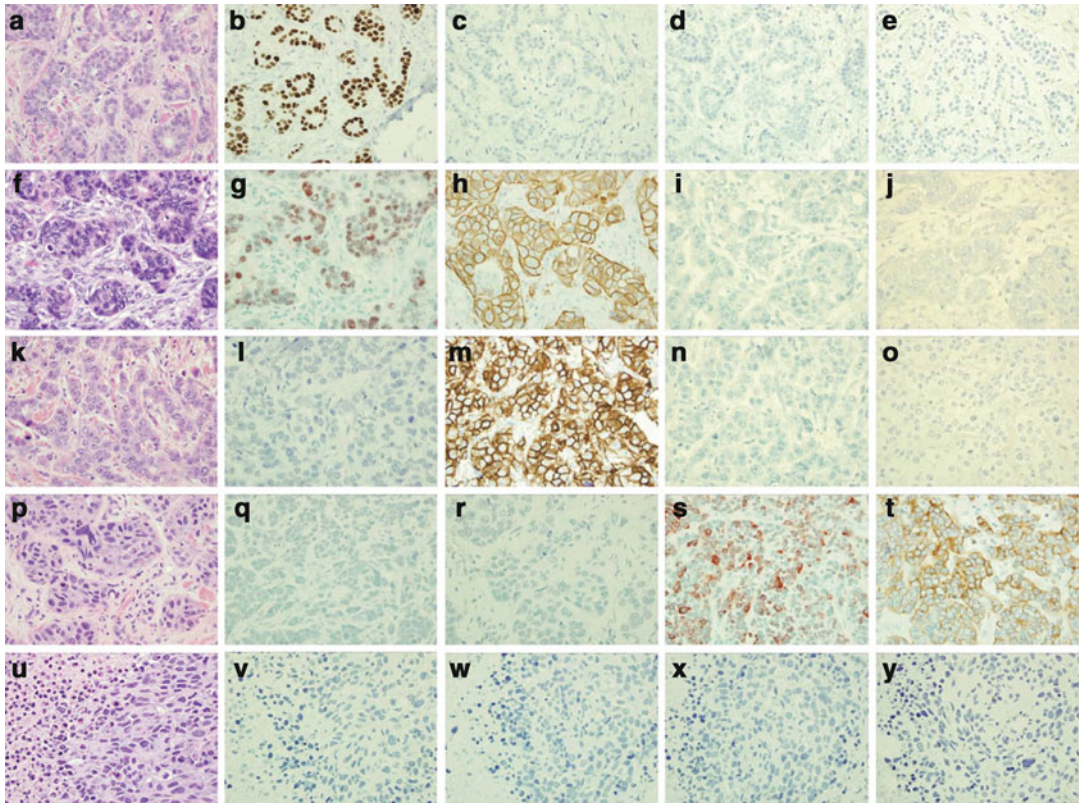


Fig. 6.11 Use of IHC in determination of molecular intrinsic subtypes. Representative cases for each molecular subtype. Hematoxylin and eosin and immunohistochemical stains of estrogen receptor, HER2, CK5/6, and epidermal growth factor receptor for luminal A (**a–e**), luminal B (**f–j**), HER2 (**k–o**), basal (**p–t**), and unclassified (**u–y**). (Adapted from Tang et al., 2009)

- 8p11.3 *FGFR1* on chromosome 8p11.3 is amplified in about 10% of breast cancers, and is associated with poor clinical outcome. Typically, it is associated with an ER+, PR+, and HER2− phenotype. In addition, *FGFR1* amplification is associated with resistance to endocrine therapy. *FGFR1* inhibitors have shown clinical response in patients with metastatic breast cancer, as an adjuvant to chemotherapy
- *MDM2 a*mplification has been reported in breast cancer and is associated with worse outcome in patients with node-negative disease
- Complex amplicons, as commonly observed with *HER2* on 17q22 (HER2) and *FGFR1* on8p11.3, typically involve a large number of adjacent genes that might also be important in the pathogenesis of breast cancer. For example, *TOP2A, RARA,* and *PPARB*. Coamplification with *TOP2A* is associated with responsiveness to anthracycline chemotherapy

Hereditary Breast Cancer BRCA1 and BRCA2

- Hereditary breast cancer (HBC) means that an alteration in a single major gene strongly contributes to the development of cancer or cancer-related conditions within the family. HBC was brought first to the medical literature by the surgeon Paul Broca, who accounted for

his wife pedigree in 1865 showing four generations of breast cancer and occurrences of cancer of the GI tract. In 1990, Hall et al. described a linkage specific site of breast cancer on chromosome 17q. *BRCA1* gene was later cloned. Subsequently, a second gene located in chromosome 13q was cloned, *BRCA2*. *BRCA1* and *BRCA2* are the major well characterized genes contributing to HBC, but others are known (but very rare), but it is likely that there are more yet to be discovered. In general, HBC is characterized by a significant earlier onset of breast cancer (average, 45, beginning at the age of 20), an excess of bilateralism, a greater frequency of multiple primary cancers (such as breast and ovary), and an autosomal dominant pattern of inheritance. In females, about 45% of HBC and 80% of hereditary breast and ovarian cancers are associated with *BRCA1* mutations. Most of the remaining HBCs are attributable to *BRCA2* mutations. The lifetime risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers is about 85%. The risk of ovarian cancer is 40–60% for *BRCA1* and 15% for *BRCA2*. It is estimated that about two-thirds of male breast cancer are linked to *BRCA2*, and one-third to *BRCA1* mutations. Overall, the prognosis of *BRCA1/2* mutated population appears to be similar to non-BRCA patients, although there is still controversy on this issue. For example, Ashkenazi Jews with *BRCA1/2* mutations appear to have relatively poor outcomes. Some new studies suggest that *BRCA1* patients may even have better survival than matched non-BRCA patients, and that *BRCA2* prognosis is worse

- *BRCA1*: 1,643 mutations have been described, of which 890 have been reported only once. For *BRCA2* approximately 1,856 mutations have been identified. BRCA shows two variants of penetrance, high (84% by 70 years of age) and low (32% by 70 years). Phenotypically, most *BRCA1* mutated tumors are basal-like breast cancers: highly proliferative, poorly differentiated, and genomically unstable. Most studies find *BRCA1* HBC to have a triple negative phenotype (ER−/PR−/HER2−). They are also associated with higher histological grade. A much higher prevalence of typical and atypical medullary carcinomas is also observed compared to sporadic breast cancers (35.3 vs. 3.4% for age matched controls and *BRCA1* mutated cancers). A lower prevalence of low grade tumors is seen in *BRCA1* mutated cancers compared to sporadic cancers, including ILC, tubulolobular, tubular, and invasive cribriform types. Indeed ILC commonly lack alterations at the *BRCA1* site. Aneuploidy is common among *BRCA1* mutated tumors. The frequency *of TP53* mutations is increased in *BRCA1* tumors compared to non-HBC and *BRCA2* tumors. Tamoxifen has been shown to be beneficial in reducing the risk of contralateral breast cancer in *BRCA1* patients, suggesting that they evolve from ER-positive precursors
- *BRCA2* mutated cancers have a more variable phenotypes than *BRCA1*, including a much higher proportion of luminal subtypes, and a much proportion of basal subtypes. Most studies show that the age of onset is older than in *BRCA1*. Some studies have shown higher prevalence of ILC associated with *BRCA2* than *BRCA1*. *BRCA2* also tend to show lesser aneuploidy and S phase. In *BRCA2*, ER/PR expression appears to be similar to non-BRCA cancer—a single study has even shown higher levels. Mutations of the *BRCA2* gene are also linked to other types of cancer, including pancreatic, prostate, and melanoma

Hereditary Breast Cancer Non-BRCA

- Non-BRCA HBC represents approximately 50% of cases in the general population. Overall, their clinical pathological features are statistically similar to sporadic breast cancer patients overall, including histological subtypes and grade, proliferation, p53 status, and intrinsic subtypes
- Germline mutations of *CDH1* (E-cadherin), which are very rare, confer a 40–70% lifetime risk of hereditary diffuse gastric carcinoma, and a 39–52% of ILC. E-cadherin is an adhe-

sion protein, which is lost in sporadic ILC through somatic mutations
- Li–Fraumeni syndrome: Lynch et al. described an extended kindred with a broad spectrum of cancers: sarcoma, breast cancer and brain tumors, lung and laryngeal cancers, leukemia, lymphoma, and adrenocortical carcinomas (SBLA syndrome). It is caused by a *TP53* germline mutation. The penetrance is variable with two age specific models: one in childhood and the second in adult life
- Cowden syndrome is a cancer associated genodermatosis, also referred as multiple hamartoma syndrome. It has an autosomal dominant pattern of inheritance, and is associated with distinctive mucocutaneous lesions and cancer of the breast, thyroid, and female genitourinary tract
- Germline mutations of the *PTEN* gene (also seen in Bannayan–Riley–Ruvalcaba syndrome). Cutaneous manifestations include trichilemmomas, which are pathognomonic. Also, multiple facial papules, acral and palmoplantar keratosis, skin tags and lipomas. Merkel cell carcinoma can occur. Thirty percent of women show breast carcinomas, and one-third shows bilateral disease. Patients with the mutation are candidates for prophylactic bilateral mastectomy

Familial Breast Cancer

- Familial breast cancer (FBC) is described as breast cancer within a family history of one or more first or second degree relatives affected. A patient with one or more first degree relatives with breast cancer in this category has a substantial excess lifetime risk of breast cancer when compared to patients in the general population. The relative risk increases from 1.80, 2.93, and 3.90 with one, two, and three first degree relatives compared to women without affected pedigree. FBC suggests a clustering of cancers that probably occurred by chance. In other words, there may be a combination of genetic and nongenetic (i.e., environmental) factors that contributed to the development of cancers within a family. In such instances, where an alteration in a single major gene is not likely or is not identified, individuals may still face elevated risks of cancer

Genome Sequencing of Breast Cancers

- Whole genome sequencing (WGS): The use of rapidly evolving techniques that combines whole genome, deep generation sequencing, and next generation sequencing have provided novel insights into the understanding of mutational analysis in breast cancer. Although these studies are in their infancy, it is already clear that essentially all breast cancers have an enormous number of mutations, far more than originally imagined—suggesting that developing widely successful targeted therapies will be extremely difficult. The seminal study by Sjoblom, based on outdated sequencing technology, found more than 100 distinct mutations in just 11 breast cancers. A more recent study Ding et al., using newer higher resolution technology, found an average of 50 somatic point mutations (including *JAK2, PTCH2, CSMD1, NRK, TP53*, *MAP3K8*), 28 large deletions, 6 inversions, and 7 translocations in a single case of basal-like breast cancer. One of the next major challenges in breast cancer research will be to determine which of the mutations are the "drivers" for developing breast cancer

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Molecular Pathology of Ovarian Cancer

7

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Introduction

- Ovarian cancer is the fifth most common cause of cancer-related death in women in the US, and the leading cause of death among gynecologic neoplasms
- The 5-year survival for ovarian cancer is only 37%
- Understanding the pathophysiology of ovarian neoplasms, particularly the molecular basis of disease, is crucial in improving diagnostic and treatment modalities

Classification of Ovarian Epithelial Neoplasms

- The traditional idea of a progression from well- to poorly differentiated carcinoma for all ovarian cancer subtypes has recently been replaced by a comprehensive, dualistic model of ovarian epithelial neoplasia based on new morphologic and molecular data (Shih and Kurman 2004) (Table 7.1)
- This classification is described below, with a more detailed description of the molecular abnormalities included in subsequent sections (Fig. 7.1).

L. Cheng and J.N. Eble (eds.), *Molecular Surgical Pathology*,
DOI 10.1007/978-1-4614-4900-3_7,

Table 7.1 Characteristics of Type I vs. Type II ovarian tumors

	Type I	Type II
Histotypes	Low-grade serous carcinoma Mucinous carcinoma Endometrioid carcinoma Clear cell carcinoma Malignant Brenner tumor	High-grade serous carcinoma Malignant mixed müllerian tumor Undifferentiated carcinoma
Precursors/origin	Benign adenomas → atypical proliferative (borderline) tumors → malignant neoplasms	Arise from tubal epithelium
Clinical behavior	Slow growing Often confined to ovary at time of diagnosis	Aggressive Rapid progression Early metastasis
Molecular abnormalities	MAPK signaling pathway (*KRAS/BRAF*) *Wnt*/β(Beta)-catenin/Cyclin D1 *PI3K/Akt2/PTEN* pathway *ARID1a* Microsatellite instability *HNF1-β(beta)* *PPP2R1A* *EGFR/HER2neu*	*TP53* *CDKN2*/p16 *BRCA1* and *BRCA2* *Akt2* (*PI3K/Akt2/PTEN* pathway) *Notch3* *HBXAP (Rsf-1)* *NAC1* HLA-G Cyclin E1 *EGFR/HER2*

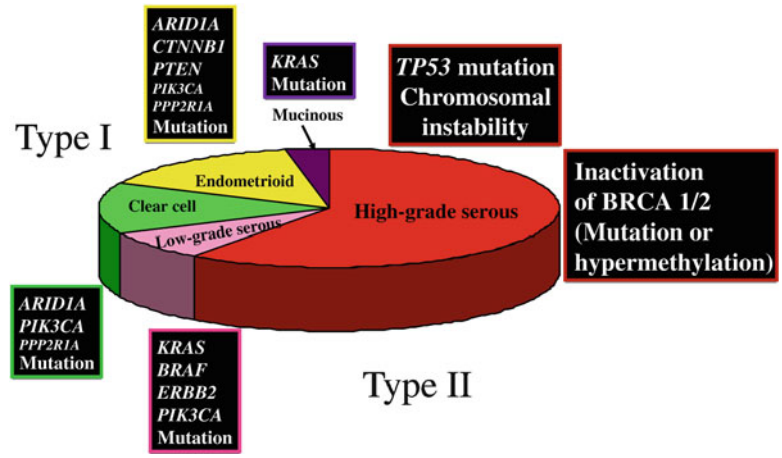

Fig. 7.1 Prevalence of epithelial ovarian cancer histotypes and their associated molecular abnormalties. (Reprinted from Human Pathology, Kurman and Shih 2011, with permission from Elsevier)

Type I Tumors

- Type I tumors are composed of several diverse histotypes including low-grade serous carcinoma (LGSC), mucinous carcinoma, endometrioid carcinoma, malignant Brenner tumor, and clear cell carcinoma
- Precursors/origin
  - These tumors are thought to develop in a stepwise fashion from benign to borderline to malignant tumors
  - Borderline tumors
    - The term "borderline tumor" refers to an entity intermediate in behavior between cystadenomas and carcinomas
    - Findings in recent years have led to refinement of this category and the histologic and behavioral spectrum it encompasses
    - For serous tumors, two categories have been defined based on behavior: atypical proliferative serous tumors (APST),

which is a typical borderline tumor with or without noninvasive implants; and micropapillary serous carcinoma (MPSC), a term synonymous with noninvasive LGSC
    - Mucinous borderline tumors of intestinal type are relatively indolent even if they contain areas of intraepithelial carcinoma or foci of microinvasion (<5 mm), and therefore are best categorized as atypical mucinous proliferative tumors (APMT), with qualification as necessary ("with intraepithelial carcinoma" or "with microinvasion")
    - Atypical proliferative seromucinous tumor, also known as mucinous borderline tumor of endocervical type, has been found to be associated with endometriosis and endometrioid tumors
  - Low-grade serous tumors are believed to progress from adenomas to borderline tumors to noninvasive micropapillary carcinoma (noninvasive LGSC), and finally to invasive LGSC
  - Mucinous tumors may arise from the tubal–peritoneal junction, based on their association with Walthard nests and Brenner tumors, and the presence of transitional metaplasia at the tubal–peritoneal junction in some salpingectomy specimens (Seidman et al. 2011)
  - Many endometrioid and clear cell carcinomas have also been found to be associated with benign or borderline-like lesions, as well as with endometriosis, which is thought to be the benign precursor of these tumors
- Clinical behavior
  - These are indolent tumors which grow to a large size while remaining confined to the ovary at diagnosis
  - Although LGSC demonstrates a pattern of spread similar to its high-grade counterpart, it behaves in a more indolent fashion and is associated with a better prognosis
- Summary of molecular findings
  - Endometrioid and clear cell carcinomas commonly have abnormalities in *ARID1A, CCND1*/β(beta)-catenin, and the *PI3K/Akt2/PTEN* pathway
  - LGSC commonly has abnormalities in the MAPK signaling pathway (*KRAS* and *BRAF*) and the *PI3K/Akt2/PTEN* pathway
  - Mucinous carcinoma commonly has mutations in *KRAS*
  - Type I tumors generally lack mutations in *TP53*, unlike type II tumors, and therefore this helps in differentiating the two categories

Type II Tumors

- This category includes high-grade serous carcinoma (HGSC), malignant mixed müllerian tumor (MMMT, carcinosarcoma), and undifferentiated carcinoma
- Histopathology
  - Tumors in this category are high grade in appearance, with complex architecture and significant nuclear atypia
  - Necrosis and high mitotic activity are common
  - HGSC is exclusively epithelial in differentiation, while MMMT displays both epithelial and stromal differentiation
- Precursors/origin
  - The majority of type II tumors appear to arise from tubal epithelium, either from intraepithelial carcinomas which shed cells that implant on the ovary, or from normal tubal epithelium that implants on the ovary to form inclusion cysts from which serous carcinoma can develop (Kurman and Shih 2010)
  - Evidence that supports an origin from the distal fallopian tube includes:
    - Gene expression profiling has found a significant correlation between serous carcinomas and the normal fallopian tube
    - Higher rates of tubal hyperplasia, dysplasia, and occult carcinoma (particularly in the distal tube or fimbriae), as well as *TP53* mutations within dysplastic foci, have been found in prophylactic salpingo-oophorectomy specimens compared to resections for other causes

- In patients with concurrent serous tubal intraepithelial carcinoma (STIC) and ovarian serous carcinoma, identical *TP53* mutations have been found in both components

- Clinical behavior
  - These tumors behave aggressively, with rapid progression and early metastasis
- Summary of molecular findings
  - Type II tumors commonly have mutations in *TP53*, chromosomal instability, and inactivation of *BRCA1* and *BRCA2*

Molecular Pathways and Alterations by Tumor Type

Type I Ovarian Tumors

Low-Grade Serous Tumors

- Introduction
  - This category of tumors includes APST and LGSC
  - Low-grade serous tumors of all types commonly demonstrate a papillary architecture and psammoma bodies
  - APST demonstrates papillary epithelial proliferation with hierarchical branching
    - Foci of invasion less than 5 mm are permitted (APST with microinvasion)
    - Extraovarian implants may be noninvasive, noninvasive desmoplastic, or invasive
  - MPSC, or noninvasive LGSC, demonstrates an appearance similar to that of APST but with a micropapillary or cribriform epithelial proliferation
  - LGSC also demonstrates cells with a higher nuclear–cytoplasmic ratio and slightly more cytologic atypia than APST
  - The following discussion focuses largely on the molecular pathology of LGSC, but also addresses findings in APST and benign serous tumors where relevant
- Genetic pathways: functions, role in pathogenesis, and frequency of abnormalities
  - *MAPK* signaling pathway
    - *MAPK* (mitogen-activated protein kinase), also known as *ERK* (extracellular signal-regulated protein kinase), is a downstream target of RAS, RAF, and MAPK/ERK kinase
    - *MAPK* responds to growth factors and other signals by promoting cell proliferation and opposing cell death, and is important in mediating drug-induced apoptosis in tumor cells
    - *KRAS* and *BRAF* are both oncogenes involved in the activation of the MAPK pathway
    - Frequency of mutations
      - *KRAS* mutations have been found in 22–36% of serous borderline tumors and up to 33% of LGSC (Mok et al. 1993)
      - BRAF mutations have been found in up to 31% of serous borderline tumors and up to 36% of LGSC (Mayr et al. 2006)
      - Overall, 60–88% of APST express mutations in either *KRAS* or *BRAF* (Ho et al. 2004)
        - With rare exceptions, these mutations are mutually exclusive
      - Mutations in *KRAS* and *BRAF* help distinguish low-grade serous tumors from HGSC, as these mutations are found in only up to 12% of HGSC (Sieben et al. 2004)
        - The V599E mutation in *BRAF* occurs exclusively in LGSC (36%)
      - Of note, serous cystadenomas adjacent to *KRAS*- or *BRAF*-mutated serous borderline tumors were found to have identical mutations in 86% of cases, suggesting that mutation of these two genes precedes progression to serous borderline tumors
        - Others have found these mutations in early APST, supporting their early role in tumorigenesis
    - Immunohistochemical findings
      - By immunohistochemical staining, activated (phosphorylated) MAPK was found to be expressed in 71% of APST, 80–81% of LGSC, and 41% of HGSC (Hsu et al. 2004)

        - In low-grade serous tumors, MAPK immunoexpression correlates with mutations in *KRAS* and *BRAF*. This is in contrast to findings in HGSC
    - *CDKN2*/p16
        - p16, encoded by *CDKN2* (*p16ink4*) on 9p21, is a tumor suppressor gene involved in the Rb pathway, and is discussed in more detail below (see section "HGSC and MMMT")
        - Although found more frequently overexpressed in HGSC, p16 has been found by some authors to be expressed in a significant percentage of low-grade serous tumors
        - Immunohistochemistry
            - Overexpression of p16 by immunohistochemical analysis has been found in 27.3% of LGSC in one study, and as many as 85% of serous borderline tumors in another (O'Neill et al. 2007; Nazlioglu et al. 2010)
                - A possible loss of expression as tumors progress from APST to LGSC has been proposed
- Clinical implications
    - *MAPK* signaling pathway
        - The constitutive activation of the *MAPK* signaling pathway in type I tumors suggests a role for MAPK kinase inhibitors in treatment
            - In fact, treatment of *KRAS*- or *BRAF*-mutated ovarian cancer cell lines with a MAPK kinase inhibitor was found to cause significant apoptosis and growth inhibition (Pohl et al. 2005)
        - Treatment with cisplatin may induce activation of MAPK, with subsequent development of cisplatin resistance
            - Furthermore, treatment with a proteasome inhibitor sensitizes cisplatin-resistant ovarian cancer cells to cisplatin-induced cell death, indicating a potential role for proteasome inhibitors along with cisplatin in *MAPK*-activated tumors (Wang et al. 2011)
        - Patients with both *MAPK* expression and paclitaxel sensitivity have significantly better 5-year survival than those without these two characteristics (74.9 vs. 31%) (Hsu et al. 2004)
- Summary
    - The most common molecular abnormalities in low-grade serous tumors are in the *MAPK* signaling pathway
        - *MAPK* expression is common in APST and LGSC, and correlates with mutations in *KRAS* and *BRAF*
    - Mutations in *KRAS* and *BRAF* are useful in distinguishing LGSC from HGSC, in which they occur much less frequently
    - A possible role exists for MAPK kinase inhibitors and proteasome inhibitors in the treatment of *MAPK*-activated cancers
    - Aberrant expression of p16 is more common in HGSC, but has also been found in a subset of LGSC, with some authors finding a correlation with lower stage and lower grade tumors

Endometrioid and Clear Cell Carcinomas

- Introduction
    - Endometrioid carcinomas of the ovary, most commonly found in woman in their 50s, demonstrate a histologic appearance similar to that of their uterine counterparts
        - Well-differentiated endometrioid carcinomas are composed of branching and confluent glands lined by tall columnar stratified cells
        - Grading is based primarily on the extent of nonsquamous solid architecture, with grade 1 having <5% solid areas, grade 2 with 5–50%, and grade 3 with >50%
        - Nuclear atypia is variable and can be significant, and may also be used as a criterion to assign a tumor one grade higher than that indicated by architecture
        - Various types of metaplasia may be seen, including squamous, secretory, and mucinous
        - Endometrioid carcinomas tend to demonstrate expansile invasion, but also may be infiltrative

- Clear cell carcinomas, which affect a similar age group, display diverse but distinct histologic appearances
  - Common patterns include papillary, tubulocystic, and solid, and these often coexist in a single tumor
    - Papillary areas commonly have hyalinized stroma
    - Tubulocystic carcinomas demonstrate tubules and cysts of varying sizes lined by tumor cells
    - Solid areas demonstrate sheets of polygonal cells with clear cytoplasm
  - A variable number of cells may demonstrate eosinophilic cytoplasm rather than the classic clear cytoplasm
    - PAS-positive hyaline globules in the cytoplasm may also be seen
  - A spectrum of nuclear atypia is observed, often within the same tumor
  - However, clear cell carcinomas are classified as high grade by definition
- Endometrioid and clear cell carcinomas are discussed together in this section due to the significant overlap of molecular pathways involved in their respective pathogeneses

• Genetic pathways: functions, role in pathogenesis, and frequency of abnormalities
- Wnt/β(beta)-catenin pathway
  - The gene *CTNNB1* on 3p22.1 encodes β(beta)-catenin, a protein involved in the Wnt signaling pathway, which plays a role in the regulation of cell proliferation and differentiation
  - Missense mutations of *CTNNB1* frequently result in constitutive activation of the Wnt signaling pathway in endometrioid carcinomas
  - Frequency of mutations
    - Mutations in *CTNNB1* have been found in 31–38% of ovarian endometrioid tumors, primarily in exon 3 of the gene (Catasús et al. 2004)
    - Although *CTNNB1* mutations have been linked to microsatellite instability in colon cancers, only rare cases of ovarian endometrioid carcinoma have been found to have both *CTNNB1* mutations and microsatellite instability
      - While *CTNNB1* mutations are always associated with nuclear staining for β(beta)-catenin, microsatellite instability is associated with a membranous β(beta)-catenin staining pattern
      - These abnormalities therefore likely represent two independent mechanisms of pathogenesis
  - Immunohistochemistry
    - Nuclear β(beta)-catenin immunohistochemical staining has been found in 38–85% of endometrioid carcinomas and up to 5.5% of clear cell carcinomas (Moreno-Bueno et al. 2001)
      - In endometrioid carcinomas, immunopositivity has been strongly correlated with the presence of *CTNNB1* mutations
    - Nuclear expression of β(beta)-catenin in endometrioid carcinomas has been associated with squamous differentiation in these tumors
- Cyclin D1
  - The protein Cyclin D1, encoded by the oncogene *CCND1* on 11q13, is a target of the β(beta)-catenin pathway, with activation of the pathway resulting in increased expression of cyclin D1
  - Cyclins are involved in the regulation of cyclin-dependent protein kinases (cdks), with cyclin D1 functioning specifically in allowing the cell to progress from G1 to S phase
  - Immunohistochemistry
    - Although some studies have found no association between cyclin D1 overexpression and histotype, others have found a more frequent association with endometrioid carcinomas, with immunohistochemical positivity being found in 32% of ovarian endometrioid carcinomas and 6% of clear cell carcinomas (Catasús et al. 2004)

- *PI3K/Akt2/PTEN* pathway
  - The *PI3K/Akt2/PTEN* pathway is involved in the regulation of apoptosis, angiogenesis, cell proliferation and growth, and cell metabolism
  - Activation of the pathway can be the result of amplification of *PIK3CA* or *Akt2*, activating mutations in *PIK3CA*, or inactivating mutations of *PTEN*
    - *PIK3CA* is an oncogene located on chromosome 3q26.32, and encodes the *PI3K* catalytic subunit
    - *Akt2* is an oncogene located on chromosome 19q13.1–13.2, and encodes a protein–serine/threonine kinase
    - *PTEN* (phosphatase and tensin homolog deleted on chromosome 10) is a tumor suppressor gene located on chromosome 10q23.3
  - Frequency of mutations/amplifications
    - Mutation or amplification of *PIK3CA* has been found in 30.5% of ovarian cancers overall, and up to 45% of endometrioid and clear cell carcinomas (Campbell et al. 2004)
    - *PTEN* is mutated in 14–31% of endometrioid carcinomas (mostly of low grade and low stage), up to 8.3% of clear cell carcinomas, and 20.6% of endometrial cysts (Sato et al. 2000; Willner et al. 2007)
      - Most of the identified mutations have been frameshift mutations
      - LOH at the 10q23.3 locus has been found in 42% of endometrioid carcinomas, 27.3% of clear cell carcinomas, 56.5% of endometrial cysts, and 0% of normal endometrium
    - Mutations in *Akt2* are more often seen in HGSC (see below)
- *ARID1A*
  - The gene *ARID1A* encodes the protein BAF250a, which binds to AT-rich DNA sequences and functions as a component of a complex (SWI/SNF) involved in regulating the expression of cell proliferation genes
  - Because many cases of *ARID1A* mutations show both alleles to be affected, it is hypothesized that *ARID1A* is a tumor suppressor gene
  - Frequency of mutations
    - Mutations in *ARID1A* have been found in 46–57% of ovarian clear cell carcinomas, as well as 71% of ovarian clear cell carcinoma cell lines (Jones et al. 2010)
    - Endometrioid carcinomas have also been found to harbor *ARID1A* mutations at a frequency of up 30% (Wiegand et al. 2010)
    - Mutations have not been found in HGSC
  - Immunohistochemical findings
    - Mutations in *ARID1A* in both endometrioid carcinomas and clear cell carcinomas have been correlated with a loss of immunopositivity for BAF250a, with 73% of *ARID1A*-mutated clear cell carcinomas and 50% of *ARID1A*-mutated endometrioid carcinomas showing this loss
      - Loss of expression is specific for these tumors vs. HGSC
    - *ARID1A* may be mutated earlier than *HNF1-β(beta)* in tumorigenesis
- *HNF-1-β(beta)*
  - HNF-1-β(beta) (hepatocyte nuclear factor-1-β(beta), also known as vHNF-1 or LFB3), along with the related protein HNF-1-α(alpha), has been shown to play a role in transcriptional activation during embryogenesis
  - *HNF-1-β(beta)* mRNA expression levels have been found to be several times higher in clear cell carcinomas vs. other ovarian tumors
    - The mechanism of upregulation may be related to CpG island hypomethylation
  - Immunohistochemical findings
    - Almost all clear cell carcinomas have been found to be immunopositive for HNF-1-β(beta), with most other tumors showing either no staining or only focal faint positivity

        - Nuclear staining is also absent in endometriosis and in normal ovarian surface epithelium (Tsuchiya et al. 2003)
- Microsatellite instability
    - Microsatellite instability refers to the inactivation of DNA mismatch repair genes, with a resulting increase in mutation frequency of oncogenes and tumor suppressor genes, and a consequently increased risk of neoplastic transformation in various tissue types
    - This is the mechanism responsible for Lynch syndrome (hereditary nonpolyposis colorectal cancer, or HNPCC), a hereditary cancer syndrome in which loss of function of the mismatch repair genes *MLH1, MSH2,* and *MSH6* is frequently found
    - In addition to colorectal cancers, patients with Lynch syndrome are at increased risk of developing cancers at numerous other sites, including the upper gastrointestinal tract, urinary system, and female genital tract, particularly the endometrium and ovary
    - Mechanisms of microsatellite instability in ovarian cancers include frameshift mutations in the coding tracts of *BAX*, *IGFIIR*, and *MSH3*, as well as *MLH-1* promoter hypermethylation
        - Loss of *hMSH2* expression has also been demonstrated
    - Frequency
        - The overall frequency of microsatellite instability in sporadic ovarian cancers was found to be 17%
        - Endometrioid tumors show a frequency of 17–50%, and clear cell carcinomas show a frequency of 6% (Fujita et al. 1995)
        - Microsatellite instability is uncommon in other ovarian tumor types
    - Immunohistochemical findings
        - Loss of hMLH-1 nuclear staining has been reported to occur in 14% of endometrioid carcinomas and 6% of clear cell carcinomas, with most tumors being of high grade but low stage (Catasús et al. 2004)
- *MAPK* signaling pathway
    - Mutations in *KRAS* and *BRAF,* both oncogenes involved in the activation of the *MAPK* pathway, are more commonly associated with mucinous tumors and LGSC, but may also be mutated in endometrioid and clear cell carcinomas. (See sections "Mucinous Tumors" and "Low-Grade Serous Tumors" for more details on these two genes and the MAPK pathway.)
    - Frequency of mutations
        - *KRAS* mutations have been found in up to 10% of endometrioid carcinomas and very rarely in clear cell carcinomas (Gemignani et al. 2003)
        - *BRAF* mutations have been found in up to 9% of endometrioid carcinomas and up to 25% of clear cell carcinomas (Mayr et al. 2006)
- *TP53*
    - Although found with the greatest frequency in serous tumors, *TP53* mutations have also been reported to occur in a subset of endometrioid and clear cell carcinomas, particularly those of advanced stage and high grade
    - Frequency of mutations
        - *TP53* mutations have been reported to occur in as many as 42% of endometrioid carcinomas and 8% of clear cell carcinomas, with the highest frequency (75%) in grade 3 endometrioid carcinomas (Willner et al. 2007)
        - In a mouse model, *TP53* mutations were found with lower frequency in tumors which had defects in the Wnt/β(beta)-catenin or *PI3K/Akt2/PTEN* signaling pathways, suggesting two separate pathways in the development of low vs. high-grade endometrioid carcinomas (Wu et al. 2007)
- *PPP2R1A*
    - The serine–threonine protein phosphatase PP2A is a family of holoen-

zymes containing a heterodimer core with a catalytic subunit and a regulatory subunit (PPP2R1A or PPP2R1B)
    - ♦ PPP2R1A acts as a scaffold in this complex, which is involved in regulating cell growth and proliferation
  - The heterozygous and clustered nature of mutations in this gene suggest that *PPP2R1A* is an oncogene in tumorigenesis
  - Frequency of mutations
    - ♦ Mutations in the *PPP2R1A* gene have been found in 7.1% of clear cell carcinomas, as well as in 3/7 ovarian clear cell carcinoma cell lines (Jones et al. 2010)
- Clinical implications
  - *Wnt/B(beta)-catenin pathway*
    - The presence of CTNNB1 mutations or β(beta)-catenin nuclear immunopositivity in endometrioid carcinomas has been associated with better differentiation, low grade, early stage, and a favorable prognosis
    - One group found cyclin D1 expression to be inversely correlated with tumor grade, suggesting a better prognosis for tumors with high cyclin D1 expression (Sui et al. 1999)
  - *HNF-1-β(beta)*
    - Silencing of *HNF-1-β(beta)* in ovarian cancer cell lines results in significantly more apoptosis compared to controls. It is therefore an important potential target for novel ovarian cancer therapies
    - Given its relative specificity for clear cell carcinomas compared to other ovarian cancer histotypes, HNF-1-β(beta) is also a useful immunohistochemical marker in diagnosis
  - Microsatellite instability
    - PCR analysis of tumor tissue using microsatellite markers as well as detecting loss of expression of mismatch repair genes by immunohistochemistry are useful methods of assessing for microsatellite instability in patients deemed to be at risk
    - Data concerning the effect of microsatellite instability on clinical behavior are somewhat inconsistent, with evidence of association with longer survival at odds with the association of microsatellite instability with undifferentiated components and aggressive behavior
- Summary
  - Endometrioid and clear cell carcinomas show significant similarities and overlap in their molecular pathology
  - The Wnt/β(beta)-catenin pathway, including cyclin D1, is most commonly aberrant in endometrioid carcinomas
    - Mutations in *CTNNB1*, which correlate with immunohistochemical positivity for β(beta)-catenin, are commonly seen, and have been linked to squamous differentiation of the tumor
    - Cyclin D1 immunopositivity may also be seen
  - The *PI3K/Akt2/PTEN* pathway is also affected in these two tumor types, with *PIK3CA* abnormalities common in both, and *PTEN* mutations more common in endometrioid carcinomas
    - Of note, endometrioid carcinomas with *PTEN* mutations have been associated with a lower grade and a better prognosis
  - *ARID1A* is a putative tumor suppressor gene, aberrations in which can be seen in both tumor types, although they are more frequently associated with clear cell carcinomas
    - In *ARID1A*-mutated carcinomas, loss of the BAF250a protein is seen by immunohistochemistry
  - Expression of *HNF-1-β(beta)* is relatively specific for clear cell carcinomas, and immunohistochemical staining for the protein therefore serves as a useful diagnostic tool
  - Microsatellite instability is particularly associated with endometrioid carcinomas, which are seen with increased frequency in patients with Lynch syndrome
    - A small percentage of clear cell carcinomas also demonstrate microsatellite instability

- *MAPK* signaling pathway defects, namely mutations in *KRAS* and *BRAF*, may also be present in endometrioid and clear cell carcinomas
- Although *TP53* mutations are more frequently associated with type II tumors, they also occur in a significant number of high-grade endometrioid carcinomas
- Clear cell carcinomas also demonstrate abnormalities in the gene *PPP2R1A*

Mucinous Tumors

- Introduction
  - The category of ovarian mucinous tumors includes APMT and mucinous carcinoma, both of which are often unilateral and may grow to a large size prior to resection
  - Two types of APMT have been described: the gastrointestinal type and the endocervical-like or seromucinous type
  - The more common subtype is the gastrointestinal type, a generally multicystic tumor with an intestinal-type mucinous lining
  - Endocervical-like or seromucinous APMT demonstrates both mucinous and serous-type lining cells, and may also demonstrate endometrioid or eosinophilic epithelium
    - These tumors are more often bilateral and small, with an architecture resembling that of APST, and are more frequently associated with endometrioid and clear cell carcinomas
  - APMT may display architectural complexity
  - The presence of marked nuclear atypia without invasion warrants a diagnosis of mucinous intraepithelial carcinoma
    - The prognosis of these tumors is still favorable
  - Mucinous carcinomas, the majority of which are of the gastrointestinal type, are uncommon compared to other types of ovarian tumors
    - A well-differentiated architecture is typical, and grading is best determined based on nuclear features
    - Invasion in these tumors may be destructive and infiltrative or expansile
    - Mucinous carcinomas often coexist with adjacent APMT
- Genetic pathways: functions, role in pathogenesis, and frequency of abnormalities
  - MAPK signaling pathway
    - *KRAS* (vi-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog) and *BRAF* (v-raf murine sarcoma viral oncogene homolog B1) are both members of the RAS–RAF–MEK–ERK–MAP kinase pathway (see section "Low-Grade Serous Tumors" above), and are also downstream activators of the EGFR pathway
    - Both KRAS and BRAF function as oncogenes
    - Frequency of mutations
      - *KRAS* mutations have been found in 13–33% of mucinous adenomas, 33–79% of mucinous borderline tumors, and 10–75% of mucinous carcinomas (Mok et al. 1993; Sieben et al. 2004)
      - Codon 12 is the most common site of mutation in the *KRAS* gene
      - *BRAF* mutations are relatively uncommon in mucinous tumors, found in up to 9% of mucinous carcinomas and not at all in mucinous borderline tumors (Mayr et al. 2006)
      - All *BRAF* mutations were found in exon 15, with most involving codon 600
      - As in other tumor types, *KRAS* and *BRAF* mutations have been found to be mutually exclusive in most cases
- Clinical implications
  - *KRAS* mutations have been demonstrated to be more common in lower stage tumors, but no association with prognosis has been found
- Summary
  - The most commonly mutated gene in mucinous tumors of the ovary is *KRAS,* with mutations found in adenomas, APMT, and carcinomas
  - *BRAF* mutations are much less common, but when present have been found to be mutually exclusive with *KRAS* mutations

Type II Ovarian Tumors

HGSC and MMMT

- Introduction
  - HGSC is the most common type of ovarian cancer, and usually occurs in the sixth and seventh decades
    - Patients often present at an advanced stage, with abdominal and pelvic dissemination of tumor
    - Architecturally, these carcinomas are complex, with papillary, glandular, cribriform, and solid patterns; necrosis is common
    - High-grade cytology is seen, with marked nuclear atypia and high mitotic activity
    - Overall survival is generally poor
  - MMMT, or carcinosarcoma, is characterized by both epithelial and stromal malignant components
    - The epithelial component may be comprised of any ovarian carcinoma type, most often HGSC or endometrioid carcinoma
    - The stromal component demonstrates a sarcomatous appearance and may contain heterologous elements
    - The frequent expression of epithelial markers in the sarcomatous component, as well as the demonstration of monoclonality in these tumors, supports the idea that these are carcinomas with sarcomatoid differentiation
      - Some have referred to them as "metaplastic carcinomas"
    - HGSC and MMMT are discussed together in this section because of similar molecular aberrations, particularly in *TP53*, as well as their putative similar origin from STIC
- Genetic pathways: functions, role in pathogenesis, and frequency of abnormalities
  - *TP53*
    - *TP53* is a tumor suppressor gene located on chromosome 17p, encoding the transcription factor p53, which is involved in apoptosis
    - *TP53* has frequently been found to be inactivated in diverse tumor types
    - Mutations in *TP53* are the most common and significant molecular abnormality found in type II ovarian carcinomas
    - Frequency of mutations
      - Mutations in *TP53* are found in 40–60% of all advanced ovarian cancer cases, and as many as 79% of all malignant ovarian or similar peritoneal epithelial tumors
      - Among pelvic (ovarian, tubal, and peritoneal) HGSC, more than 96% were found to have *TP53* mutations, including tumors of low stage
        - Most were missense mutations in exons 4–8 (Cancer Genome Atlas Research Network 2011)
      - MMMT has also been found to have *TP53* mutations, with identical mutations and LOH patterns in the carcinoma and sarcoma components
      - In two cases of MMMT arising in serous carcinoma, the MMMT was found to have the same *TP53* mutation as the serous carcinoma, supporting the idea of the sarcomatous component arising from the carcinoma (Gallardo et al. 2002)
    - Immunohistochemical findings
      - The majority of malignant ovarian cancers are p53-immunopositive, with a significant correlation between immunopositivity and *TP53* gene mutations
  - *CDKN2*/p16
    - The gene *CDKN2* (also known as *p16ink4* or *MTS1*), located on 9p21, encodes the cyclin-dependent kinase inhibitor p16
    - p16 is a tumor suppressor which binds cdk4 and cdk6, inhibiting the activity of the cdk4-6/cyclin D enzyme complex, which is required for the phosphoryla-

tion of Rb and resulting progression of the cell cycle
    - Immunohistochemical findings
        - Overexpression of p16 by immunohistochemical analysis has been found in the majority of HGSC, with 83.3% of these showing diffuse positivity, compared to less than 30% of LGSC (O'Neill et al. 2007)
        - Some groups have found differing results (see section on LGSC above); however, the diffuse expression of p16 by immunohistochemistry is still generally most consistent with HGSC
- Telomere length
    - Telomeres are the noncoding ends of eukaryotic chromosomes, consisting of guanine-rich simple tandem repeats
    - Telomeres function to protect the chromosome from end to end fusions, exonuclease activity, and other damage
    - In normal cells, telomeres shorten with each replication cycle, eventually contributing to the onset of replicative senescence
    - In immortal cell lines, such as tumor cells, the enzyme telomerase is activated, functioning to maintain telomere length and allowing the cell to continue dividing indefinitely
    - Frequency of abnormalities
        - STIC has been found to have telomeres shorter than those of normal tubal epithelium in 82% of cases
            - This shortening of telomeres may be due to ovulation-induced oxidative stress, resulting in chromosomal instability and contributing to the development of STIC
            - Those STICs which acquire the ability to maintain telomere length may then progress to HGSC (Kuhn et al. 2010)
        - Most HGSC demonstrate shorter telomeres than the associated normal tubal epithelium
            - This is also true of metastatic malignant cells in ascites specimens compared to the accompanying benign cells (Counter et al. 1994)
        - Telomerase activity is seen more frequently and to a greater degree in invasive carcinomas compared to normal ovaries and benign and borderline serous tumors
        - Telomerase activity also helps distinguish malignant from benign cells in ascites specimens
- Familial ovarian cancer: *BRCA1* and *BRCA2*
    - Both *BRCA1* and *BRCA2* are tumor suppressor genes involved in DNA double-strand break repair
    - *BRCA1*, located on 17q21, and *BRCA2*, located on 13q12, have both been linked to a hereditary predisposition for breast cancer
    - Mutations in *BRCA1* and *BRCA2* have also been linked to ovarian cancers
        - It has been found that the majority of breast-ovarian cancer families carry *BRCA1* mutations, and most of the remainder carry *BRCA2* mutations
    - Frequency of abnormalities
        - Approximately 10–17% of ovarian cancer occurs in patients with a known predisposing genetic mutation, primarily *BRCA1* and *BRCA2*, as well as Lynch syndrome (see above)
        - *BRCA1* mutations particularly predispose to serous carcinoma, which comprises 90% of ovarian cancers in *BRCA1* mutation carriers
        - These two genes and their related pathways may also play a role in sporadic cancers, which can be categorized into "*BRCA1*-like" and "*BRCA2*-like" based on gene expression profiles
            - Epigenetic effects or changes in downstream effectors of *BRCA1* and *BRCA2* may be responsible (Jazaeri et al. 2002)
        - Recent results from The Cancer Genome Atlas project have demonstrated somatic mutations in *BRCA1* or *BRCA2* in 3% of HGSC

- 11–31% of lost *BRCA1* expression has been found to be due to DNA hypermethylation rather than mutation (Wang et al. 2004)
- Alterations have also been found in other homologous recombination genes, with approximately half of all HGSC found to have homologous recombination defects

– *PI3K/Akt2/PTEN* pathway
- Abnormalities in this pathway are more commonly found in endometrioid and clear cell carcinomas (discussed previously)
- However, the oncogenes *Akt2* and *PIK3CA* are more frequently amplified in HGSC than in other tumor types
- Frequency of abnormalities
  - HGSC is *Akt2*-amplified in 18.2–29% of cases (Park et al. 2006)
    - Normal ovarian tissue, benign tumors, borderline tumors, and LGSC show no amplification of the gene
  - Although *PIK3CA* mutations are infrequently seen in HGSC, *PIK3CA* amplifications are seen in approximately 13% of these tumors (Nakayama et al. 2006b)

– *MAPK*
- *MAPK* (mitogen-activated protein kinase) is most frequently expressed in low-grade serous tumors (see above) but has also been found in HGSC
- Immunohistochemical findings
  - By immunohistochemical staining, activated (phosphorylated) MAPK was found to be expressed in 41% of HGSC (Hsu et al. 2004)
  - In contrast to LGSC, HGSC demonstrating *MAPK* expression all had wild-type *KRAS* and *BRAF*

– *Notch3*
- Notch receptors are membrane receptors which play a role in cell fate regulation, cell proliferation, and cell death during development
- The *Notch3* gene, located at 19p13.2, encodes one such Notch receptor
- Frequency of abnormalities
  - Overexpression of *Notch3* is more common in HGSC (amplification frequency of 19.5%, overexpression in 66%) vs. low-grade serous tumors and nonneoplastic epithelium (Park et al. 2006)
- Immunohistochemical findings
  - Immunohistochemical staining for Notch3 (both nuclear and cytoplasmic) has been found in 55% of ovarian carcinomas but not in normal ovarian surface epithelium
    - The intensity of staining is correlated with the DNA copy ratio

– *HBXAP (Rsf-1)*
- The gene *Rsf-1* (*HBXAP*, Hepatitis B virus x-associated protein), located at 11q13.5, encodes a protein which partners with hSNF2H to form the RSF complex; this complex is involved in chromatin remodeling
- Frequency of abnormalities
  - Amplification of the 11q13.5 locus has been found in 13.2–15.7% of HGSC, with *Rsf-1* found to have the most significantly amplified mRNA expression among genes at this locus
  - No amplification is seen in low-grade tumors and normal ovaries (Shih et al. 2005)
- Immunohistochemical findings
  - Immunohistochemical staining for Rsf-1 correlates with the presence of gene amplification
  - A correlation has also been found between the intensity of Rsf-1 nuclear immunostaining and that for hSNF2H, with evidence suggesting that Rsf-1 may stabilize the hSNF2H protein (Sheu et al. 2008)

– *NAC1*
- NAC1 (nucleus accumbens 1), encoded by the gene *NAC1* on 19p13, is a member of the BTB/POZ domain family, and contains a domain which may play a role in chromatin organization and transcription
- The role of NAC1 in ovarian cancer pathogenesis may also be partly mediated

by its negative regulation of the growth inhibitor Gadd45GIP1 (DNA-damage-inducible 45-gamma interacting protein)
    - Immunohistochemical findings
        - Immunopositivity for NAC1 is stronger in serous carcinomas than in benign tumors or normal ovaries, with high immunointensity seen more frequently in HGSC compared to LGSC
        - Higher staining intensity and mRNA levels were also found in recurrent tumors compared to primary tumors (Nakayama et al. 2006a)
- *HLA-G*
    - HLA-G (human leukocyte antigen G) is a major histocompatibility (MHC) protein, the expression of which has been shown to facilitate evasion of immunosurveillance by tumor cells and has been linked to multiple nonovarian cancers
    - Immunohistochemical findings
        - By immunohistochemical analysis, 61% of HGSC have been found to express HLA-G, with a discrete membranous staining pattern
        - Expression has not been found in low-grade serous tumors or normal ovarian surface epithelium
    - By PCR analysis, the HLA-G isoforms 1 and 5 were found to predominate in HGSC (Singer et al. 2003a, 2003b)
- Cyclin E1 (*CCNE1*)
    - Cyclin E, encoded by the gene *CCNE1* at 19q13, is involved in promoting the progression of the cell cycle from S1 to G phase
    - Frequency of abnormalities
        - Amplification of the *CCNE1* locus has been found to be specific for HGSC vs. LGSC or normal ovarian tissue, with a frequency of 32.2–36.1% (Nakayama et al. 2007a)
    - Immunohistochemical findings
        - High cyclin E1 expression by immunohistochemical analysis has been correlated with amplification of the *CCNE1* gene (Farley et al. 2003)

- Clinical implications
    - *TP53*
        - *TP53* gene mutations and overexpression have been linked to cisplatin resistance, resulting from the inability of the mutated protein to activate apoptosis (Perego et al. 1996)
        - The evidence for the prognostic significance of *TP53* mutations is still somewhat contradictory, with some evidence pointing to these mutations as a negative prognostic factor, others finding no correlation, and one study even finding a short-term survival benefit
            - Additional data is needed to more definitively define the role of p53 in prognosis
        - Immunohistochemical staining for p53 is useful in differentiating type I and type II tumors
    - Telomere length and telomerase activity
        - The increased telomerase activity seen in HGSC suggests a potential utility for telomerase inhibitors in treatment; several studies have explored this potential
            - The cytokine interferon-β(beta) (IFN-β(beta)), which inhibits tumor cell growth, was found to suppress telomerase activity in ovarian cancer cells (Lee et al. 2010)
            - Inhibition of hTERT (human telomerase reverse transcriptase), the major site of transcriptional regulation of the enzyme, has demonstrated rapid inhibition of growth in ovarian cancer cell lines (Luo et al. 2009)
    - *BRCA1* and *BRCA2*
        - In comparison to women with sporadic ovarian cancer, those with *BRCA1*- and *BRCA2*-mutated cancers have better outcomes.
            - Patients with epigenetically silenced *BRCA1* have survival similar to those with wild-type *BRCA1*
        - *PARP1* (poly-ADP-ribose-polymerase) is a nuclear enzyme required for base excision repair of single-strand breaks

        - ♦ *PARP* inhibitors may have potential in treating patients with defects in DNA repair, including those with *BRCA1* and *BRCA2* mutations
    - *PI3K/Akt2/PTEN* pathway
        - ◦ Amplification of *Akt2* has been associated with undifferentiated histology and with age over 50 years; a trend towards higher mortality is also observed
    - *MAPK*
        - ◦ Greater expression of *MAPK* is seen in high-grade tumors from younger patients (Hsu et al. 2004)
    - *Notch3*
        - ◦ Tumors with *Notch3* overexpression may be amenable to targeted therapy, either by γ(gamma)-secretase inhibitors or by disruption of Notch3 and ligand binding
        - ◦ γ(gamma)-secretase inhibitors prevent activation of Notch3, and are found to inhibit proliferation and promote apoptosis in *Notch3*-expressing cancer cell lines
    - *HBXAP* (*Rsf-1*)
        - ◦ *Rsf-1* may have prognostic significance, as patients with HGSC and *Rsf-1* amplification demonstrate shorter overall survival compared to those with nonamplified tumors (Shih et al. 2005)
        - ◦ Implications for treatment also exist, with silencing of Rsf-1 in overexpressing cell lines resulting in a significant inhibition of growth, and expression of *Rsf-1* in cell lines being associated with paclitaxel resistance (Choi et al. 2009)
    - NAC1
        - ◦ The intensity of NAC1 immunostaining was found to be predictive of recurrence within 1 year in patients with advanced stage HGSC status post optimal debulking and standard chemotherapy (Nakayama et al. 2006a)
        - ◦ *NAC1* expression may also be associated with resistance to paclitaxel and resulting shorter survival in paclitaxel-treated patients
            - ♦ This resistance may be mediated by Gadd45GIP1, which is also a potential target for treatment (Jinawath et al. 2009)
    - HLA-G
        - ◦ An HLA-G-specific ELISA test has been developed to measure sHLA-G, a product of the HLA-G5 isoform
            - ♦ Using this test, sHLA-G was found in almost all malignant ascites samples and at significantly higher levels than in benign samples (Singer et al. 2003b), indicating potential use as a diagnostic tumor marker
        - ◦ HLA-G may also have prognostic implications, with an association between the presence of HLA-G-expressing tumor cells in effusions and better survival (Davidson et al. 2005)
    - Cyclin E1
        - ◦ High cyclin E1 expression has been associated with shorter median survival and an increased relative risk of death in women with advanced stage ovarian cancer status post suboptimal debulking (Farley et al. 2003)
- Summary
    - Mutations in *TP53* are the most common genetic abnormality in HGSC and in ovarian cancer overall
        - ◦ The presence of these mutations is helpful for diagnosis, and is thought to confer a worse prognosis, although data on the latter are somewhat contradictory
    - In MMMT, identical *TP53* mutations have been found in both the epithelial and stromal components
    - p16 is commonly overexpressed in HGSC
    - The enzyme telomerase, expressed in a wide variety of tumors, is also found in ovarian carcinomas, and has demonstrated potential as a target for therapy
    - Expression of *MAPK*, although more common in type I tumors, may also be seen in HGSC
        - ◦ *MAPK*-expressing HGSC are wild type for *KRAS* and *BRAF*

- Within the *PI3K/Akt2/PTEN* pathway, amplification of *Akt2* is most associated with HGSC, and has been associated with undifferentiated histology and a worse prognosis.
  - *PIK3CA* amplification is also seen in HGSC
- *BRCA1* and *BRCA2* are the genes responsible for most familial cases of breast and ovarian cancer
  - Patients with ovarian carcinomas in this setting have better outcomes than those with sporadic cancers
  - These genes and their related pathways may also be involved in sporadic cancers
- Several other genetic abnormalities have also been described in HGSC, including overexpression of *Notch3, HBXAP, NAC1, HLA-G,* and *Cyclin E*
  - Many of these abnormalities have diagnostic and therapeutic relevance, as discussed above

Molecular Abnormalities not Associated with Specific Histology

- EGFR family
  - The EGFR (epidermal growth factor receptor) family, also known as the ERBB or HER family, is a group of transmembrane receptors which include both EGFR and HER2 (ERBB2)
  - Activation of EGFR family receptors by ligand binding leads to the activation of multiple different signaling pathways, including *MAPK* and *PI3K/Akt2/PTEN*, with resulting effects on cell survival, proliferation, and differentiation (Fig. 7.2)
  - Frequency of abnormalities
    - Amplification of *EGFR* has been found in up to 22% of ovarian cancers overall (Stadlmann et al. 2006)
      - Activating mutations may also be seen
    - Amplifications of *HER2* have been found in 23% of borderline tumors and 8–66% of ovarian carcinomas, both of various subtypes (Ross et al. 1999)
      - No correlation has generally been found between gene amplification and tumor type, stage, or grade
  - Immunohistochemical findings
    - EGFR expression by immunohistochemistry has been found in as many as 64.5% of invasive ovarian carcinomas overall, including mucinous, serous, and endometrioid carcinomas (Vermeij et al. 2008)
  - Clinical implications
    - There may be a role for EGFR inhibitors in patients with *EGFR* mutations, although the evidence remains unclear
      - The response rate has been relatively low (Gordon et al. 2005), possibly due to the fact that the mutations most often found in ovarian cancers are not the same as those found in non-small cell lung cancers
    - EGFR expression has been associated with poor outcome in multiple studies, as well as with higher tumor grade, abnormal *TP53* expression, larger residual tumor size, and a higher proliferation index
    - The finding of a greater frequency of *HER2* amplification in higher stage tumors in one study suggests that this may be a marker of poor prognosis (Afify et al. 1999)
    - Trastuzumab and pertuzumab are monoclonal anti-HER2 antibodies commonly used in the treatment of *HER2*-amplified breast cancer
      - In ovarian cancer, overall response rates for these two drugs individually have been relatively low, but recent evidence suggests a potentially greater response rate using both in combination (Faratian et al. 2011)
- DNA methylation
  - DNA methylation is an epigenetic alteration which has been shown to occur aberrantly in a wide variety of human neoplasms
  - Global hypomethylation results in the activation of oncogenes, while tumor suppressor genes can be silenced via

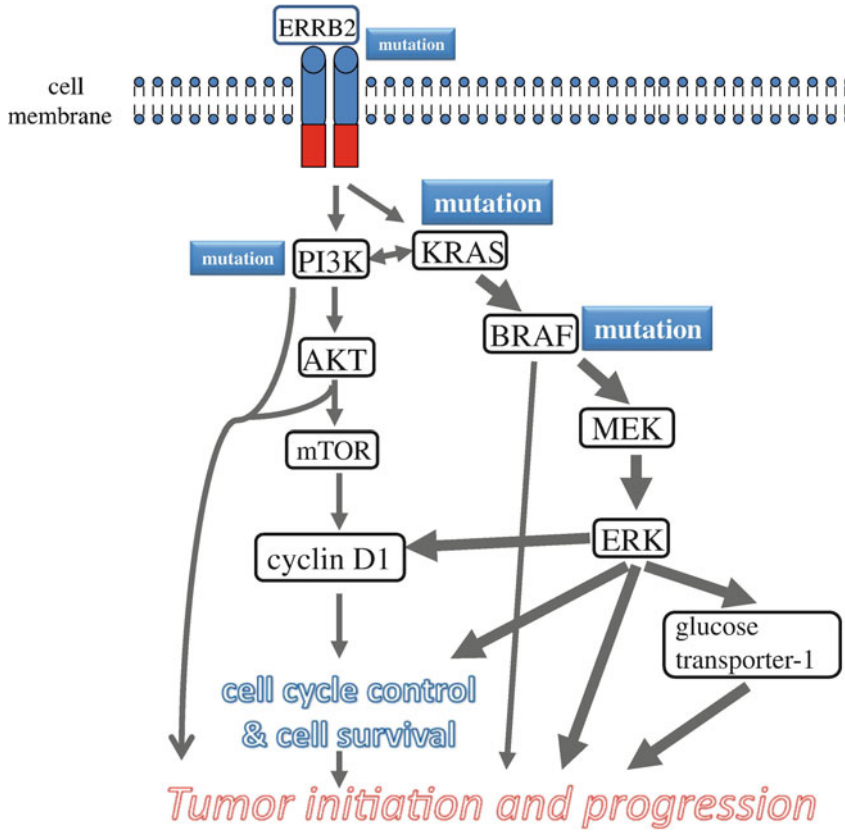

Fig. 7.2 Interaction of pathways involved in the pathogenesis of LGSC and other type I tumors. (Reprinted from Human Pathology, Kurman and Shih (2011), with permission from Elsevier)

hypermethylation of CpG islands within their promoter regions
- Hypermethylation of various genes has been described in ovarian cancers
  - For example, promoter hypermethylation of specific genes (*CDKN2, E-cadherin, RAR-β(beta), H-cadherin, APC, GSTP1, MGMT,* and *RASSF1A*) increases in frequency from benign cystadenomas to invasive carcinomas
- Hypomethylation has also been shown to progressively increase from nonneoplastic ovarian tissue to carcinoma
- Other examples of aberrant methylation status have been discussed earlier in relation to specific genetic loci
- Clinical implications
  - The detection of DNA methylation status has promising potential as a screening tool, particularly with the development of sensitive assays to detect the methylation status of multiple genes, and the potential to detect biomarkers in fluids draining the tumor site
  - Strong hypomethylation in ovarian cancer tissue has been associated with advanced stage and high grade

Conclusions

- Studies of the molecular characteristics of ovarian cancer have led to numerous new insights into the pathophysiology of this disease
- Differences in the involved molecular pathways have supported the division of epithelial ovarian cancers into type I tumors, which are slow-growing tumors thought to develop

in a stepwise manner from benign and borderline precursors, and type II tumors, which are more aggressive and arise in a de novo fashion
- Although there is much overlap in the molecular pathways involved in the various ovarian tumor types, each histologic type is associated with certain characteristic abnormalities
- These insights into the specific aberrations present in each ovarian cancer histotype have translated into insights on new diagnostic, therapeutic, and prognostic modalities
- Continued efforts to better understand the molecular characteristics of ovarian cancer promise to offer further insights into its pathophysiology and best clinical management, with the hopes of ultimately reducing the burden of this high-mortality disease

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Molecular Pathology of Endometrial Cancer

8

Xavier Matias-Guiu and Jaime Prat

Contents

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Introduction

Overview of Endometrial Carcinoma

- In Western countries, endometrial carcinoma (EC) is the most common cancer of the female genital tract accounting for 10–20 per 100,000 person-years
- EC occurs in peri- and postmenopausal women, although it may also develop in premenopausal women, particularly in the setting of hyperestrogenism and hereditary nonpolyposis colon cancer (HNPCC) syndrome
- Etiological factors include unopposed estrogenic stimulation (anovulatory cycles, estrogen administration), obesity, tamoxifen treatment, or insulin resistance
- From a clinical viewpoint, EC falls into two different types (types I and II) (Tables 8.1 and 8.2)

L. Cheng and J.N. Eble (eds.), *Molecular Surgical Pathology*,
DOI 10.1007/978-1-4614-4900-3_8, © Springer Science+Business Media New York 2013

Table 8.1 Clinicopathological features of types I and II endometrial carcinomas

	Type I	Type II
Age	Pre- and perimenopausal	Postmenopausal
Unopposed estrogen	Present	Absent
Hyperplasia precursor	Present	Absent
Grade	Low	High
Myometrial invasion	Minimal	Deep
Histologic type	Endometrioid	Nonendometrioid
Behavior	Stable	Progressive

Table 8.2 Genetic alterations of endometrial carcinomas

Type I	Type II
Microsatellite instability	*TP53*
PTEN	LOH
KRAS	*p16*
Beta-catenin (*CTNNB1*)	E-cadherin
PIK3CA	*c-erb B2*
ARID1A	*STK15*

  - Type I tumors are low-grade and estrogen-related endometrioid endometrial carcinomas (EECs) that usually develop in perimenopausal women and coexist with or are preceded by endometrial hyperplasia
  - Type II tumors are high-grade nonendometrioid endometrial carcinomas (NEECs) (mainly serous and clear cell carcinomas), unrelated to estrogen stimulation, which may arise in endometrial polyps or from precancerous lesions that develop atrophic endometrium, and tend to occur in older women (Fig. 8.1)

- Whereas the vast majority of type I carcinomas are cured by hysterectomy, type II carcinomas are very aggressive tumors that require adjuvant therapy

Type I Pathology

- Type I tumors usually develop in perimenopausal women in the setting of hyperestrogenism, obesity, and diabetes. Pathologically, these tumors are EECs, variants of EECs, and mucinous carcinomas
- Although a proportion of EECs arise from endometrial hyperplasia, in many cases the nonneoplastic endometrium appears atrophic or weakly proliferative
- EECs are the most common histological type of EC (80%) (Fig. 8.1a)
  - EECs show a wide spectrum of morphological features including villoglandular pattern, squamous differentiation, secretory change, ciliated cells, or other appearances (sertoliform, microglandular, with small nonvillous papillae, mucin-rich, and oxyphilic type)
- Well-differentiated EEC contains complex glandular structures that resemble to those of the normal proliferative endometrium but are closely packed (back to back) (Fig. 8.1a)
  - Complexity of the glandular elements increases in high-grade tumors which may show gland fusion and cribriforming or may grow in sheets
- EECs with squamous differentiation account for 25–50% of EECs
  - The presence of squamous differentiation does not affect prognosis
- The villoglandular variant accounts for 15–30% of all EEC
  - These tumors are low-grade neoplasms composed of long, slender, delicate papillae with thin fibrovascular cores
  - Although tumors can be purely villoglandular, they often contain areas of typical EEC in the myoinvasive front
- The secretory variant of EEC, also called, secretory adenocarcinoma, is very uncommon. The tumor glands are composed of cells with large subnuclear vacuoles, similar to those of early secretory phase endometrium
  - Prognosis is similar to that of well-differentiated EEC
- Mucinous carcinoma of the endometrium accounts for less than 10% of all EC

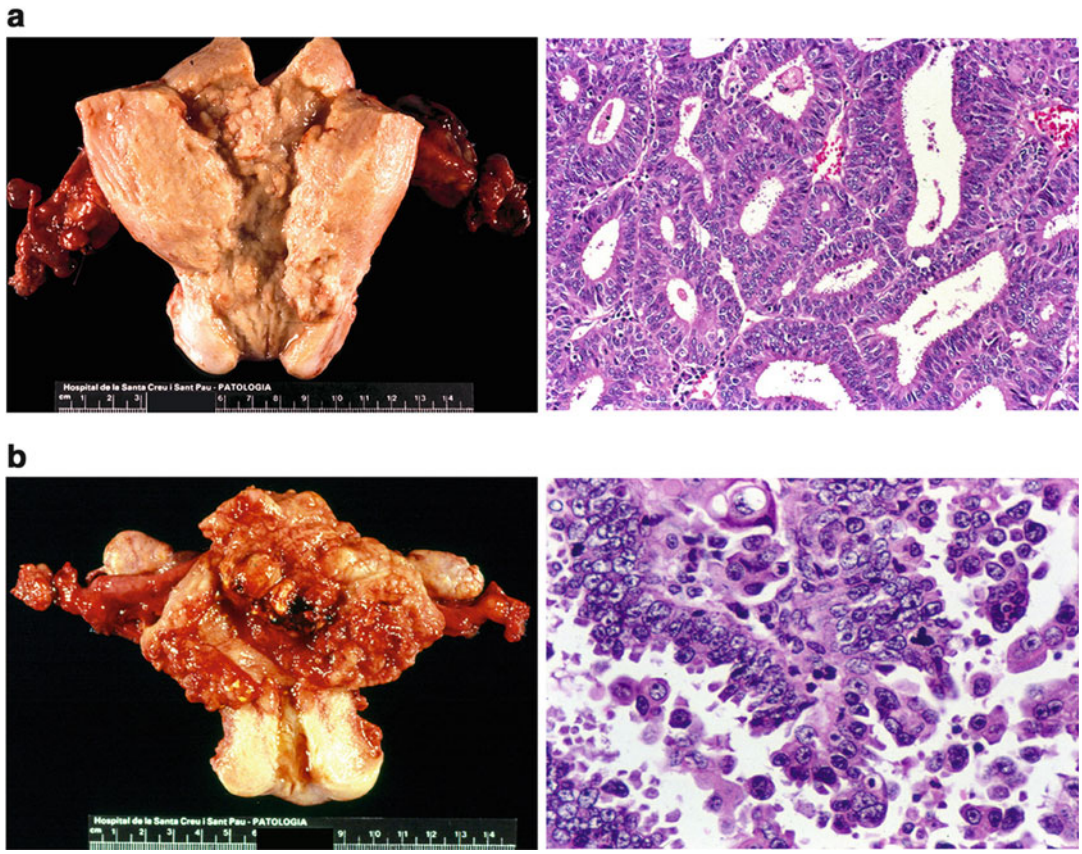

Fig. 8.1 (**a**) Endometrioid carcinoma. (*Upper left*) Polypoid tumor with only superficial myometrial invasion. (*Upper right*) Well-differentiated (grade 1) adenocarcinoma. (**b**) Nonendometrioid carcinoma. (*Lower left*) Large hemorrhagic and necrotic tumor with deep myometrial invasion. (*Lower right*) Serous carcinoma (grade 3) exhibiting stratification of anaplastic tumor cells and abnormal mitoses. (Reprinted from Diagnostic Histopathology, Catasus et al. (2009b), pp. 556–563, with permission from Elsevier)

  - These tumors share clinical features with EEC, which almost always contain mucin-producing cells
  - Mucinous carcinoma shows intracytoplasmatic mucin in at least 50% of the tumor cells

Type II Pathology

- Type II tumors (NEEC) are high-grade invasive serous and clear cell carcinomas
- Serous carcinoma, which is the prototype of NEEC, accounts for 5–10% of ECs
  - Histologically, it shows thick, fibrotic, or edematous papillae with prominent stratification of tumor cells and cellular budding
  - There are often anaplastic cells with large, eosinophilic cytoplasm (Fig. 8.1b)
  - The tumor usually invades the myometrium deeply and there is extensive lymphovascular space invasion
  - Multicentric involvement of other parts of the female genital tract and extrauterine spread may be found at the time of diagnosis
- NEECs are not preceded by endometrial hyperplasia
  - The "precursor" of serous carcinoma is thought to be the so-called *endometrial intraepithelial carcinoma* (SEIC). However, SEIC has metastatic potential.

- Clear cell adenocarcinoma is also considered a type of NEEC
  - The endometrial tumor is similar to clear cell carcinomas of the ovary or cervix and comprises about 5% of all EC
  - Microscopically, clear cell adenocarcinomas are characterized by a variety of patterns such as solid, papillary, glandular, and tubulocystic
  - Tumor cells may exhibit a prominent clear appearance, with abundant glycogen, and a hobnail configuration

Distinction Between Type I and Type II

- Distinction between EEC and NEEC is usually done by microscopic examination
  - Differential diagnosis may be difficult and subjected to interobserver variation in some cases
  - Immunohistochemistry can be of help
- The typical immunohistochemical profile of EEC includes positive immunoreaction for cytokeratins, vimentin, and estrogen and progesterone receptors
- The typical immunohistochemical profile for serous carcinoma includes strong immunoreaction for p53 and p16
  - Whereas p53 is expressed in only 10–35% of EEC—usually in high-grade tumors—70–90% of serous carcinomas show p53 immunoreaction
  - Similarly, p16 shows patchy, weak to moderate staining in EEC but diffuse and strong immunoreaction in 95% of serous carcinomas
- Other potentially useful markers include beta-catenin, PTEN, and E-cadherin
  - Nuclear immunoreaction for beta-catenin and inactivation (lack of immunoreaction) of the PTEN tumor suppressor gene are seen in EEC
  - Lack of membranous immunoreaction for E-cadherin is seen in serous carcinomas
  - The squamous morules of complex atypical hyperplasia and well-differentiated adenocarcinoma typically show nuclear immunoreaction for beta-catenin

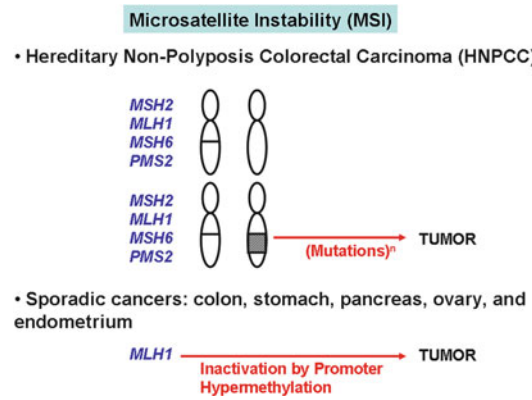

Fig. 8.2 Microsatellite instability in hereditary and sporadic endometrial carcinoma

  - Negative beta-catenin immunoreaction in curettings is frequently followed by the finding of invasive carcinoma (with *PTEN*, *RAS*, and *PIK3CA* mutations/alterations) in the corresponding hysterectomy specimen

- Recently, IMP2 has been proposed as a marker of serous carcinomas of the endometrium

Type I Molecular Features

- The molecular alterations involved in the development of EEC differ from those of NEEC. cDNA analysis has clearly shown that EEC and NEEC exhibit different gene expression profiles (Fig. 8.2)
- EEC show microsatellite instability (MI), and mutations in the *PTEN, KRAS, PIK3CA*, and beta-catenin genes
- NEECs exhibit alterations of *TP53*, loss of heterozygosity (LOH) on several chromosomes (chromosomal instability), as well as other molecular alterations (*STK15, p16*, E-cadherin, and *C-erbB2*)

Microsatellite Instability

- MI has been demonstrated in 75% of EC associated with hereditary nonpolyposis colon cancer (HNPCC) as well as in 25–30% of sporadic EC

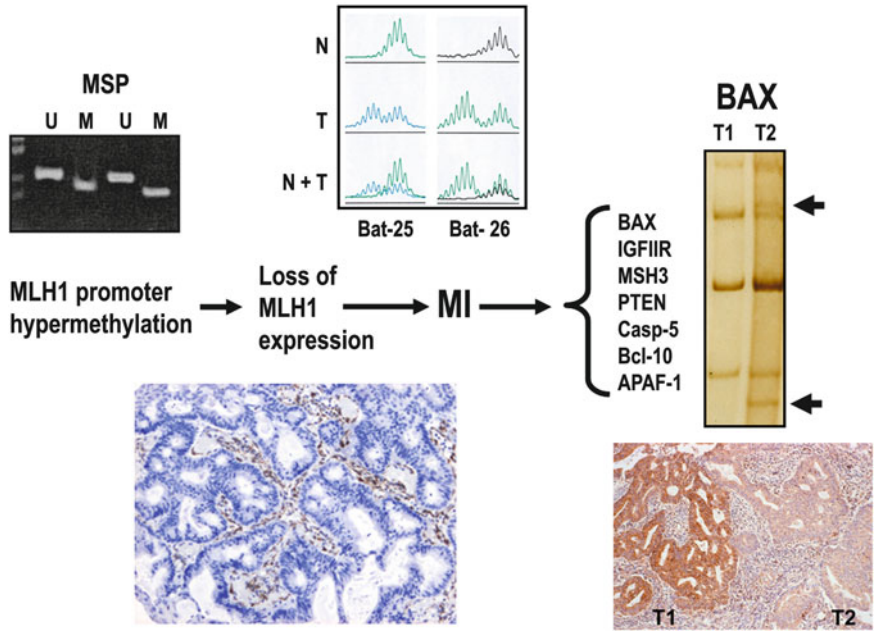

Fig. 8.3 *MLH1* inactivation by promoter hypermethylation is the most common cause of the MI phenotype in endometrial carcinoma. Progressive accumulation of alterations secondary to MI affects important regulatory genes, and promotes carcinogenesis. *BAX* somatic frameshift mutations are heterogeneously distributed throughout the tumor and provide selective growth advantage. (Reprinted from Diagnostic Histopathology, Catasus et al. (2009b), pp. 556–563, with permission from Elsevier)

- The MI-associated mismatch repair deficiency leads to accumulation of mutations in repetitive DNA sequences; i.e., microsatellites
- EC patients from HNPCC kindreds have an inherited germline mutation in *MLH-1, MSH-2, MSH-6*, or *PMS-2*; nevertheless, EC only develops after instauration of a deletion or mutation in the contralateral *MLH-1, MSH-2, MSH-6,* or *PMS-2* allele in endometrial cells (Fig. 8.2)
- In sporadic EC, MI occurs more frequently in EEC (30%) than in NEEC
  - In sporadic tumors, the main cause of mismatch repair deficiency is *MLH-1* inactivation by promoter hypermethylation
  - Abnormal methylation of *MLH-1* may also be detected in atypical hyperplasia, suggesting that it may be an early event in the pathogenesis of EEC that precedes the development of MI (Fig. 8.2)
- The prognostic significance of MI is controversial
  - There is convincing evidence for its association with adverse prognostic factors such as high histological grade
- Mutations in some repetitive mononucleotide tracts located within the coding sequence of some genes involved in cell proliferation, cell differentiation, DNA-repair, and apoptosis, such as *BAX, IGFIIR, hMSH3, hMSH6, MBD4, CHK-1, CASP-5, ATR, ATM, BML, RAD-50, BCL-10*, and *APAF-1*, are secondary events in EC with MI (Fig. 8.3)
- EEC is frequent in patients with HNPCC
  - Characteristic microscopical features of EEC arising in this setting are
    - Poor differentiation
    - Crohn-like lymphoid reaction
    - Lymphangioinvasive growth
    - Tumor infiltrating lymphocytes

- Immunoreaction for *MLH-1, MSH-2, PMS-2,* or *MSH-6* may be helpful in the evaluation of cases
- Occasionally, NEEC has been described in HNPCC patients, but these tumors usually show mixed areas, combining NEEC with EC

Phosphatase and Tensin Homolog *(PTEN)*

- *PTEN* is frequently abnormal in EC
  - LOH at chromosome 10q23 occurs in 40% of EC
  - Somatic *PTEN* mutations are common in EC
  - They are almost exclusively restricted to EEC and occur in 37–61% of cases (Fig. 8.4)
- Concordance between MI status and *PTEN* mutations suggests that *PTEN* could be a likely candidate to be targeted for mutations in the MI-positive EC
- *PTEN* mutations have been detected in endometrial hyperplasias with and without atypia (19% and 21%, respectively), both regarded as precursors of EEC
- Although the prognostic significance of *PTEN* mutations in EC is controversial, their association with favorable prognostic factors has been reported
  - It has been suggested that EECs with *PTEN* mutations have genomic instability, which is the rational for administrating PARP inhibitors
- In agreement with Knudson's two-hit proposal, LOH at 10q23 frequently coexists with somatic *PTEN* mutations
  - The coexistence of both alterations leads to activation of the PI3K/AKT pathway, which plays a key role in the regulation of cellular homeostasis (Fig. 8.5)

Phosphatidylinositol-4,5-Bisphosphate 3-Kinase, Catalytic Subunit Alpha *(PIK3CA)*

- Mutations in *PIK3CA* (p110α) (alfa) contribute to the alteration of the *PI3K/AKT* signaling pathway in EC (Fig. 8.5)
  - They are predominantly located in the helical (exon 9) and kinase (exon 20) domains, but can also occur in exons 1–7
  - *PIK3CA* mutations are infrequent in endometrial hyperplasia

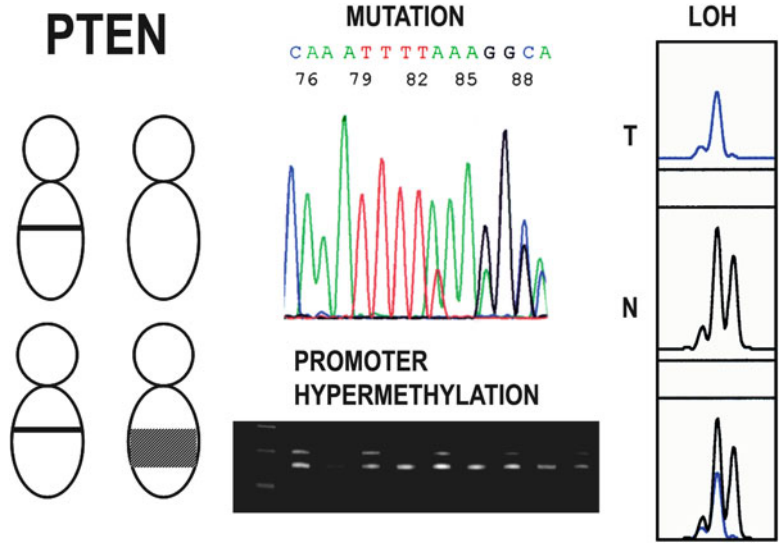

Fig. 8.4 *PTEN* inactivation may occur by several mechanisms such as mutation, LOH at 10q23, and promoter hypermethylation. (Reprinted from Diagnostic Histopathology, Catasus et al. (2009b), pp. 556–563, with permission from Elsevier)

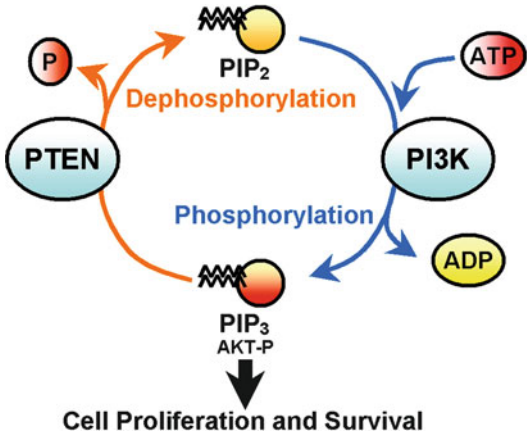

Fig. 8.5 PI3K/PTEN function. Phosphorylation of PI3K converts phosphatidylinositol biphosphate (PIP2) into phosphatidylinositol triphosphate (PIP3) promoting cell proliferation and survival. PTEN negatively regulates PI3K signaling by dephosphorylation of PIP3. (Reprinted from Pathology, Prat et al. (2007), with permission from Wolters Kluwer Health)

- *PIK3CA* mutations occur in 24–39% of the cases, and frequently coexist with *PTEN* mutations
  - *PIK3CA* mutations, particularly in exon 20, have been associated with adverse prognostic factors such as high histological grade and myometrial invasion Catasus et al. (2008)
  - Simultaneous alterations in both *PI3K/AKT* and the *TP53* pathways have a negative effect on prognosis and are associated with lower survival
- Although initially described in EEC, *PI3KCA* mutations also occur in NEEC, and also in mixed EEC–NEEC
- Gene expression profile differences in the *PI3K/AKT* signaling pathway identify two subgroups of high-grade endometrial carcinomas with different molecular alterations (PI3K/AKT pathway vs. p53 alterations) that may have distinct roles in endometrial carcinogenesis (Fig. 8.6) Catasus et al. (2010)
- Mutations in *PIK3RI* (p85α) (alfa), the inhibitory subunit of *PI3K*, have been detected in 43% of EEC, and 12% of NEEC
  - Distribution of *PIK3R1* mutations is nonrandom; most mutations are localized to the p85α-nSH2 (alfa) and -iSH2 domains that mediate binding to p110α (alfa)
- In EEC, *PIK3R1* mutations frequently coexist with *PTEN* and *KRAS* mutations but tend to be mutually exclusive with PIK3CA mutations

RAS–MAPK Pathway

- The RAS–RAF–MEK–ERK signaling pathway plays an important role in endometrial tumorigenesis (Fig. 8.7)
- The frequency of *KRAS* mutations in EC ranges between 10 and 30%
  - In some series, *KRAS* mutations are more frequent in EEC with microsatellite instability
- *RASSF1A* inactivation by promoter hypermethylation may contribute significantly to increase the activity of the RAS–RAF–MEK–ERK signaling pathway
- EC frequently shows inactivation of *SPRY-2* by promoter methylation
  - *SPRY2* is involved in the negative regulation of the FGFR pathway
  - Reduced SPRY2 immunoexpression is seen in almost 20% of EC, and is strongly associated with increased cell proliferation
- Somatic mutations in the receptor tyrosine kinase *FGFR2* have been recently found in 10–12% of EC, particularly in EEC (16%)
  - *FGFR2* mutations and *KRAS* mutations are mutually exclusive events

Beta-Catenin

- The beta-catenin gene (*CTNNB1*) maps to 3p21
  - Appears to be important in the function of both APC and E-cadherin
  - A component of the E-cadherin–catenin unit, important for cell differentiation and maintenance of the normal tissue architecture
  - Important in signal transduction
  - Increased cytoplasmic and nuclear levels result in transcriptional activation through the *LEF/Tcf* pathway

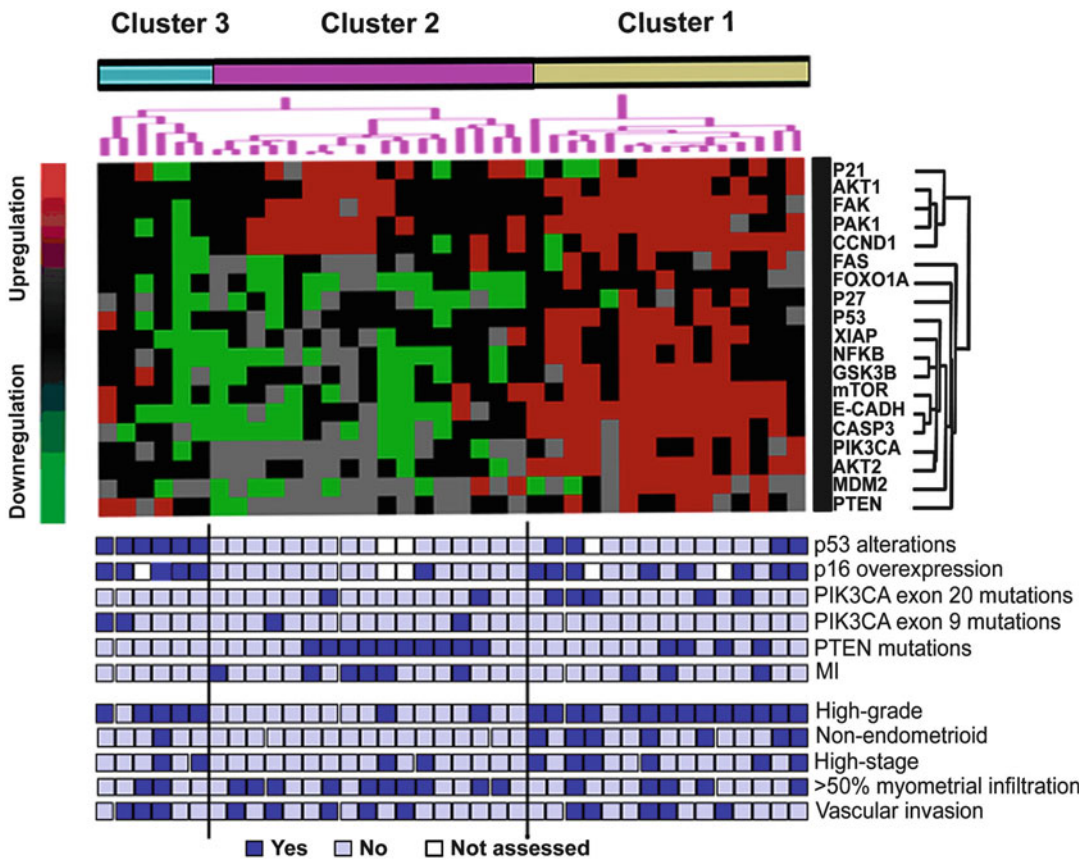

Fig. 8.6 Hierarchical clustering analysis of mRNA expression of 19 genes in 38 endometrial carcinomas. Upregulated and downregulated expression is indicated as *red* and *green cubes*, respectively. Genes that did not vary in their expression level are shown in black and genes with unsatisfactory results are labeled in gray. Enclosed in the clustering image, results of *TP53*, p16, PIK3CA, PTEN, and MI analysis as well as clinicopathological parameters, such as high-grade (grade 3), nonendometrioid, high-stage (stage 2 or higher), myometrial invasion (450%), and vascular invasion, are graphically represented for each case

- Mutations in exon 3 of *CTNNB1* occur in 14–44% of EC and result in stabilization of the protein, cytoplasmic, and nuclear accumulation (Fig. 8.8), and participation in signal transduction and transcriptional activation through the formation of complexes with DNA-binding proteins. *CTNNB1* mutations appear to be independent of MI and mutational status of *PTEN* and *KRAS*
- Although, there is a good correlation between *CTNNB1* mutations and beta-catenin nuclear immunoreaction, other genes of the *Wnt/beta-catenin/LEF-1* pathway may be responsible for the stabilization and putative transcription activator role of beta-catenin in EEC
- Beta-catenin alterations have been described in endometrial hyperplasias and grade 1 EECs that contain squamous metaplasia (morules)
  - They are typically absent in metastatic EC and help in the identification of synchronous independent primary EECs of uterus and ovary
- Prognostic significance of beta-catenin mutations in EC is controversial; however, tend to occur in tumors with favorable prognosis

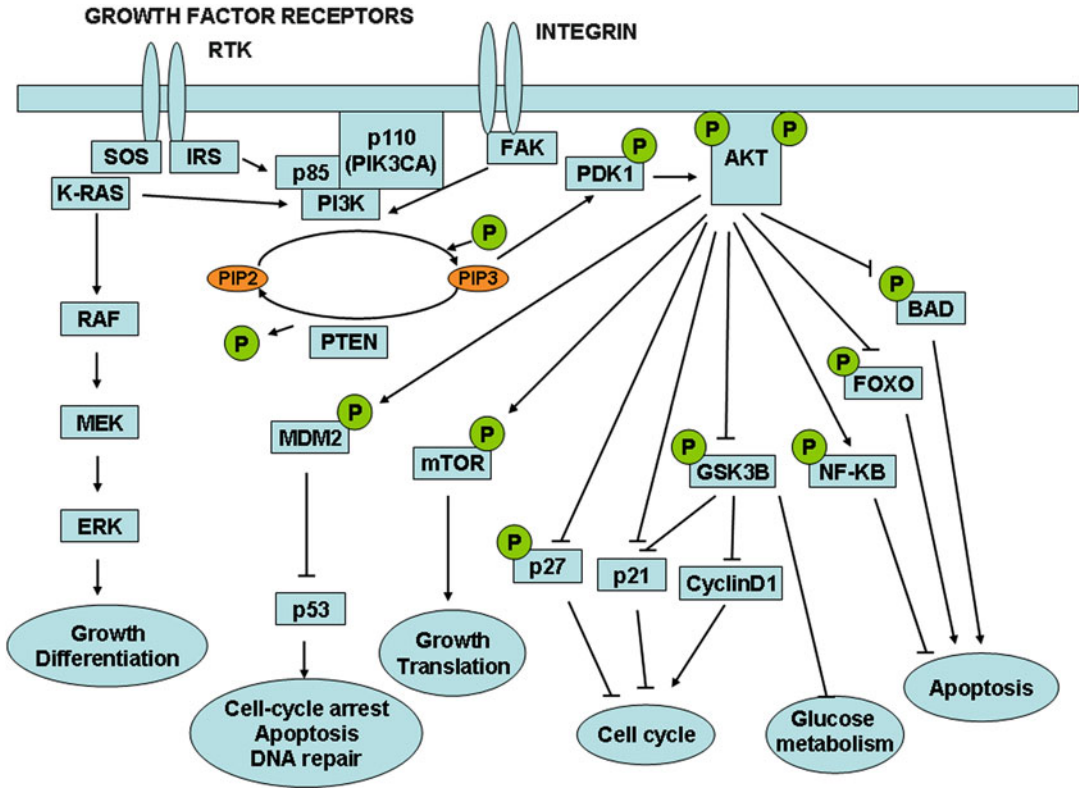

Fig. 8.7 PI3K-AKT, and RAS-MAPK signaling pathways (Reprinted from Diagnostic Histopathology, Catasus et al. (2009b), pp. 556–563, with permission from Elsevier)

Type II Molecular Features

TP53

- Whereas *TP53* mutations occur in over 90% of serous carcinomas, they are present in only 10–20% of EEC, mostly grade 3 tumors (Fig. 8.9)
  - *TP53* mutations are infrequent (<5%) in clear cell carcinomas
- P53 protein can induce apoptosis or prevent a cell from dividing if there is DNA damage
  - Mutation of the *TP53* gene diminishes the cell's ability to repair DNA damage before entry to S-phase, leading to a greater chance that mutations will be fixed in the genome and passed to successive generations of cells

Other Alterations

- Inactivation of the cell cycle regulator *p16* is also more frequent in NEEC (40%) than in EEC (10%)
  - Although the underlying mechanism is unclear, it probably involves deletion and promoter hypermethylation
- Reduced expression of E-cadherin is frequent in EC, and may be caused by LOH or promoter hypermethylation
  - LOH at 16q22.1 is seen in almost 60% of NEEC but only in 22% of EEC
- *C-erbB2* overexpression and amplification are seen more frequently in NEEC (43%) than in EEC (29%)
- NEEC shows chromosomal instability, widespread chromosomal gains and losses, and aneuploidy

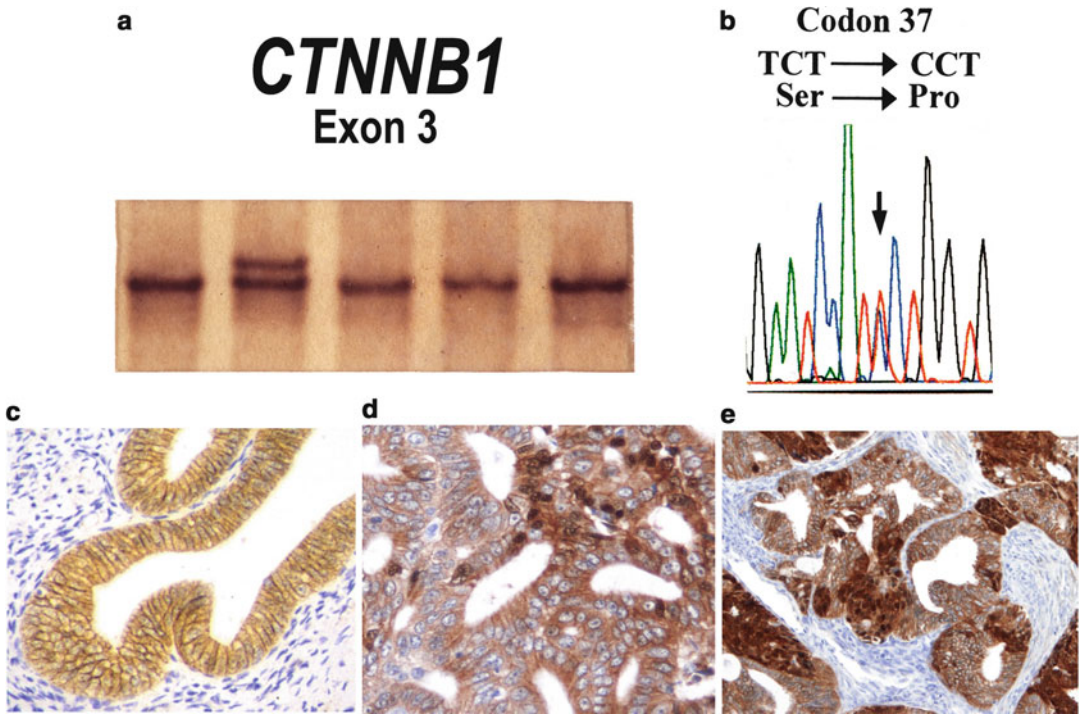

Fig. 8.8 (**a**) CTNNB1 (b-catenin gene) mutations shown by SSCP with abnormal extra band and (**b**) corresponding partial representative nucleotide sequence demonstrating a missense mutation in exon 3. Different patterns of b-catenin immunostaining in endometrioid carcinoma; (**c**) membranous immunoreaction, (**d**) membranous immunostaining with occasional positive nuclei, and (**e**) membranous and nuclear immunostaining in squamous morules. (Reprinted from Pathology, Prat et al. (2007), with permission from Wolters Kluwer Health)

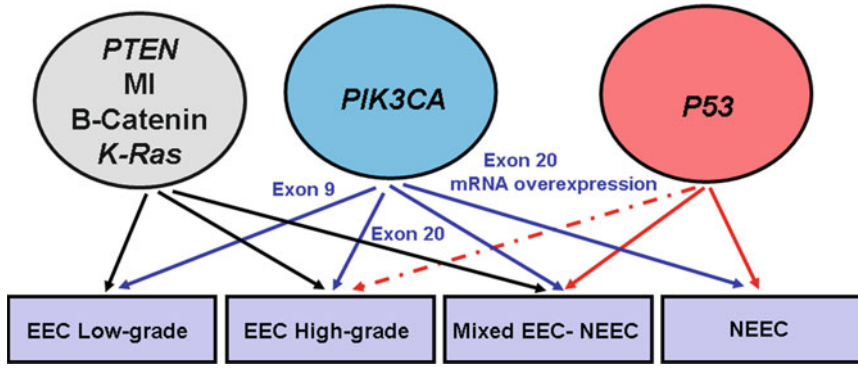

Fig. 8.9 MI, and *PTEN*, *PIK3CA*, *KRAS*, *CTNNB1* (beta-catenin), and *TP53* mutations are the most common molecular genetic alterations in endometrial carcinomas; *EEC* endometrioid endometrial carcinoma; *NEEC* nonendometrioid endometrial carcinoma. (Reprinted from Diagnostic Histopathology, Catasus et al. (2009b), pp. 556–563, with permission from Elsevier)

- cDNA arrays have demonstrated that NEECs usually show overexpression of genes (*STK-15, BUB1, CCNB2*) involved in the regulation of the mitotic spindle checkpoint
  - One of these genes, *STK-15*, essential for chromosome segregation and centrosome functions, is frequently amplified in NEEC (60%)
- New biomarkers of serous carcinoma are EpCAM, claudin-3, and claudin-4 receptors, serum amyloid A, folate-binding protein, mesothelin, LRP-1, and IMP2
- Clear cell carcinomas (NEECs) show specific features including lack of *TP53* alterations and, possibly, mutations in *PIK3CA* and *PTEN*, as well as immunoreactivity for hepatocyte nuclear factor (HNF1β) (beta)
- Mutation of the AT-rich interactive domain-containing protein 1A (*ARID1A*) gene and loss of the corresponding protein BAF250a have recently been described as a frequent event (almost 50% of cases) in clear cell and endometrioid carcinomas of the ovary
  - This mutation has also been found in 29% of grade 1 or 2, and 39% of grade 3, endometrioid carcinomas of the endometrium; 18% of uterine serous carcinomas, and 26% of uterine clear cell carcinomas
  - Uterine low-grade endometrioid carcinomas frequently exhibit loss of *ARID1A* expression (26%)

Molecular Features of Tumors not Fitting in the Dualistic Model

- Classification of EC into type I and type II is artificial and the dualistic model has recently been challenged
  - In daily practice, pathologists are faced with ECs showing combined or hybrid morphologic and molecular characteristics (often EEC and serous carcinomas) (Fig. 8.10)
  - Furthermore, even though serous and clear cell carcinomas have been classified within the same category of tumors (NEECs), recent studies have shown that these are in fact distinct tumor types that exhibit different clinical, immunohistochemical, and molecular features

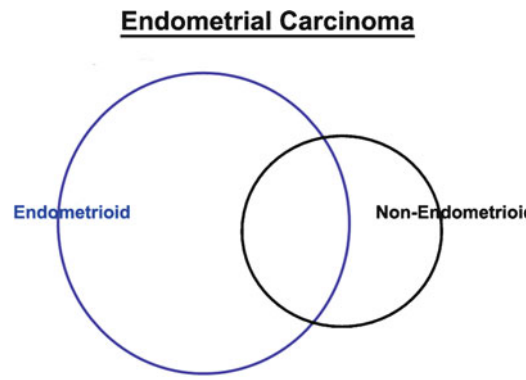

Fig. 8.10 A significant number of EC show combined morphologic and molecular features of EEC and NEEC

Mixed Endometrioid–Nonendometrioid Adenocarcinomas

- ECs showing an admixture of EEC and NEEC (serous and/or clear cell carcinomas) with the minor component representing at least 10% of the neoplasm are classified as a mixed carcinomas and prognosis depends on the proportion of the most aggressive component
- It has been suggested that, in mixed carcinomas, the NEEC component develops as a result of tumor progression—through *TP53* and *PIK3CA* mutations—from a preexisting EEC, since these tumors frequently retain the molecular alterations of typical EEC (Fig. 8.11)
  - This hypothesis would explain not only the existence of mixed EEC–NEEC, but also the presence of MI, as well as alterations in *PTEN, KRAS*, or beta-catenin, in NEEC
- Mixed EEC–NEEC may exhibit overlapping features with EEC (mixed EEC–NEEC morphology, early age at presentation, evidence of estrogen stimulation or preexisting hyperplasia, coexistence of *TP53* mutations, and MI or *PTEN* mutations)

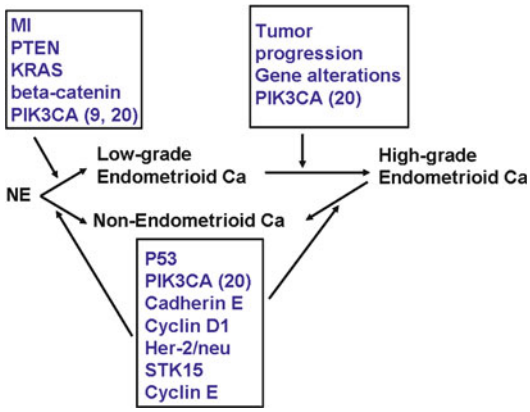

Fig. 8.11 Pathogenesis of endometrial carcinoma: an alternative to the dualistic model. Ca, carcinoma; NE, normal endometrium. (Reprinted from Diagnostic Histopathology, Catasus et al. (2009b), pp. 556–563, with permission from Elsevier)

Endometrioid Carcinomas with Ambiguous Features

- Some ECs exhibit overlapping or intermediate features between EEC and NEEC
  - In these tumors, it is not possible to delineate two different components; i.e., one of EEC, and another of NEEC
  - The term "EC with ambiguous features" has been proposed for such cases
- Although the features of these tumors need to be better defined, the term EC with ambiguous features can be applied to ECs that exhibit architectural pattern typical of low-grade EEC, but high-grade nuclear features, as seen in NEEC
  - These tumors tend to behave more aggressively than conventional EEC and occasionally show the molecular alterations of NEEC
- Another controversial issue is the gray zone between high-grade (predominantly solid) EEC, and NECC
  - Differential diagnosis between these two tumor types is difficult
  - Some high-grade EECs exhibit the typical molecular alterations of NEEC, such as *TP53* mutations

Undifferentiated Carcinoma and Dedifferentiated Carcinoma

- The World Health Organization (WHO) defines undifferentiated carcinoma (UC) of the endometrium as an epithelial tumor that fails to show evidence of either glandular or squamous differentiation
  - UC represents 1–10% of ECs
- UC is characterized by a monotonous proliferation of medium-sized, epithelial cells growing in solid sheets
  - The tumor cells have enlarged nuclei with prominent nucleoli
  - UC is associated with poor prognosis in the vast majority of the cases
    - Over 50% of cases present with advanced stage disease and 75% of patients die of tumors
- Occasionally, UC arises from preexisting well- or moderately differentiated EEC
  - The term *dedifferentiated carcinoma* (DDC) has been used to designate this type of EC
  - Prognosis of DDC is identical to that of UC
  - In DDC, the undifferentiated component is regarded as a result of tumor progression from the coexisting low-grade EEC
- Microsatellite instability is the predominant molecular feature of DDC
  - *TP53* mutations may also be found

Malignant Mixed Müllerian Tumors

- Malignant mixed müllerian tumors (MMMT), also called uterine carcinosarcomas, represent less than 5% of ECs
  - Composed of a biphasic pattern, with malignant epithelial elements and a sarcomatous component
  - Epithelial elements have usually the features of NEEC, but may also have the appearance of EEC
  - Sarcomatous component may be either homologous or heterologous

- Even if MMMT is currently classified as ECs, it should be regarded as a special type since it is associated with worse prognosis than that of the ordinary EC
  - The metastatic pattern of MMMT supports the theory of a neoplasia driven by the epithelial component
    - Myometrial infiltration, lymphovascular involvement, and metastases display more often the epithelial elements than the sarcomatous components of the tumor
- Immunohistochemical and molecular genetics studies support the clonal nature of the two—epithelial and mesenchymal—components in MMMT, supporting the hypothesis that they represent in fact metaplastic (sarcomatoid) carcinomas
  - Expression of epithelial markers in the sarcomatous components occurs in a large proportion of cases
- MMMT cell lines are capable of differentiating into epithelial, mesenchymal, or both components
  - Chromosome X inactivation studies, LOH, and gene mutation analyses all have shown that the epithelial and mesenchymal elements share common genetic alterations
- MMMT probably occurs through epithelial to mesenchymal transition (EMT) in ECs
  - EMT is a process of cellular trans-differentiation in which epithelial cells lose polarity and cell–cell contacts, reorganize their cytoskeleton, and acquire expression of mesenchymal phenotype
- MMMT show expression of genes that repress epithelial markers (E-cadherin) and enhance expression of mesenchymal markers, including proteins involved in skeletal muscle development
  - MMMT have revealed a microRNA signature typical of EMT Castilla et al. (2011)

cDNA Array Results

- cDNA array studies have demonstrated that the expression profiling of EEC differs from that of NEEC
  - In one study, 191 genes exhibited greater than twofold differences between 10 EECs and 16 NEECs
    - One of the genes, *TFF3*, was significantly upregulated in EECs, while increased expression of *FOLR* was seen in NEECs
- In another study, different expression profile involving 66 genes was seen in EEC and NEEC
  - Estrogen-regulated genes were upregulated in EEC
  - NEEC showed increased expression of genes involved in the regulation of the mitotic spindle checkpoint
- Differential expression of 1,055 genes between EECs and serous carcinomas was seen in another investigation
  - Genes upregulated in serous carcinomas were *IGF2, PTGS1*, and *p16*
  - Genes upregulated in EEC included *TFF3, FOXA2*, and *MSX2*
- Another analysis identified 315 genes that statistically distinguished EEC from NEEC
- ECs with microsatellite instability and stable ECs also have different gene expression profiles
  - Two members of the secreted frizzled related protein family (SFRP1 and SFRP4) are downregulated more frequently in EC with microsatellite instability
- Ovarian and uterine tumors with beta-catenin alterations show similar gene expression profile
- The gene expression profiles of similar histological subtypes of ovarian and endometrial carcinomas show that clear cell carcinomas have similar profile regardless of the organ of origin
  - Differences were seen when comparing endometrioid and serous carcinomas of ovarian and endometrial origin

Molecular Alterations in Myometrial Invasion

Overview on Myometrial Invasion

- Deep myometrial invasion is an important prognostic factor of EC

  - Usually correlates with high histological grade, vascular invasion, cervical involvement, and lymph node metastasis
  - Associated with high risk of recurrence
- EEC may exhibit various patterns of myometrial invasion, including diffuse infiltration or expansile-type invasion
  - A distinctive pattern of myometrial invasion designated *microcystic, elongated, and fragmented* (MELF) change shows glands lined by attenuated epithelium with luminal neutrophilic infiltrate resembling endothelium
  - MELF most likely represents EMT and is highlighted by the low-molecular-weight cytokeratin CK19

Epithelial to Mesenchymal Transition

- EMT is a process whereby epithelial cells lose polarity and cell–cell contacts, undergo remodeling of the cytoskeleton, acquire migratory abilities and a mesenchymal-like gene expression program
- Although EMT is a well-known mechanism for interconversions between epithelium and mesenchyme during embryonic development, EMT has recently been recognized as an important phenomenon that participates in invasion and metastasis
- EMT can be induced by different signals and pathways, such as those mediated by TGFβ (beta), tyrosine kinase receptors, and/or Wnt, depending on the specific cellular context
  - Activation of one or more of these pathways frequently converges in a group of transcription factors such as *SNAIL1, SLUG, ZEB1, ZEB2, E47, E2-2*, and *TWIST*, most of them capable of repressing E-cadherin, a master regulator of cell adhesion and polarity
- SNAIL protein expression is increased, and correlates inversely with E-cadherin immunoreactivity, in metastatic EC but not in the corresponding primary tumors
- By protein microarray analysis, a significantly negative correlation between E-cadherin protein decrease and SNAIL expression has also been demonstrated in primary EEC
  - High TWIST expression occurs in invasive EC and affects patient survival
- Comparison between EC samples from the most superficial tumor and the myoinvasive front has shown increases in *SNAIL, SLUG, HMGA2*, and *TWIST* mRNA expression, and decrease in E-cadherin expression, at the myoinvasive front Montserrat et al. (2011)
- EMT features are particularly evident in EECs exhibiting MELF pattern of myometrial invasion at the invasive front

ETS Transcription Factors

- ETS transcription factors activate matrix-degrading proteases and are related to EMT
  - Upregulation of ERM/ETV5, an ETS transcription factor, is associated with early myometrial invasion and correlates with increased matrix metalloproteinase (MMP)-2
- Higher expression of matrix metalloproteinases (MMP-2, MMP-9) in EC is associated with invasive and aggressive behavior in NEEC
  - Increases of MMP-7 have been seen, as a result of beta-catenin nuclear accumulation, in EC with *CTNNB1* mutations
- Transcription factor RUNX1/AML1 is upregulated in EC during invasion
  - A cooperative role of ERM/ETV5 and RUNX1/AML1 has been proposed

Proteomics

- Proteomic analysis shows differential protein expression in superficial and invasive areas of EC
  - Some of the proteins expressed in the invasive front, like Fascin1, have been associated with promotion of the acquisition of migratory and invasive phenotypes
- Different enzymes involved in oxidative stress (ROS), such as SOD1 and BLVRB, are preferentially expressed at the myoinvasive front
  - In the initial stages of EMT and cell migration, ROS is generated and targets downstream molecules which trigger tumor metastasis

Apoptosis–Resistance

Overview of Apoptosis

- Cell death is a phenomenon that commits irreversibly to loss of cellular functions
  - Morphologically, cells that undergo apoptosis frequently show cell volume shrinking, chromatin condensation, nuclear fragmentation, and plasma membrane blebbing
- Commonly, cell death occurring during menstrual phase has been associated to a hormone-dependent ischemic-derived necrosis
  - Several evidences point to apoptosis as another phenomenon with important implications in endometrium tissue remodeling by removing cells from functional layer
- Deregulation of apoptosis plays an important role in development and progression of cancer
  - Cells resistant to apoptosis are likely to have survival advantage, escape the immune surveillance, and may also be resistant to therapy
- Apoptosis can be initiated by two main mechanisms
  - Intrinsic pathway—originated in the mitochondria
  - Extrinsic pathway—triggered by the activation of death receptors in the cell surface (Fig. 8.12)

Members of the Bcl-2 Family

- Members of the *Bcl-2* family of genes are abnormal in EC
  - Compared to normal tissue, there is upregulation of *Bcl-xL* and *Bcl-2*, which is important for development of metastasis
- Pathways that control Bcl-2 expression like the PI3K/AKT pathway are abnormal in EC
  - Other "noncanonical" molecular events, such as NF-κB pathway, which plays an important role in EEC tumorigenesis, correlate with strong immunoreactivity for Bcl-xL (Fig. 8.12)
- In normal endometrium, both BAX and BAK are modulated during menstrual cycle and reach their higher levels in apoptotic epithelial

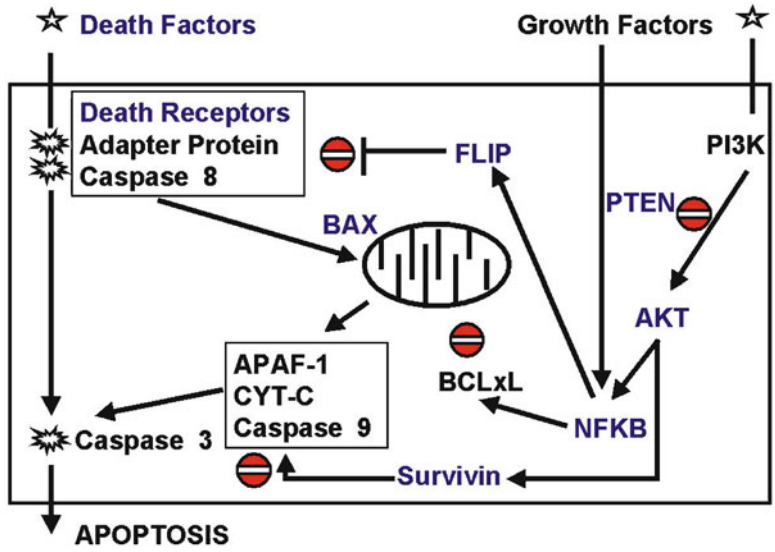

Fig. 8.12 Apoptosis: intrinsic (mitochondrial), and extrinsic (death receptor-initiated) pathways. (Reprinted from Pathology, Prat et al. (2007), with permission from Wolters Kluwer Health)

cells at menstruation, indicating a potentially ovary-derived hormone-dependent regulation
  - Lower to undetectable Bcl-2 protein levels are detected in late secretory through menstrual phases suggesting a direct relationship between anti-apoptotic Bcl-2 proteins and BAX-like proapoptotic members in decision of cell fate
- *BAX* is a target gene for mutations in EEC with microsatellite instability and may have a role in resistance to apoptosis in these tumors

Extrinsic Pathway

- One of the most important regulators of death receptor signaling is *FLIP*, which shares high homology to caspase-8 but lacks proteolytical activity (Fig. 8.12)
  - Transfection of EC cell lines with *FLIP* siRNA results in a marked decrease in cell viability after *TRAIL* exposition
  - In EEC, *FLIP* may be regulated by a cellular complex containing casein kinase 2 and kinase suppressor of RAS1 (CK2–KSR1)
  - CK2 beta regulatory subunit is overexpressed in EC and regulates cell proliferation
  - The kinase suppressor of RAS1 (KSR1) is considered a scaffold protein that regulates the intensity and duration of the MAP kinase pathway
  - KSR1 interacts with different kinases of the Raf/MEK/ERK signaling pathway to enhance its activation
  - KSR1 expression is increased in EC Llobet et al. (2011)

Resistance to Hypoxia and Radiation Therapy

- EC is treated by surgery and adjuvant radiation
  - Recurrence postradiotherapy is usually associated with poor prognosis and increased risk of metastases
  - These tumors are treated by chemotherapy (doxorubicine, cisplatin, paclitaxel)
  - Understanding the molecular and genetic mechanisms underlying resistance to either radio and/or chemotherapy is crucial for the establishment of new therapeutic targets that improve outcome in EEC
- Ionizing radiation (IR) causes lesions at the DNA level, known as DNA single-strand breaks and double-strand breaks (DSBs)
  - IR can rapidly prevent DNA replication by activation of cell cycle checkpoints to avoid formation of toxic DNA replication lesions
  - The main signaling molecules involved in the DNA damage response are the serine threonine kinases ATM and ATR
    - ATM responds primarily to DNA DSBs
    - ATR acts mainly in response to replication fork stalling
    - Ionizing radiation also activates ATR
- In response to DSB, ATM and DNA-protein kinases phosphorylate H2AX. γ-H2AX plays an essential role in the repair process regulating the recruitment of repair factors, such as Nbs1, 53BP1, and BRCA1, to foci located at DSB sites
- Unrepaired DNA damage is measured by H2AX phosphorylation (γ-H2AX), and γ-H2AX is used as a standard marker of unrepaired double-strand DNA damage
- Tumor hypoxia renders tumors more resistant to IR treatment
  - By reacting with the radiation-created broken ends of DNA, oxygen fixes the damage and thus enhances radiation-induced cell death
  - The "oxygen enhancement effect" renders oxygenated cells three times more radiosensitive than hypoxic cells
  - Under hypoxic conditions, the oxygen enhancement effect is lost, and cells become more radio resistant
- Low PR expression is associated with recurrence
  - PR expression in ECs from patients carrying one specific DNA polymorphism (so-called PROGINS allele) is predictive of the risk of recurrence

- *MLH-1* promoter methylation and decreased MLH-1/MSH-2 expression are not predictive of recurrence in stage I EC
  - De novo *MLH-1* promoter methylation is occasionally found during EC progression in patients receiving radiation therapy
- p53 overexpression is significantly predictive of recurrence in EC
  - It does not correlate with *TP53* mutations
- Absence of E-cadherin expression predicts distant metastasis but not local recurrence
  - Nuclear immunoreaction of beta-catenin does not predict recurrence
- Immunohistochemical comparison of postradiation recurrent tumor and primary EC reveals increased nuclear expression of beta-catenin in the recurrent tumors
- HIF-1α (alfa) is another candidate molecule for conferring radio resistance to EC cells
  - HIF-1 is the most important mediator of hypoxia and controls the expression of over 100 genes
  - Compared to levels in primary EC, HIF-1α (alfa) expression increases in postradiation recurrences
  - HIF-1α (alfa) controls classical NF-κβ (beta) activation pathway and survival under hypoxia through RelA (p65) nuclear accumulation
  - Possibly, HIF-1α (alfa) expression results in increased radio resistance

Stem Cells

- Somatic stem cells (SSC) are undifferentiated cells present in most adult tissues
  - SSC are defined by their functions: high proliferative potential, self-renewal, and differentiation into one or more lineages
  - Another property of SSC is the retention of a DNA synthesis label (bromodeoxyuridine—BrdU) for prolonged periods of time
  - The Hoechst dye exclusion is a good method for the identification and isolation of stem cells in adult tissues
  - A side population (SP) can be identified by dual-wavelength flow cytometry after incubating the target cells with the DNA-binding dye Hoechst 33342 (Fig. 8.13) Cervello et al. (2011)
- Cancer stem cells (CSC) have been defined in analogy to SSC as cells that have the capacity to self-renew
  - Undergo divisions that allow the generation of more identical CSC and give rise to the variety of more differentiated cells found in the tumor

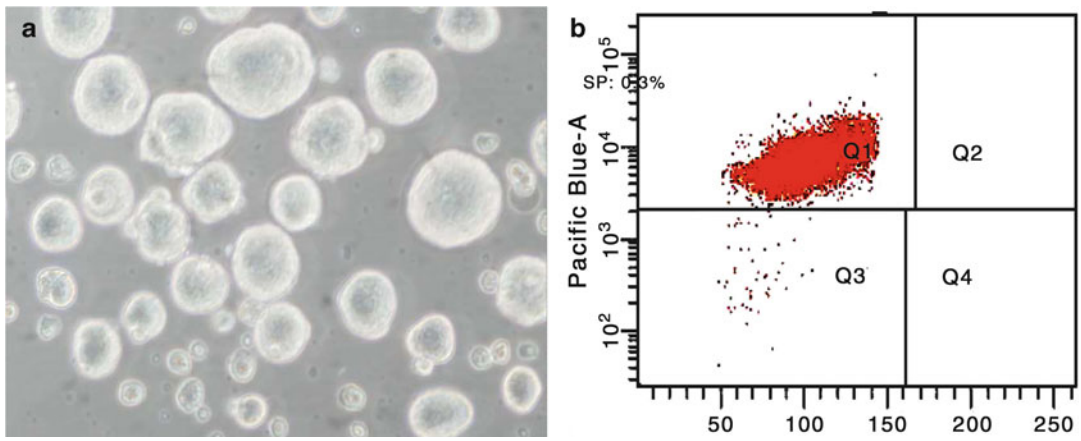

Fig. 8.13 The endometrial carcinoma cell line Ishikawa shows cancer stem cell features. Ishikawa cells can grow as floating spheres (**a**). This cell line contains a side population that excludes Hoescht stain. Q1, stained region; Q3, unstained region, side population (**b**). (Reprinted from International Journal of Gynecological Pathology, Cervello et al. (2011), with permission from Wolters Kluwer Health)

- An epithelial and stromal SP has been identified in human endometrium
  - The mean percentage of SP cells in the epithelial fraction is 0.21% during the menstrual phase, 0.15% during the proliferative phase, and 0.02% in the secretory phase
  - Currently, there are no markers for endometrial epithelial stem cells and they cannot be distinguished from their mature progeny
- SP cells have been identified in both human primary ECs and some cell lines
  - In vitro studies showed that AN3CA-SP cells proliferate slower than the corresponding non-SP fraction
    - Cells showed long-term self-renewal properties and accumulated in the G1 phase of the cell cycle, suggesting they are in a dormant quiescent state
  - AN3CA-SP cells are resistant to paclitaxel treatment compared to the non-SP subset
    - No differences were observed with cisplatin
- Investigations with Hec-1 SP cells revealed long-term proliferating and self-renewal capacity in vitro
  - These cells initiate larger tumors than the non-SP population and, more important, they undergo EMT during tumor development
- To date, only one surface marker, the CD133/1 epitope, has been proposed for identification and isolation of endometrial CSC
  - CD133+ cells have been isolated from Ishikawa, Hec1-A, RL-95, AN3CA, MFE280, and MFE296 cell lines
  - The ratio of positive cells differs between lines, ranging from 0.38% to 15.5%
  - CD133+ cells showed higher proliferative potential and tumorigenicity than the negative subset
  - There is no conclusive evidence that CD133 is the universal marker for endometrial CSC

Targeted Therapies

- The importance of the PI3K/PTEN/AKT survival pathway in EC raises the possibility that PI3K inhibitors, such as Wortmannin and derivatives, may be used as potential anticancer agents
  - Decrease of Akt phosphorylation and increased apoptosis are seen in mutated *PTEN* human endometrial cancer cells in the presence of PI3K inhibitor
- ECs with *PTEN* mutations show a high level of genetic instability similar to the one seen in breast and ovarian cancers with *BRCA-1* and *BRCA-2* alterations
  - PARP inhibitors are used in the treatment of patients with *PTEN*-mutated Ecs (Table 8.3)
- mTOR is the downstream effector of AKT
  - Upon activation, mTOR-Raptor activates S6K and inhibits 4EBP1 to accelerate mRNA translation
  - Tumors associated with *PTEN* inactivation are particularly susceptible to the therapeutic effects of mTOR inhibitors (Table 8.3)
  - Several mTOR inhibitors are available for clinical trials: CCI-779 (temsirolimus), RAD001 (everolimus), and AP23573
  - Pharmacological inhibition of mTOR by CCI-779 in $PTEN^{+/-}$ mice has shown reduced neoplastic proliferation, tumor size, and S6K activity
- Dual PI3K–mTOR inhibitors may also be used as a targeted therapy in EC
  - The p110 subunits of PI3K and mTOR share similar structures
  - The dual PI3K–mTOR inhibitors may target p110α (alfa), β (beta), and δ (delta) isoforms, mTORC1 and mTORC2
  - BEZ235, a dual PI3K and mTOR inhibitor, suppresses cell growth in EC cell lines, especially in cells with *PIK3CA* and/or *PTEN* mutations

Table 8.3 Endometrial carcinoma (targeted therapies)

Target	Predictive marker
mTor (temsirolimus, everolimus, deferolimus)	*PTEN* loss, *PIK3CA, KRAS* mutations?
HER2 (trastuzumab)	*c-erbB2* amplification
EGFR (Erlotinib)	EGFR expression
VEGF (bevacizumab/ thalidomide)	VEGFR expression?
Tyrosine kinases (sorafenib/ sunitinib)	NFkB, MCL-1, FLIP?
PARP	*PTEN* loss
FGFR2	*FGFR2* mutations

- EGFR, which is highly expressed in normal endometrium, is also overexpressed in EC—a finding associated with poor prognosis
  - In these cases, GW572016 (Lapatinib) has been used as a single agent (Table 8.3)
- Medroxiprogesterone acetate is frequently used in EC patients
  - Two large GOG trials evaluating oral progestins in these patients showed an overall response rate or 15–25% with median progression-free survival of less than 4 months and overall survival of less than 11 months
- Proteasome inhibitors trigger cell growth arrest or apoptosis on several tumors
  - Bortezomib causes cell death by blocking NF-κB activity in some tumors
  - In EC, proteasome inhibitors induce cell death by increasing NF-κB transcriptional activity
- Sorafenib (BAY 43-9006, Nexavar) is a potent tyrosine kinase inhibitor with antiproliferative and antiangiogenic activities
  - Although originally described as a B- and c-RAF kinase inhibitor, it has also shown activity against vascular endothelial growth factor receptor 2 (VEGFR2), platelet-derived growth factor receptor, FLT3, RET, and c-Kit
  - Sorafenib sensitizes EC cells to TRAIL-induced apoptosis by downregulating FLIP and Mcl-1 (Table 8.3) Ortega et al. (2008)
- Histone acetylation is one of the mechanisms involved in the epigenetic control of gene expression
  - Histone deacetylase inhibitors (HDACI) are promising anticancer drugs
  - HDACI cause derepression of genes whose reactivation would promote an antiproliferative effect
  - Examples of genes upregulated by HDACI are *p21, TRAIL-R2, p19ARF, Bmf*, and *Rap1*
  - Paradoxically, HDACI cause downregulation of important genes such as thymidylate synthetase, *Bcr-Abl* and *c-Myc*
  - HDACI have growth inhibitory effect on EC cell lines, by decreasing the proportion of cells in S phase, and increasing the proportion of cells in the G_0–G_1 and or G_2–M phases of the cell cycle
  - HDACI upregulates *p21*, *p27*, and E-cadherin, and downregulates Bcl-2, and cyclin D1 and cyclin D2
  - The growth-suppressor effects seem to be irrespective of the *TP53* gene status

Summary of Keypoints

- There are two clinicopathologic variants of endometrial carcinomas (endometrioid and nonendometrioid) that show specific molecular features and different gene expression profiles
- The main molecular features of endometrioid carcinomas are: microsatellite instability and mutations of *PTEN, PIK3CA, KRAS*, and beta-catenin genes
  - Although the clinical and prognostic relevance of each of these alterations has not been fully elucidated, mutations in *PTEN* and beta-catenin gene seem to be associated with a favorable outcome
- In HNPCC patients, microsatellite instability analysis and immunoreactivity of mismatch repair proteins are important to confirm the diagnosis of hereditary endometrial carcinoma
- The main features of nonendometrioid carcinomas are:
  - *TP53* mutations
  - Inactivation of *p16* and E-cadherin, *c-erbB2* amplification
  - Alterations in genes involved in the regulation of the mitotic spindle checkpoint (*STK-15*)
  - LOH at multiple loci indicating chromosomal instability
- Some nonendometrioid carcinomas probably arise from preexisting endometrioid carcinomas
  - This is the most likely reason why some tumors exhibit combined or mixed features at the clinical, pathological, and molecular levels
- Some EC do not fit in the dualistic (type I vs. type II) model
  - Mixed endometrioid–nonendometrioid tumors (*TP53*)
  - Dedifferentiated carcinomas (microsatellite instability)
  - Malignant mixed müllerian tumors (epithelial to mesenchymal transition)

- EMT, Ets transcription factors, and enzymes involved in oxidative stress may have a role in myometrial invasion
- In endometrial carcinoma, apoptosis–resistance may play a role in tumor progression (FLIP under CK2 and KSR1 regulation)
- Molecular pathology is important for identifying biomarkers as predictive factors for success in targeted therapies
  - Candidate pathways are PI3K, mTOR, EGFR, apoptosis, and histone acetylation

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Molecular Pathology of Kidney Tumors

9

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Contents

Introduction

- Renal cancer is the sixth leading site of new cancer diagnosis for men and eighth for women, with an estimated 60,920 cases diagnosed in 2011, according to American Cancer Society statistics
  - Carcinoma of renal tubular origin (92%) comprises the majority of malignancy, followed by carcinoma of the renal pelvis (7%) and nephroblastoma (Wilms tumor, 1%)
- Renal neoplasms are a distinctive and heterogeneous group of entities
  - Clinical behavior varies significantly between lesions
    - Sometimes histologic appearance is deceptively bland

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L. Cheng and J.N. Eble (eds.), *Molecular Surgical Pathology*,
DOI 10.1007/978-1-4614-4900-3_9,

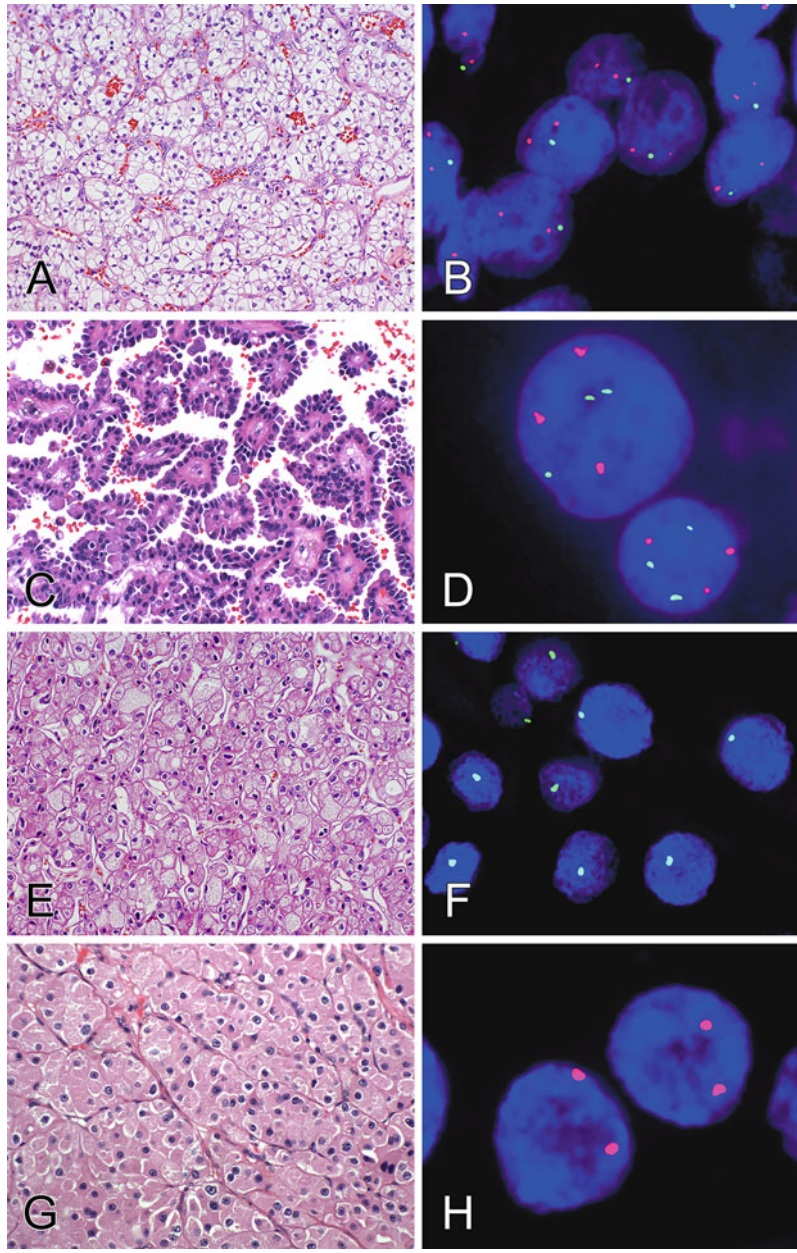

Fig. 9.1 Histopathologic and cytogenetic features of renal cell neoplasms by FISH. Clear cell renal cell carcinoma (**a**) frequently shows chromosome 3p deletion (**b**) indicated by the presence of a single 3p signal (*green*) in cells with two chromosome 3 centromere signals (*red*) per cell. Papillary renal cell carcinoma (**c**) consistently shows trisomy 7 (three *green signals*) and 17 (three *red signals*) (**d**). Most chromophobe renal cell carcinomas (**e**) show a complex multichromosomal deletion with monosomy of 1, 2, 6, 10, and 17. In (**f**), each chromophobe tumor cell possesses only a single chromosome 10 signal. Despite its morphological similarity to chromophobe renal cell carcinoma (especially eosinophilic variant), oncocytoma (**g**) does not exhibit multichromosomal deletions; it retains a disomic chromosomal profile. Each of the two nuclei in (**h**) shows two fluorescent signals for chromosome 17 (disomic pattern) (from Cheng et al. 2009; with permission from Elsevier).

Table 9.1 Genetics and manifestations of familial renal carcinoma syndromes

Syndrome	Inheritance	Chromosome location	Gene	Protein	Mechanism	Pathologic manifestations	
						Kidney lesions	Nonkidney lesions
von Hippel–Lindau	AD	3p25	*VHL*	pVHL	Mutation	Clear cell renal cell carcinoma, multiple bilateral cysts	Pheochromocytoma, pancreatic cyst and neuroendocrine tumor, CNS and retinal hemangioblastoma, endolymphatic sac tumor, epididymal cystadenoma
Hereditary papillary renal cell carcinoma	AD	7q31	*MET*	Hepatocyte growth factor receptor	Mutation	Papillary carcinoma (type I), multiple/ bilateral with late onset	
Hereditary leiomyomatosis and renal cell carcinoma	AD	1q42	*FH*	Fumarate hydratase	Mutation Loss	Papillary carcinoma, similar to type II, with unique morphologic features	Cutaneous and uterine leiomyomas, rarely leiomyosarcoma
Birt–Hogg–Dubé	AD	17p11	*FLCN*	Folliculin	Mutation	Oncocytoma Chromophobe carcinoma Clear cell carcinoma Hybrid oncocytic tumor	Benign cutaneous tumors, pulmonary cysts, medullary carcinoma of thyroid
Tuberous sclerosis	AD	9q34/16q13	*TSC1/TSC2*	Hamartin/tuberin	Mutation	Angiomyolipomas Renal cysts Oncocytoma	Facial angiofibroma, periungual fibroma, shagreen patch, hypopigmented macules, cardiac rhabdomyoma, retinal hamartomas, giant cell astrocytoma, pulmonary lymphangioleiomyomatosis

(continued)

Table 9.1 (continued)

Syndrome	Inheritance	Chromosome location	Gene	Protein	Mechanism	Pathologic manifestations	
						Kidney lesions	Nonkidney lesions
Constitutional chromosome 3 translocation	AD	3p and 3q	*FHIT*, others	FHIT and others	Translocation	Clear cell carcinoma	
Succinate dehydrogenase germline mutation	AD	1p36	*SDHB*	Succinate dehydrogenase subunit B	Mutation	Renal tumors with unique morphologic characteristics	Paraganglioma, papillary thyroid carcinoma

AD autosomal dominant

Table 9.2 Molecular-genetic alterations in renal neoplasms

Tumor type	Chromosomes	Genes	Mechanism and manifestations	Other genetic alterations
Clear cell renal cell carcinoma	3p14.2 3p21 3p25 Others	*FHIT* *RASSF1A* *VHL*	Deletion, mutation, methylation	+5q22, −6q, −8p12, −9p21, −9q22, −10q, −14q
Clear cell papillary renal cell carcinoma	7 17	?	Low copy number gains of chromosomes 7 and 17, less frequent than papillary carcinoma	Upregulation of *HIFα* by non-*VHL* pathway
Multilocular cystic renal cell carcinoma	3p	*VHL*	Mutation of *VHL*, 3p deletion	
Papillary renal cell carcinoma	7 17 7q31.1 7q31 Y	*FRA7G* *MET* *LRRK2* ?	Trisomy of chromosomes 7, 17 Gain of 17q31 Loss of Y	+3q, +8, −9p21,+12, −14q, +16, +17q21, +20,
Chromophobe renal cell carcinoma	1, 2, 6, 10, 13, 17, 21, Y	?	Multiple chromosome loss	−5q22, −8p, −9p23, −18q22
Oncocytoma	1, 14 11q13	?	Loss Translocation	−1p, −8p, −11q13, 14q, −19q, −21q, −X/Y der(13)t(13;16)(p11;p11)
Collecting duct carcinoma	1, 2, 6, 10, 13q, 13, 14, 15, 22	? *BCR-ABL* *Rb*	Deletion t(9:22)(q34;q11)	−1q32, −6p, −8p, −9p, −13q, −19q32, −21q
Renal carcinoma associated with Xp11.2 translocation	1p34 1q21 17q23 17q25 Xq11.2 3q23 19q13.1 10q23	*PSF-TFE3* *PRCC-TFE3* *CLTC-TFE3* *ASPL-TFE3* *NONO-TFE3* ? ? ?	t(X;1)(p11.2;p34) t(X;1)(p11.2;q21) t(X;17)(p11.2;q23) t(X;17)(p11.2;q25) inv(X)(p11.2;q12) t(X;3)(p11;q23) t(X;19)(p11.2;q13.1) t(X;10)(p11.2;q23)	

(continued)

Table 9.2 (continued)

Tumor type	Chromosomes	Genes	Mechanism and manifestations	Other genetic alterations
Renal carcinoma associated with t(6;11)	6p21 11q12–13	*TFEB-alpha*	t(6;11)(p21.1;q12–13)	
Mucinous tubular and spindle cell carcinoma	1, 4, 6, 8, 9, 11, 13, 14, 15, 18, 22	? ?	Multiple chromosome loss Deletion	−8p,−9p, −11q, +12q, +16q, +17, +20q
Metanephric adenoma	2p13 2p		Partial monosomy	Inv(9)(p12q13), t(1;22)(q22;q13), t(15;16)(q21;p13)

? = unknown genes

    - Cytologic/histologic similarity can be significant across both benign and malignant neoplasms
- Molecular and cytogenetic alterations have been of great historical importance in establishing the classification of renal neoplasms
  - Modern molecular methodologies are already playing a significant role in classification
    - Subdivision of new diagnostic entities
    - Resolution of challenging differential diagnoses (Fig. 9.1)
    - Direction of targeted therapy
- A number of genetic syndromes with predisposition to development of renal tumors have been carefully studied, resulting in increased understanding of underlying cytogenetic and molecular events

Genetic Renal Neoplasia Syndromes

- Although most renal neoplasms are sporadic, approximately 3–5% are associated with inherited syndromes (Table 9.1)
- Investigation into their molecular and genetic mechanisms has led to improved understanding of both the syndromes themselves and sporadic renal neoplasms (Table 9.2)

von Hippel–Lindau Disease

- von Hippel–Lindau (VHL) disease is a genetic syndrome with autosomal dominant inheritance, associated with germline inactivating mutation of *VHL* tumor suppressor gene
  - Greater than 90% penetrance at 65 years of age
  - Estimated incidence of 1 in 35,000 live births
  - The *VHL* gene is located at chromosome 3p25.3
  - Patients with VHL disease generally have one mutated copy of the *VHL* gene
    - Tumorigenesis is thought to occur with inactivation of the second copy, via loss of heterozygosity (LOH), promoter hypermethylation, or mutation, in a "two-hit" model (Fig. 9.2)
    - Fewer total steps are required in the development of a renal tumor, compared to sporadic cases
- The VHL syndrome is characterized by the following:
  - Renal manifestations
    - Multiple, bilateral renal cysts (66% of patients)
    - Clear cell renal cell carcinoma
      - Cysts have been hypothesized to represent a potential precursor for clear cell carcinoma, as some small carcinomas can be identified arising in a cyst
      - However, VHL patients also develop small, solid carcinomas without demonstrable association with a cyst (Fig. 9.3)
      - Clear cell renal cell carcinoma is variably reported to develop in 24–70% of patients, with metastatic disease representing a significant cause of death in VHL patients
  - Nonrenal manifestations
    - Capillary hemangioblastomas of the central nervous system and retina (60–84% of patients)
    - Pheochromocytomas (18% of patients)
    - Pancreatic cysts (70%) and neuroendocrine tumors (9% of patients)
    - Epididymal cystadenomas (54% of male patients) or broad ligament cystadenomas (in women)
    - Endolymphatic sac tumors of the inner ear (14% of patients)
- The *VHL* gene codes for pVHL protein, a 213-amino acid member of the ubiquitin ligase family
  - *VHL* is key in cell cycle control and gene regulation, particularly in regulation of hypoxia-inducible factor (*HIF*)
    - *HIFα*
      - A transcription factor involved in the response to changes in oxygen supply in the cellular environment

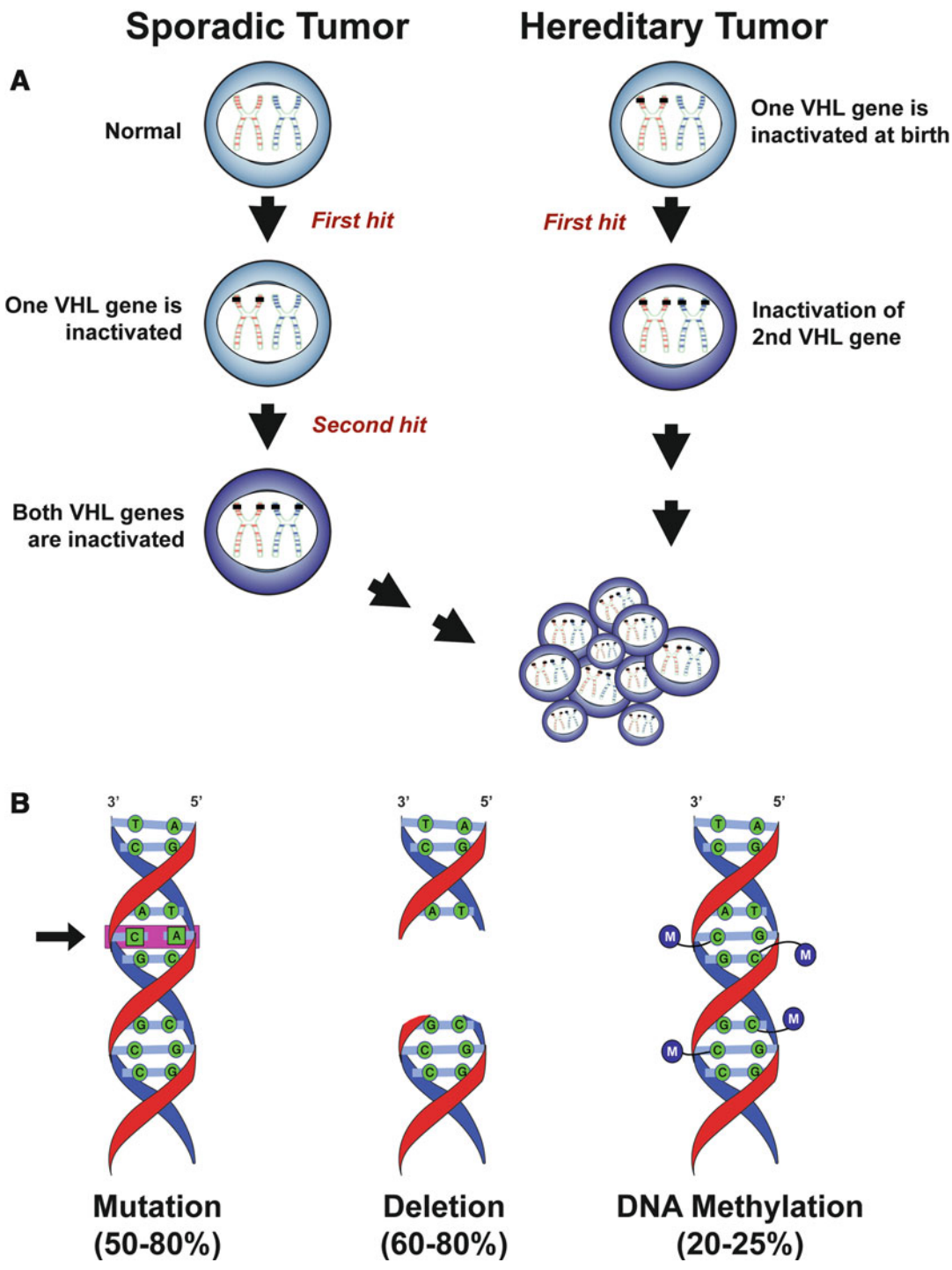

Fig. 9.2 Pathways of *VHL*-associated carcinogenesis. (**a**) The *VHL* tumor suppressor gene plays a critical role in both sporadic and hereditary renal cancers. Both copies of the *VHL* gene must become inactivated to initiate tumor development. In familial clear cell renal carcinoma, one copy of the *VHL* gene has already been inactivated at birth; consequently, a single additional *VHL* gene inactivation initiates tumorigenesis. Sporadic tumors require two steps to inactivate both *VHL* alleles to initiate tumorigenesis. Further genetic alterations may also be required for initiating the development of a tumor. (**b**) *VHL* gene inactivation may occur through one of several different pathways, including genomic mutation (50–80%), deletion (60–80%), or abnormal DNA methylation (20–25%). Different mechanisms are frequently involved in carcinogenesis. Mutation of one allele and deletion of the remaining wild-type allele are often seen in clear cell renal carcinomas (from Cheng et al. 2009; with permission from Elsevier).

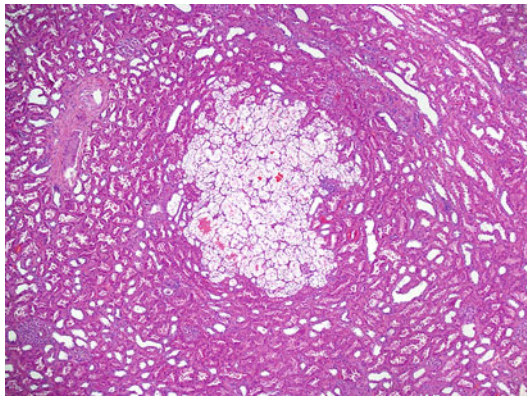

Fig. 9.3 A small, poorly circumscribed, and unencapsulated focus of clear cell carcinoma in a patient with VHL disease, arising without an identifiable cyst.

- Important in physiologic response to ischemia and hypoxia, as well as tumor growth and angiogenesis
- Normal pVHL forms a ubiquitin–ligase complex with Elongin B, Elongin C, Rbx1, NEDD8, and Cullin-2, targeting HIF1α for ubiquitin-mediated degradation in cells under normal oxygen conditions
- In hypoxic or iron-deficient conditions, HIF1α is not degraded and instead binds to hypoxia-response element sequences in the nucleus, resulting in activation of several downstream genes, including: *VEGF*, *PDGF*, *GLUT1*, and *TGFα* (Fig. 9.4)
- Loss of *VHL* in tumor cells therefore leads to accumulation of HIF1α and upregulation of these downstream genes, with key roles that promote tumor growth, support, and spread, such as the following:
  - Cell proliferation
  - Angiogenesis
  - Metastatic potential
  - Glucose transportation
- Carbonic anhydrase IX (CA-IX)
  - A membrane protein whose expression is also VHL–HIF1α-dependent
  - CA-IX is involved in regulation of intracellular and extracellular pH, with expression induced by hypoxic conditions
  - As such, expression is seen in clear cell renal cell carcinoma, even in high grade and sarcomatoid forms
    - Immunohistochemical expression has proposed utility in resolving the differential diagnosis of challenging cases
    - In VHL patients, single renal tubular epithelial cells and cells within renal cysts have been found to show expression
- Mammalian target of rapamycin (mTOR)
  - Inactivation of *VHL* also upregulates the mTOR pathway
  - mTOR is a serine/threonine protein kinase involved in monitoring of cellular and environmental nutrition and energy status, with effects in protein translation, cell growth, angiogenesis, and apoptosis
  - Downstream targets include phosphatidylinositol 3 kinase, involved in cell survival, proliferation, and neovascularization
- Primary cilium
  - pVHL has also been found to play a role in structure of the primary cilium, a cellular sensory mechanism involved in inhibition of epithelial proliferation
  - pVHL functions in association with phophatidylinositol 3 kinase and glycogen synthase kinase 3β (GSK3β) in maintenance of the primary cilium
  - Defects of the primary cilium have been implicated in formation of renal cysts
  - Cyst-dependent and cyst-independent pathways of development for clear cell carcinoma have been proposed

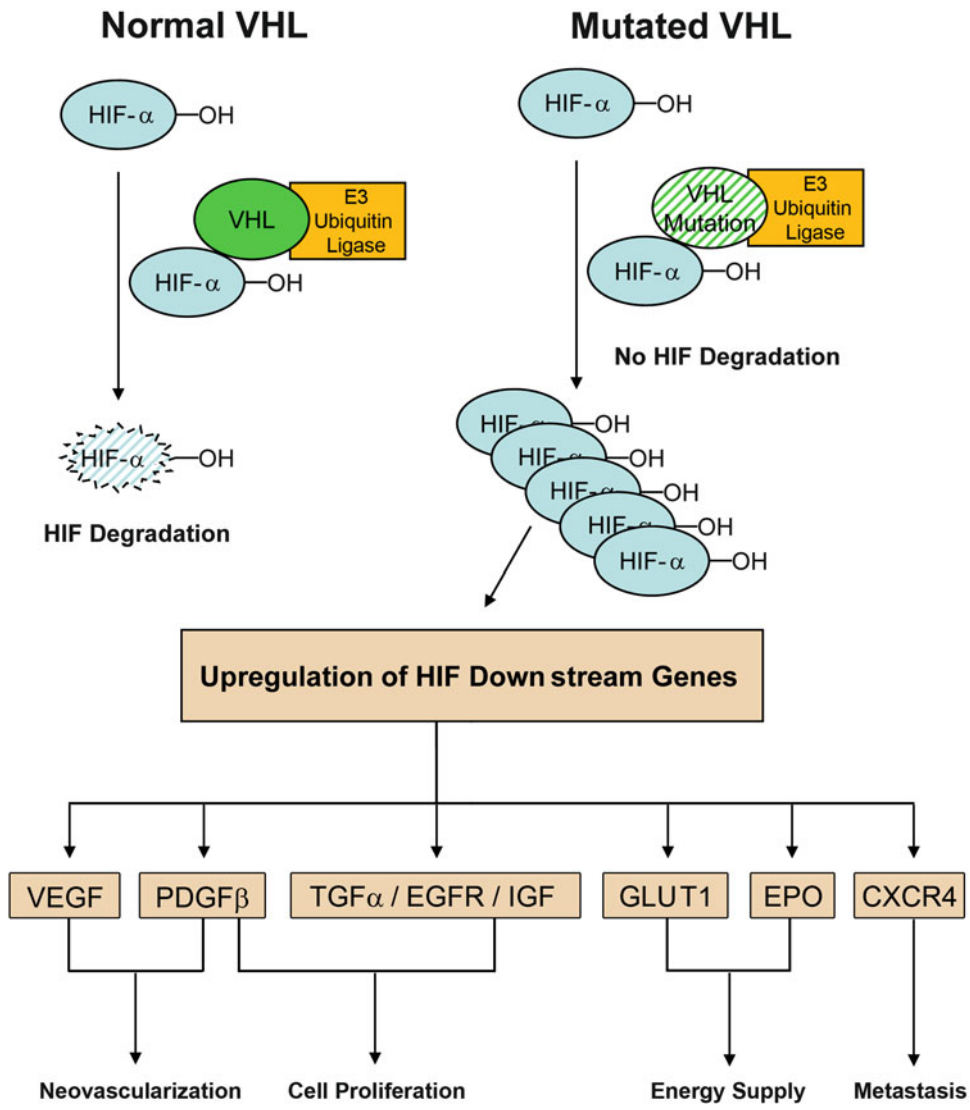

Fig. 9.4 Mechanisms of *VHL*-associated carcinogenesis. The normal *VHL* gene product forms a complex with E3 ubiquitin ligase, which induces the degradation of HIFα to regulate the activity of genes downstream from *HIF*. When both copies of the *VHL* gene are inactivated, HIF degradation is retarded and HIF accumulates to higher levels, triggering upregulation of HIF downstream gene activities. The major upregulated factors include *VEGF*, *PDGFβ*, *TGFα*, *EGFR*, *IGF*, *GLUT1*, *EPO*, and *CXCR4*, resulting in enhanced neovascularization, stimulation of cell growth, enhanced glucose transportation, and cancer progression. HIF downstream upregulation is associated with tumorigenesis in both familial and sporadic clear cell renal cancers. *VHL* von Hippel–Lindau; *VEGF* vascular endothelial growth factor; *PDGF* platelet-derived growth factor; *TGF* transforming growth factor; *EGFR* epidermal growth factor receptor; *IGF* insulin-like growth factor; *GLUT1* glucose transporter protein 1; *EPO* erythropoietin; *CXCR4* chemokine (C-X-C motif) receptor 4 (from Cheng et al. 2009; with permission from Elsevier).

- CXCR4
  - CXCR4 is a chemokine receptor involved in metastasis of renal carcinomas, as well as tumors of other organs
  - Regulation occurs through HIF1α and mTOR
  - Inhibition of this pathway may represent a potential therapeutic target in the management of renal cell carcinoma patients

Hereditary Papillary Renal Carcinoma

- Hereditary papillary renal carcinoma is a genetic syndrome with autosomal dominant inheritance and high penetrance, associated with (typically) late onset of multiple, bilateral, type I papillary renal cell carcinomas
  - Renal tumors
    - Patients have approximately a 90% likelihood of developing renal cell carcinoma by 80 years of age
    - Generally, onset in fifth to seventh decades
    - Numerous tumors often present within the same kidney
    - A form with early onset has also been described (second to third decades)
- Activating mutation of *c-MET* protooncogene (mesenchymal–epithelial transcription factor) at chromosome 7q31.3
  - 126 kb genomic region with 20 exons, encoding the 1,390 amino acid MET protein
  - MET is a receptor in the tyrosine kinase family, whose ligand is HGF (hepatocyte growth factor)
    - Increased *c-MET* implicated in papillary tumors of other organs, including thyroid, ovary, and colon carcinomas
  - Mutation of the tyrosine kinase domain results in constitutive activation without ligand binding
    - Results in cell proliferation, neovascularization, and cell motility
  - Duplication of the mutated chromosome 7 is frequently seen (trisomy 7)
  - Activating mutation of *MET* seen in only a small subset of sporadic papillary renal cell carcinoma cases (13%)

Hereditary Leiomyomatosis and Renal Cell Carcinoma

- Hereditary leiomyomatosis and renal cell carcinoma (HLRCC) is a genetic syndrome with autosomal dominant inheritance and incomplete penetrance
  - Renal manifestations
    - Papillary renal cell carcinoma (similar in appearance to type II tumors, with unique morphologic features)
    - Renal carcinoma develops in only a subset of patients (15–20%) and is often unifocal/unilateral, with early onset
    - Tumors are aggressive, often presenting at advanced stage, with many patients dying of metastatic disease
    - Morphologically, tumors have features of type II papillary carcinoma, including papillary, solid alveolar, tubular, glandular, and sheet-like architecture
      - Unique morphologic features include the prominent, inclusion-like eosinophilic nucleolus, surrounded by a clear halo (similar to the inclusions seen in cytomegalovirus infection)
  - Nonrenal manifestations
    - Leiomyomas of the skin and uterus
      - In contrast to renal carcinoma, most patients (85–98%) develop uterine and cutaneous leiomyomas
      - Development of uterine leiomyomas at a young age (under 30 years) may prompt consideration of the diagnosis of HLRCC
      - Morphologically, the leiomyomas may show similar nuclear features to the kidney tumors (prominent eosinophilic nucleolus with "halo")
    - Leiomyosarcoma occurs in a small subset of cases
- The syndrome is associated with mutation of *FH* (fumarate hydratase) gene at chromosome 1q42.3–q43
  - *FH* includes ten exons and codes for a 500-amino acid peptide
  - The FH protein is involved in the conversion of fumarate to malate in the Krebs cycle
  - Biallelic inactivation is found in the majority of tumors, suggesting a role of *FH* as a tumor suppressor gene

Tuberous Sclerosis

- Tuberous sclerosis (tuberous sclerosis complex or TSC) is an autosomal dominant, inherited tumor syndrome, with near-complete penetrance and variable expressivity
  - Estimated to affect 1 in 6,000–12,500 people
    - Cases thought to be more frequent than historically detected, due to improvement in imaging modalities and increased utilization of imaging
  - Renal manifestations
    - Renal cystic disease
      - Variable cyst formation in approximately 45% of patients, ranging from microcystic disease, undetectable by imaging, to a polycystic kidney phenotype
    - Renal tumors (50–80% of patients)
      - Angiomyolipomas (multifocal and bilateral), present in 75–80% of affected children greater than 10 years of age
      - Multifocal clear cell renal cell carcinoma and oncocytoma (in a subset of patients)
  - Nonrenal manifestations
    - Skin lesions
      - Facial angiofibromas ("adenoma sebaceum")
      - Periungual fibromas
      - Shagreen patches (peau chagrin)
      - Hypopigmented macules
    - Central nervous system lesions
      - Cortical tubers
      - Subependymal nodules
      - Subependymal giant cell astrocytomas
    - Tumors of other organs
      - Cardiac rhabdomyomas (in up to 50% of patients)
      - Pulmonary lymphangioleiomyomatosis
      - Retinal astrocytic hamartomas ("phakomas")
- Tuberous sclerosis is associated with germline mutation of *TSC* genes 1 or 2 (*TSC1* or *TSC2*)
  - A significant number of cases (70%) result from sporadic germline mutations, while 30% are inherited
  - *TSC1*
    - Located at chromosome 9q34
    - Encodes 130 kD protein hamartin
      - Involved in cell adhesion through ezrin–radixin–moesin family of actin-binding proteins and GTPase Rho
      - Proposed tumor suppressor function, due to tumor formation with loss of both wild-type alleles
  - *TSC2*
    - Located at 16p13.3
    - Encodes tuberin protein with GTPase-activating activity for RAS-related protein Rap1
    - *PKD1* is adjacent to *TSC2* on chromosome 16p13 and mutations disrupting both genes are associated with severe, early-onset polycystic kidney disease
      - Sometimes referred to as the *TSC2–PKD1* contiguous gene syndrome
  - Since hamartin and tuberin form a complex together, similar syndromic phenotypes are seen as a result of mutation of either gene
    - The TSC1/TSC2 complex regulates mTOR activity through RHEB, a RAS-like small GTPase
      - Loss of function of the complex by mutation of either gene leads to uninhibited cell growth signaling through the mTOR pathway
      - Targeted therapy with inhibitors of the mTOR pathway (rapamycin/sirolimus, everolimus) have shown promise in control of angiomyolipoma growth, though tumors potentially resume growth after cessation of therapy
    - TSC2 also regulates *VEGF* expression (a component of the VHL/HIF pathway)
- The majority of tumors demonstrate a benign clinical course; however, some cases show

progressive growth or outright aggressive behavior, suggesting that additional genetic events may be involved

Birt–Hogg–Dubé Syndrome

- The Birt–Hogg–Dubé syndrome is an autosomal dominant tumor syndrome with incomplete penetrance, associated with the development of the following:
  - Renal manifestations
    - Multicentric renal epithelial tumors with unusual features and varied histologic subtypes, even within the same kidney
      - Chromophobe carcinoma
      - Clear cell carcinoma
      - "Hybrid" tumors with overlapping features between chromophobe carcinoma and oncocytoma
        - A single tumor may include multiple subpopulations of cells, resembling both chromophobe carcinoma and oncocytoma
        - The renal cortex may include microscopic foci of oncocytic cells (oncocytosis)
      - Renal cysts
  - Nonrenal manifestations
    - Benign cutaneous tumors
      - Fibrofolliculomas
        - Circumscribed proliferation of specialized connective tissue surrounding dilated hair follicles
        - Forehead, scalp, face, neck
      - Trichodiscomas
      - Acrochordons
    - Pulmonary cysts
      - Spontaneous pneumothorax
    - Medullary carcinoma of the thyroid
- Associated with mutation of the *FLCN* or *BHD* gene at 17p11.2, which codes for folliculin, a 64-kD protein (579 amino acids) with proposed tumor suppressor function
  - More than 50 different germline mutations have been described
  - In the majority of cases, mutation results in truncation and a dysfunctional gene product
  - Folliculin interacting protein, FNIP1, is a partner of folliculin and interacts with 5′ AMP-activated protein kinase (AMPK)
    - AMPK negatively regulates mTOR activity, suggesting a role for these two signaling pathways in the development of the Birt–Hogg–Dubé syndrome
    - Upregulation of mitochondrial gene expression has been described with deregulation of the *PGC1α–TFAM* signaling axis

Constitutional Chromosome 3 Translocation

- Constitutional chromosome 3 translocation outside of the setting of VHL disease has also been found to be associated with an increased risk of developing bilateral, multifocal clear cell renal cell carcinoma
- A variety of break points involved in translocations or insertions have been described, including the following:
  - t(3:6): t(3:6)(p13:q25.1), t(3:6)(q12:q15), t(3;6)(q11;q13), and t(3;6)(q22;q16.1)
  - t(3;8): t(3;8)(p14;q24) and t(3;8)(p13;q24)
    - t(3;8)(p14;q24) results in disruption of *FHIT* and *TRC8* genes, both with proposed tumor suppressor function
  - t(3:4): t(3:4)(p13:p16) and t(3;4)(p13;p15)
    - t(3;4)(p13;p15) results in disruption of *KCNIP4* gene
  - t(2:3)
    - t(2:3)(q33:q21) disrupts *DIRC1* gene
    - t(2:3)(q35:q21) disrupts *DIRC2* and *DIRC3* genes
  - t(1:3)(q32:q13.3)
    - Disrupts *NORE1A* and *LSAMP* genes
  - t(3;15)(p11;q21)
  - t(3;12)(q13;q24)
  - ins(3;13)(p24.2;q32q21.2)
    - Also associated with development of lung and prostate cancers

- A "three-hit" model of carcinogenesis has been proposed, in contrast to the "two-hit" model of VHL disease
  - First: Germline balanced translocation
  - Second: Somatic loss of the chromosome 3 translocate
  - Third: Somatic mutation of the remaining *VHL* gene allele
  - Tumors often have later onset compared to VHL disease patients, perhaps due to longer time course of progression through the three events

SDHB Germline Mutations

- Similar to fumarate hydratase in HLRCC, succinate dehydrogenase is an enzyme involved in the Krebs cycle, whose mutations have been implicated in the development of tumors, particularly pheochromocytoma and paraganglioma
- Germline mutations of the subunits of succinate dehydrogenase (mitochondrial complex II genes) are associated with pheochromocytoma/paraganglioma syndromes (PHEO/PGL) *SDHB* gene (PGL4), *SDHC* (PGL3), and *SDHD* (PGL1); sometimes called "Carney–Stratakis" syndrome when combined with gastrointestinal stromal tumor (GIST)
  - Mutation of *SDHAF2* (formerly *SDH5*) may be the cause of PGL2 syndrome
- Renal manifestations
  - Renal tumors are seen in some patients, particularly those with *SDHB* mutations (estimated 14%)
  - Distinctive morphologic features include the following:
    - Bubbly eosinophilic cytoplasm with intracytoplasmic inclusions and indistinct cell borders
    - Cystic areas with a lobulated/circumscribed tumor border and entrapment of normal tubules/glomeruli
    - Some cases may be poorly differentiated or sarcomatoid
  - Prognosis appears to be good in nonsarcomatoid cases
- Nonrenal manifestations
  - Pheochromocytoma
  - Paraganglioma
  - GIST, so-called type 2 (*SDHB*-negative GIST)
- Immunohistochemical staining for *SDHB* can be performed and reveals a strong positive granular cytoplasmic reaction (mitochondrial) in normal tissues, with loss of staining in the associated tumors

Malignant Neoplasms

Clear Cell Renal Cell Carcinoma

- The most common subtype of renal neoplasm, comprising approximately 60–75% of surgically removed tumors
  - Believed to originate from cells of the proximal tubule
- Light microscopy
  - Tumor cells with variably abundant clear cytoplasm (due to loss of cytoplasmic lipid/glycogen during histologic processing) forming nested, tubular, and alveolar/cystic architecture with a prominent fine vascular network
  - Nuclei range from small and lymphocyte-like to large and hyperchromatic, with prominent large nucleoli and bizarre, irregular nuclear contours
  - Classification often readily accomplished based on the presence of typical clear cell histopathologic features
    - However, higher-grade tumors may have confounding morphology, such as the following:
      - Eosinophilic cytoplasm
      - Pseudopapillary architecture (loss of cohesive growth)
    - Application of molecular techniques may aid the differential diagnostic process in challenging cases
- Chromosome 3p
  - Deletion of chromosome 3p is believed to represent one of the key events in carcinogenesis

  - Seen in 70–90% of clear cell renal cell carcinoma cases
    - Detection by fluorescence in situ hybridization (FISH; Fig. 9.1a, b), LOH studies, or comparative genomic hybridization (CGH)
  - Less commonly found in other subtypes of renal neoplasms
  - 3p25, 3p12–14, and 3p21 frequently involved
  - Introduction of normal chromosome 3p into tumor cell lines results in suppression of tumorigenesis
- *VHL* gene
  - Specifically, *VHL* gene loss (at chromosome 3p25.3) is common in up to 75% of sporadic clear cell renal cell carcinoma cases (as seen in VHL disease, where germline inactivation of *VHL* is present)
  - Inactivation may occur through mutation, deletion, or abnormal DNA-methylation
  - In sporadic cases, one gene copy may be inactivated by mutation (as in patients with VHL disease) with the second copy lost through deletion in a similar "two-hit" model
    - Mouse xenografts of clear cell carcinoma with homozygous *VHL* loss of function mutation result in tumor formation
      - Introduction of wild-type *VHL* gene copies into the mice results in markedly decreased or absent tumor formation
    - However, additional steps beyond *VHL* mutation also appear to be required in models of tumor development
  - Prognostic significance of *VHL* inactivation is unclear
    - Nuclear and cytoplasmic expression of pVHL by immunohistochemistry associated with lower nuclear grade and lower tumor stage
  - Crucial in tumorigenesis via the *HIF* and mTOR pathways (*see* section "von Hippel–Lindau Disease")
  - Deletion of regions flanking the *VHL* gene suggests a role for potential additional tumor suppressor genes in the pathogenesis of clear cell carcinoma
- 3p14
  - In addition to abnormalities of the *VHL* gene, loss of *FRA3B*, the fragile site locus, is present in some tumor cell lines
    - *FRA3B* lies within the *FHIT* (fragile histidine triad) gene, located at 3p14.2, which is commonly deleted in sporadic clear cell renal cell carcinoma
      - Thought to represent an early event in the carcinogenesis of clear cell carcinoma, perhaps by *VHL*-independent mechanisms
- 3p12 and 3p21
  - Alterations of 3p12 and 3p21 are additional sites affected by LOH on chromosome 3p
    - Allelic loss or promoter hypermethylation of the RAS association domain family 1A gene (*RASSF1A*) has been implicated in clear cell carcinoma, a potential tumor suppressor gene located at chromosome 3p21.3
    - Additional genes
      - *DRR1* (downregulated in renal cell carcinoma), located at 3p21.1
      - *NRC1* (nonpapillary renal carcinoma 1), located at 3p12
- Other chromosomal regions affected in clear cell carcinoma
  - 5q
    - Second most common chromosomal region involved in clear cell renal cell carcinoma, after chromosome 3p
      - Allelic duplications at 5q31.1
      - Trisomy or partial trisomy of chromosome 5, including 5q22–qter
      - Chromosome 3 and 5 translocation, leading to loss of 3p13–pter and duplication of 5q22–qter
    - Gain of 5q may be associated with better prognosis in clear cell carcinoma
  - 9p and 14q
    - Loss is seen in a subset of cases and has been associated with poorer prognosis

- Associated with higher histologic grade and tumor stage
- 9p loss occurs primarily at 9p21 (the site of *p16*) and 9p22–23 (*PTCH* gene)
- 8q gain and LOH at 8p may also participate in tumor progression

– Other chromosomal sites sometimes involved in clear cell carcinoma
- 6q, 9q, 10q, 13p, 17p

• Multifocality
– Up to 25% of patients undergoing nephrectomy for renal cell carcinoma have multifocal tumors
- Some studies of clear cell carcinoma have found a common clonal origin by LOH analysis, raising the possibility of "satellite" tumors representing intrarenal metastases
- However, multifocality is not necessarily associated with increased risk of progression and metastasis, suggesting independent origin, perhaps by "field-effect"
- Other studies have found discordant patterns of 3p deletion, X chromosome inactivation, and LOH analysis, supporting independent origin in a significant number of cases (46%)

• Molecular differential diagnosis
– A spectrum of other renal neoplasms may have significant overlap in light microscopic appearance with clear cell carcinoma, leading to potential utility for molecular diagnostic studies
– Clear cell papillary renal cell carcinoma
- A recently described renal neoplasm with overlapping morphologic and immunohistochemical features of clear cell and papillary carcinoma (*see* section "Clear Cell Papillary Renal Cell Carcinoma")
- Thus far, clear cell papillary renal cell carcinoma has been found to lack chromosome 3p and *VHL* gene abnormalities, including promoter hypermethylation
- However, the *HIF* pathway may be upregulated by other mechanisms

– Xp11 translocation carcinoma
- Can include a prominent component of cells with clear cytoplasm, resembling clear cell carcinoma at the light microscopic level (sometimes intermingled with components including eosinophilic cytoplasm and papillary architecture; *see* section "Xp11 Translocation Carcinoma")
- Tumors characteristically harbor fusions of involving the *TFE3* gene at the Xp11.2 breakpoint, with a variety of fusion partners, including *ASPL*, *PRCC*, *PSF*, *NONO*, and *CLTC*
- By immunohistochemistry, epithelial markers are often underexpressed in translocation carcinoma
  - Although HIF1α expression may be present, the downstream target CA-IX is only focally present (6%)

• Implications of molecular genetic alterations on therapy
– Low expression of CA-IX by immunohistochemistry has been associated with poor clinical outcome and poor response to interleukin therapy
– Fragile histidine triad protein (FHIT) expression has been inversely correlated with tumor aggressiveness
- Reduced in carcinoma compared to adjacent uninvolved tubular epithelium

– Targeted therapy directed against the downstream pathways of *HIF1α* have shown promise in therapy, such as inhibitors of *VEGF* or, alternatively, inhibitors of the mTOR pathway

Papillary Renal Cell Carcinoma

• Papillary renal cell carcinoma is the second most common surgically removed renal tumor (approximately 10–15%), following clear cell carcinoma
– Believed to originate from cells of the proximal or distal convoluted tubule

- Light microscopy
  - Papillary renal cell carcinoma is characterized by a predominant pattern of tubulopapillary architecture, often including foamy macrophages within papillae and/or psammoma bodies
  - Immunohistochemical features include positivity for cytokeratin 7 and alpha-methylacyl-CoA racemase (AMACR), with less frequent expression of CA-IX in contrast to clear cell renal cell carcinoma
  - Type I
    - Papillae are lined by a single layer of smaller cells with pale cytoplasm and round to ovoid nuclei, imparting a basophilic overall appearance
    - Seen in the hereditary papillary renal carcinoma syndrome (*see* section "Hereditary Papillary Renal Carcinoma")
    - Associated with better prognosis and survival when compared to type II tumors
  - Type II
    - Larger, pseudostratified cells with more abundant eosinophilic cytoplasm
    - Similar tumors are seen in the setting of HLRCC, although characteristic, distinctive nuclear features may be present, distinguishing them from typical type II tumors (*see* section "Hereditary Leiomyomatosis and Renal Cell Carcinoma")
- Polysomy 7 and 17
  - Trisomy 7
    - Commonly present in papillary renal carcinoma, although a nonspecific finding
      - Seen in a number of human neoplasms, including those of epithelial, mesenchymal and neural origins
      - Also identifiable in benign conditions, such as nodular prostatic hyperplasia and benign renal epithelial cells
    - Approximately 75% of sporadic papillary carcinomas exhibit trisomy of chromosome 7 (Fig. 9.1c, d)
      - Contains genes for both *MET* and ligand *HGF*
      - However, activating *MET* mutation at chromosome 7q31 is only seen in a smaller proportion of sporadic type I papillary carcinoma cases, in contrast to tumors of the hereditary papillary renal carcinoma syndrome
  - Trisomy 17 (Fig. 9.1c, d)
    - Common in papillary carcinoma (80–90% by FISH)
    - Less commonly found in other human tumors and other subtypes of renal cell carcinoma
    - Characterized by the following:
      - Full trisomy of chromosome 17
      - Isochromosome 17q
      - Duplication of the 17q21–qter region (Fig. 9.5)
- Significant molecular differences have been reported between type I and type II papillary carcinomas, utilizing CGH methods and microsatellite studies
  - Type I
- Increased frequency of allelic imbalance on 17q and DNA gains of 7p and 17p
- More frequent trisomy 17
  - Type II
    - Increased frequency of allelic imbalance on 9p in type II cases
      - Deletion of 9p21 and allelic loss at D9S171 locus on 9p13 associated with tumor progression and shorter survival
    - Loss of chromosomes 1p and 3p and gain of 5q
  - Type I tumors (with fewer genetic alterations) have been hypothesized to evolve into type II tumors upon acquisition of additional chromosomal abnormalities
- Chromosome 3
  - Although abnormalities of chromosome 3p are generally associated with clear cell carcinoma, deletions are also less commonly seen in papillary carcinoma
  - Abnormalities involving 3q may be seen in both clear cell and papillary carcinoma
    - Unbalanced translocation with reduplication of the normal chromosome 3

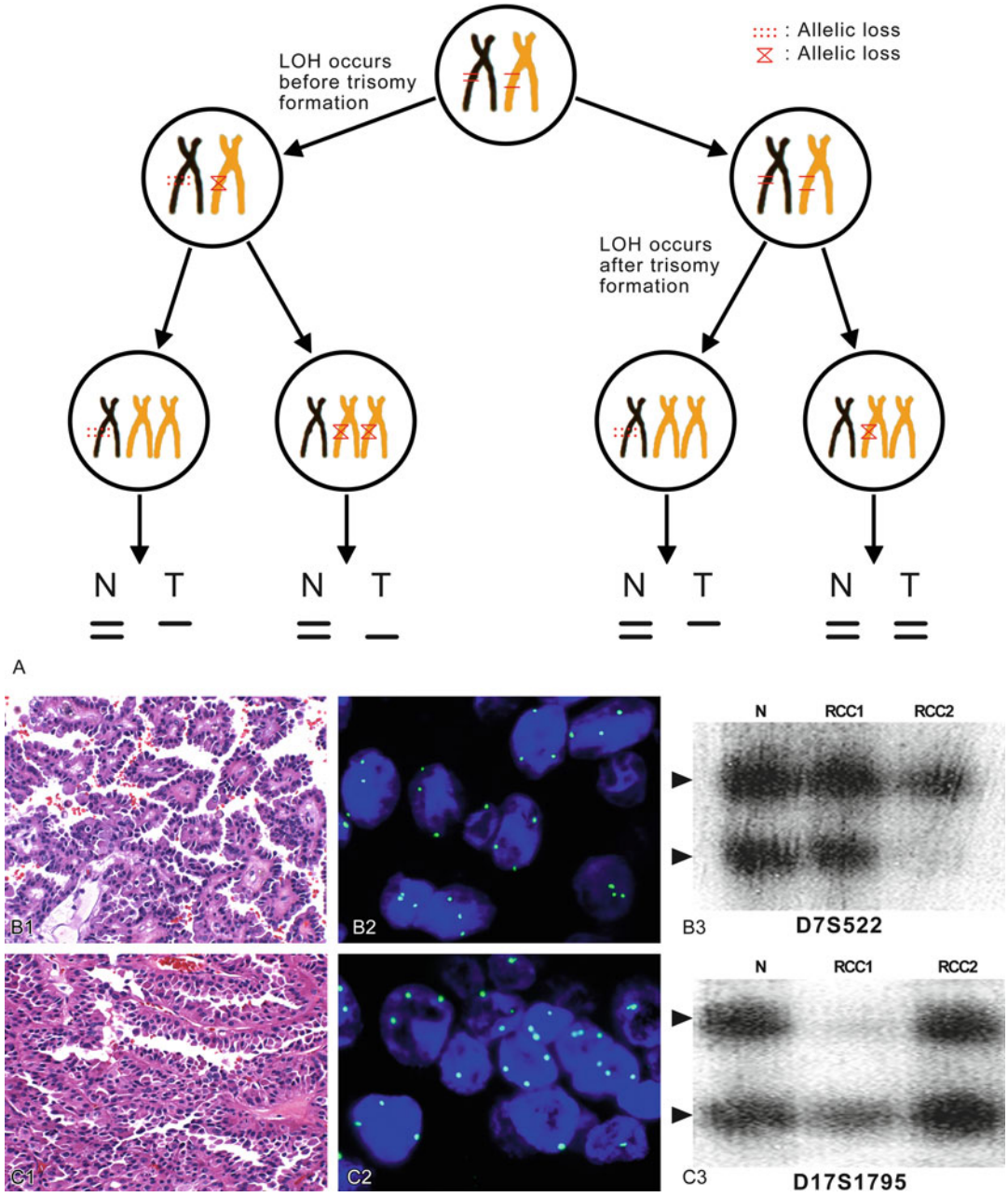

Fig. 9.5 (**a**) Proposed mechanism resulting in trisomic chromosomes 7 and 17. Acentromeric misdivision results in nondisjunction of the pair of chromosomes during cell mitosis and trisomic daughter cells. Loss of heterozygosity (LOH) could occur before trisomy formation (**a**, *left*) in which case allelic loss of either the upper or lower allele could occur (**a**, *bottom left*). LOH could occur after trisomy formation (**a**, *right*), in which case only allelic loss on the nonduplicated chromosome could be detected. Allelic loss on only one copy of the duplicated chromosome would not be detectable by LOH analysis (**a**, *bottom right*). (**b**, **c**) Histopathologic, FISH, and LOH features of papillary renal cell carcinoma. (**b1**–**b3** and **c1**–**c3**) Two cases of papillary renal cell carcinoma. (**b1** and **c1**) Papillary renal cell carcinoma composed of branching papillae and covered with a single layer of cells with eosinophilic cytoplasm. (**b2** and **c2**) FISH of the corresponding tumor with a centromeric probe of chromosomes 7 and 17 showing groups of tumor cells with trisomic chromosome 7 (**b2**) or 17 (**c2**; *green signals*). (**b3** and **c3**) Microsatellite analysis on chromosomes 7 (D7S522) and 17 (D17S1795) revealed different LOH patterns between different tumor foci (from Jones et al. 2005a; with permission)

resulting in partial trisomy of 3q is seen more often in papillary carcinoma
- Other chromosomal regions
  - Allelic loss at 7q31.1–31.2 is seen in some cases
    - Contains the aphidicolin-inducible fragile site FRA7G
      - Fragile sites are thought to be involved in tumorigenesis due to chromosomal breakage and resulting translocation, deletion, or amplification
  - Gains of 8, 12q, 16q, and 20q
    - Duplication of 20q11.2 and 20q13.2 thought to contain genes involved in development of papillary carcinoma
  - Loss of 1p, 4q, 6q, 11p, 13q, 14q, 18, 21q, X and Y
- Other genes
  - Leucine-rich repeat kinase 2 (*LRRK2*) has been found to be amplified and overexpressed in papillary renal cell carcinoma, particularly type I tumors
    - Downregulation of LRRK2 compromises MET activation and downstream signaling, suggesting a cooperative role with MET in tumor growth and survival of papillary carcinoma
- Multifocality
  - Multifocality is most common in papillary renal cell carcinoma, compared to other subtypes
    - However, concordant patterns of allelic loss are usually not seen between multifocal tumors (5%)
    - Multifocal and bilateral tumors may show cytogenetic heterogeneity between lesions, supporting independent origin, rather than intrarenal metastasis
- Molecular differential diagnosis
  - Clear cell papillary renal cell carcinoma
    - Areas of clear cell change are sometimes present within papillary renal cell carcinoma, bringing clear cell papillary renal cell carcinoma into the differential diagnosis by light microscopy, particularly in the setting of a small biopsy specimen
    - Copy number abnormalities of chromosomes 7 and 17 are sometimes seen in clear cell papillary renal cell carcinoma, although less frequently than papillary carcinoma (*see* section "Clear Cell Papillary Renal Cell Carcinoma")
    - Coexpression of CA-IX and CK7 is seen in clear cell papillary renal cell carcinoma, in contrast to papillary carcinoma, which usually lacks CA-IX expression and shows coexpression of CK7 and AMACR
  - Mucinous tubular and spindle cell carcinoma
    - Characterized by extensive tubular architecture with morphologic similarity to papillary carcinoma, particularly type I
    - However, gains of chromosomes 7 and 17 and loss of chromosome Y are not found
    - Multiple other genetic abnormalities are present including losses of chromosomes 1, 4, 6, 8, 9, 10, 12q, 13, 14, 15, 16q 17, 20q, and 22
  - Metanephric adenoma
    - Areas of solid growth in papillary renal cell carcinoma may show morphologic overlap with metanephric adenoma
    - In contrast, metanephric adenoma usually shows a normal karyotype by cytogenetics, with infrequent abnormalities of chromosomes other than 7 and 17, making molecular studies useful in resolving the differential diagnosis
  - Clear cell renal cell carcinoma with pseudopapillary change
    - Areas of dyshesive growth in clear cell carcinoma may impart a papillary appearance
    - FISH analysis for abnormalities of chromosome 7, 17, and 3p may be helpful in resolving the differential diagnosis, combined with other typical light microscopic features
  - Xp11 translocation carcinoma
    - Prominent papillary architecture is sometimes present in renal cell carci-

noma associated with Xp11 translocation
    - Nuclear labeling for TFE3 protein by immunohistochemistry or confirmation by molecular testing supports the diagnosis of translocation carcinoma
    - Chromosome 7, 17, and Y abnormalities absent
- Implications of molecular genetic alterations on therapy
  - *MET*: Use of tyrosine kinase inhibitors, targeting the tyrosine kinase domain of MET has been proposed as a potential therapy in both hereditary and sporadic papillary renal cell carcinoma

Chromophobe Renal Cell Carcinoma

- Less common than clear cell carcinoma or papillary carcinoma, comprising approximately 5% of renal tumors (similar in incidence to oncocytoma)
  - Believed to originate from intercalated cells of the collecting ducts
- Light microscopy
  - Chromophobe carcinoma is composed of solid nests, sheets, and trabecular bands of generally large, polygonal tumor cells with flocculent to eosinophilic cytoplasm and prominent ("plant cell"-like) cell borders
  - The eosinophilic variant of chromophobe carcinoma is composed predominantly of cells with eosinophilic cytoplasm, leading to considerable differential diagnostic overlap with oncocytoma and potential utility for molecular genetic studies
    - Both classic and eosinophilic areas may be intermixed within the same tumor
- Chromophobe carcinoma often exhibits multiple complex losses of chromosomes Y, 1, 2, 6, 10, 13, 17, and 21 by cytogenetics, restriction fragment length polymorphism (RFLP) analysis, CGH, FISH (Fig. 9.1e, f), and microsatellite analysis
  - The specific genetic abnormalities resulting from these changes are largely unknown
    - Although loss of 17 is seen, *TP53* gene mutation is seen in a minority of cases
    - Similarly, *PTEN* mutation is not seen, despite loss of chromosome 10
    - The folliculin (or *BHD*) gene is mutated in the setting of the Birt–Hogg–Dubé syndrome; however, such abnormalities are not seen in sporadic cases
  - In contrast to the multiple complex losses in chromophobe carcinoma, oncocytoma is characterized by usually very few abnormalities (sometimes rearrangement/translocation of 11q13, partial or complete loss of 1, 14, X, or Y)
    - The granular, eosinophilic cytoplasm in oncocytoma is associated with accumulation of mitochondria
      - Somatic mutation of mitochondrial DNA (mtDNA) can be found, resulting in decreased activity of the respiratory chain complex I
    - In contrast, chromophobe carcinoma has been found to have distinct heteroplasmic mtDNA mutations
      - Downregulation of ATP5A1, the alpha subunit of complex V of the respiratory chain, has been demonstrated by 2D gel electrophoresis of mitochondrial proteins
    - As such, the possibility that oncocytoma represents a precursor to chromophobe carcinoma has been entertained (with the eosinophilic variant constituting an intermediate form)
    - However, both classic and eosinophilic types show similar complex losses of multiple chromosomes, while oncocytoma more frequently has a normal karyotype of loss of chromosome 1
- LOH has also been identified at 9p23 (43%), 18q22 (30%), 5q22 (28%), and 8p (28%)
- Quantitative reverse transcription PCR (RT-PCR) studies reveal differential expression of *AP1M2*, *MAL2*, *PROM2*, *PRSS8*, and *FLJ20171* genes, leading to effective separation of chromophobe carcinoma from oncocytoma

  - By immunohistochemistry, MAL2 and CLDN8 staining highlights the distal nephron
  - CLDN8 staining is seen in both entities; however, MAL2 is limited to chromophobe carcinoma
- RT-PCR and immunohistochemistry studies identify *CD82* and *S100A1* as markers of chromophobe carcinoma and *AQP6* as a marker of oncocytoma
- Molecular differential diagnosis
  - Oncocytoma
    - Losses of chromosomes 2, 6, 10, and 17 by FISH studies may be helpful in excluding the diagnosis of oncocytoma and supporting the diagnosis of chromophobe carcinoma
    - Virtual karyotyping and microRNA expression studies have been proposed as a diagnostically useful modality for differentiating chromophobe carcinoma from oncocytoma and eosinophilic variants of clear cell carcinoma

Hybrid Oncocytic Chromophobe Tumor

- Hybrid tumors with overlapping morphologic features between oncocytoma and chromophobe carcinoma represent a controversial entity that has been described in the setting of the Birt–Hogg–Dubé syndrome and renal oncocytosis (*see* sections "Birt–Hogg–Dubé Syndrome" and "Renal Cell Neoplasms of Oncocytosis")
  - However, sporadic cases have also been reported, unassociated with either of these conditions
    - Tumors have been found to have frequent monosomy of chromosome 20 and multiple numerical aberrations (monosomy and polysomy) of chromosomes 1, 2, 6, 9, 10, 13, 17, 21, and 22
      - Although multiple complex losses of many of these chromosomes are seen in chromophobe carcinoma, monosomy of chromosome 20 in particular is unusual in chromophobe carcinoma and renal tumors in general
      - Likewise, multiple polysomies are not characteristic of chromophobe carcinoma, leading to the hypothesis that hybrid oncocytic chromophobe tumors represent a distinct neoplasm, separate from chromophobe carcinoma
    - Mutations of the *VHL*, *c-kit*, *PDGFRA*, and *FLCN* genes appear to be lacking in such sporadic cases
    - Although numbers of studied tumors are limited, they appear to have indolent behavior or low malignant potential

Sarcomatoid Renal Cell Carcinoma

- Sarcomatoid transformation in renal cell carcinoma represents an end stage of dedifferentiation and therefore does not constitute a histologic subtype of its own accord
  - May arise from clear cell, papillary, chromophobe, collecting duct carcinomas, unclassified renal cell carcinoma, etc.
  - Estimated 5–10% of renal cell carcinomas, variable between histologic subtypes
- Characterized by highly aggressive/malignant behavior and poorer response to therapy
- Although some molecular alterations are preserved in the epithelial and sarcomatoid components (such as chromosome 3p abnormalities in clear cell carcinoma with a sarcomatoid component), other alterations appear to be unrelated to the original neoplasm
  - Strong expression of clear cell carcinoma-specific markers by immunohistochemistry (such as HIF1α, CA-IX, and GLUT1) is often preserved in sarcomatoid cases, compared to sarcomatoid carcinoma originating from other subtypes of renal cell carcinoma (nonclear cell), which lack these markers (although VEGF expression may be present)
    - These markers may be of utility in establishing a clear cell carcinoma origin for challenging cases

  - X chromosome inactivation studies generally reveal similar patterns of X chromosome inactivation in clear cell carcinoma and its sarcomatoid component
    - However, LOH patterns may have significant differences between the two components in the same patient, supporting the hypothesis that tumors arise from the same progenitor cell but undergo genetic divergence over their evolution
  - Although typical chromophobe carcinoma (containing an epithelial component only) is characterized by multiple complex losses of Y, 1, 2, 6, 10, 13, 17, and 21, sarcomatoid cases have been found to have frequent gains of several of these chromosomes, including 1, 2, 6, 10, and 17, by FISH
    - DNA ploidy studies have demonstrated hypodiploid complements of chromosomes in the chromophobe carcinoma component and aneuploidy in the sarcomatoid component
  - Other studies have found little or no similarity in the pattern of genetic alteration between the epithelial and sarcomatoid components
  - Recent studies reveal E- to N-cadherin switching, dissociation of β-catenin from the cell membrane, and increased expression of Snail and secreted protein acidic and rich in cysteine (SPARC), supporting interpretation of sarcomatoid renal carcinoma as an example of epithelial–mesenchymal transition
    - Gain of mesenchymal characteristics is thought to be associated with increased ability to migrate and metastasize
- Although a great deal of variability exists in the pattern of genetic abnormalities in sarcomatoid components, molecular studies may be helpful in a subset of cases for determining the histologic subtype of the original carcinoma
- Implications of molecular genetic alterations on therapy
  - Expressions of c-kit and PDGFRA have been identified by immunohistochemistry in sarcomatoid and high-grade renal cell carcinoma; however, gene mutations are not found by molecular studies, failing to support use of tyrosine kinase inhibitor therapy

Multilocular Cystic Renal Cell Carcinoma

- Multilocular cystic renal cell carcinoma is a unique variant of renal cell carcinoma with excellent prognosis, characterized histologically by multiple cystic spaces lined by clear cells, generally with low nuclear grade and small aggregates of clear cells within the fibrous septa
  - Aggregates of clear cells do not expand the septa or otherwise form a significant solid component, differentiating the lesion from clear cell carcinoma with a cystic component
- Although molecular studies in this uncommon variant are limited in number, the presence of chromosome 3p deletion in a significant number of cases (74%) supports its interpretation as a variant of clear cell carcinoma
  - *VHL* gene mutation has been identified in approximately 25% of cases, somewhat lower than the rates found in clear cell carcinoma in general, perhaps due to difficulty in obtaining cellular areas for analysis
  - Similar expressions of PAX2, pGSK3β, PTEN, and CA-IX are identifiable by immunohistochemistry, supporting a similar pathogenesis to that of clear cell carcinoma
    - However, strong nuclear expression of p27 is preserved in multilocular cystic renal cell carcinoma, suggesting an area of distinction from clear cell carcinoma

Clear Cell Papillary Renal Cell Carcinoma

- Clear cell papillary (or tubulopapillary) renal cell carcinoma is a recently described neoplasm,

characterized by tubular/ductular, cystic, and branched papillary architecture, composed of cells with clear cytologic features and generally low nuclear grade
  - Tumors were originally described in the setting of endstage renal disease and acquired cystic kidney disease, but may occur in kidneys unaffected by these abnormalities
  - By immunohistochemistry, tumors express cytokeratin 7 and CA-IX (generally lacking staining for AMACR and CD10), a phenotype that overlaps between clear cell renal cell carcinoma and papillary renal cell carcinoma
- Molecular studies have found abnormalities of 3p or the *VHL* gene (including promoter hypermethylation) to be absent in clear cell papillary renal cell carcinoma, supporting distinction from clear cell carcinoma
  - However, coexpression of CA-IX, HIF1α, and GLUT1 suggests upregulation of the HIF pathway by a non–VHL-dependent mechanism
- Array CGH studies have failed to reveal a characteristic chromosomal imbalance for the unique tumor
- A minority of cases show low copy number gains of chromosome 7 and/or 17, in contrast to the more frequent gains in papillary carcinoma
  - Recent studies of the genomic profile of renal cell carcinoma in endstage renal disease, including clear cell papillary renal cell carcinoma, by analysis of genomic copy number aberrations reveal similar genomic profiles to papillary renal cell carcinoma
- Molecular differential diagnosis
  - Clear cell renal cell carcinoma
    - Perhaps the most likely entity to be considered in the differential diagnosis, due to the prominent clear cell cytology and areas of compact tubular/ductular growth, resembling and sometimes nearly identical to the solid areas of clear cell carcinoma
    - Absence of 3p or *VHL* gene abnormalities by molecular methods may be helpful in supporting the diagnosis of clear cell papillary renal cell carcinoma for challenging cases, combined with the coexpression of cytokeratin 7 and CA-IX by immunohistochemistry
  - Papillary renal cell carcinoma
    - May also be considered in the differential diagnosis of clear cell papillary renal cell carcinoma, particularly when the branched papillary component is prominent
    - Papillary carcinoma cases may have areas of clear cell change that may raise the possibility of clear cell papillary renal cell carcinoma, particularly in biopsy specimens
    - Although a specific genetic alteration to differentiate the two lesions is lacking, papillary carcinoma more frequently exhibits gains of chromosomes 7 and 17, compared to clear cell papillary carcinoma, in which low copy-number gains of these chromosomes are reported in a minority of cases
  - Multilocular cystic renal cell carcinoma
    - May be a significant consideration in the differential diagnosis, as clear cell papillary renal cell carcinoma may have a very prominent cystic component with only a minimum of ductular/tubular architecture and small, focal branched papillae
    - Similar to clear cell carcinoma in general, 3p abnormalities by FISH can be detected in the majority of cases of multilocular cystic renal cell carcinoma, while they have not been demonstrated in clear cell papillary renal cell carcinoma

Mucinous Tubular and Spindle Cell Carcinoma

- Mucinous tubular and spindle cell carcinoma is a unique neoplasm with distinctive morphologic features, including small cuboidal and

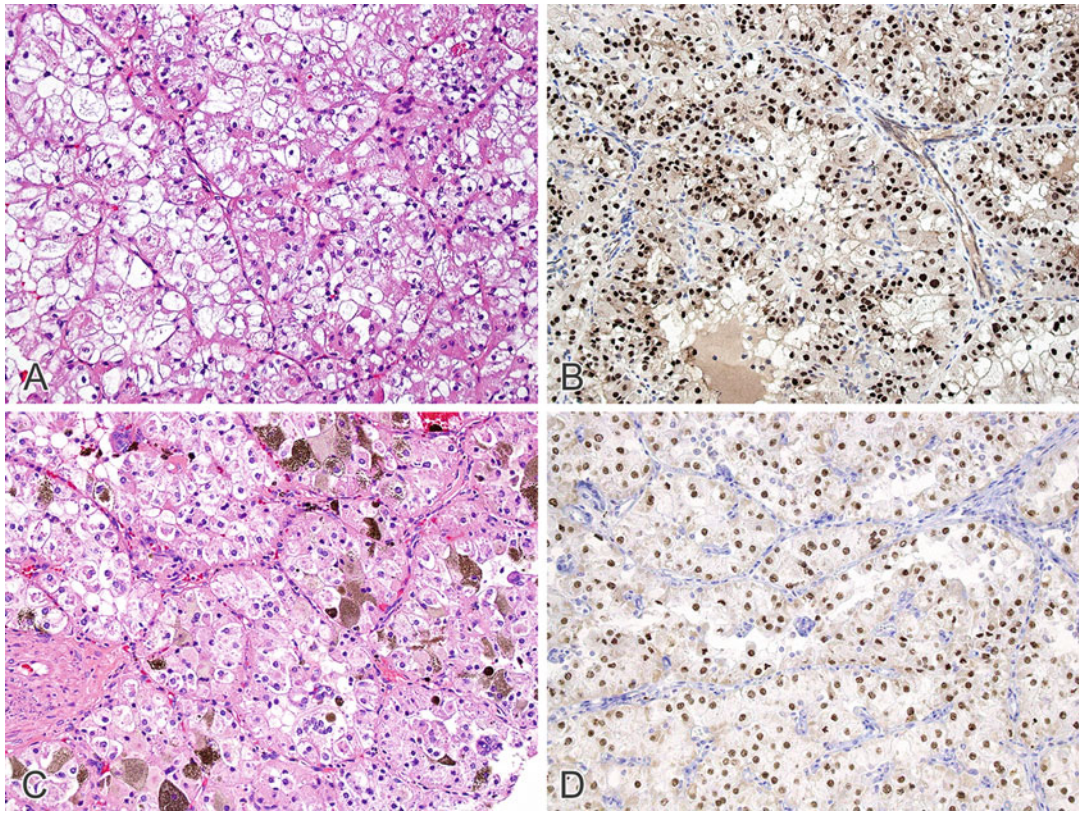

Fig. 9.6 (**a**) Xp11 translocation carcinoma, characterized by a mixed population of cells with clear and eosinophilic cytoplasm. Strong nuclear overexpression of TFE3 protein can be detected by immunohistochemistry (**b**). (**c**) Melanotic Xp11 translocation carcinoma, demonstrating scattered cells with prominent cytoplasmic pigmentation and (**d**) nuclear overexpression of TFE3 by immunohistochemistry

spindle cells, arranged in elongated tubules or sheets with a mucinous background
  - Lesions are generally low grade and low stage with infrequent metastasis
- Significant morphologic overlap with type I papillary renal cell carcinoma has raised a challenging differential diagnosis and speculation that the two entities are related
  - However, gains of chromosomes 7 and 17 and losses of chromosome Y are generally lacking by FISH studies
  - CGH and cytogenetics have demonstrated losses of chromosomes 1, 4, 6, 8, 9, 13, 14, 15, and 22 and gain of chromosomes 12q, 16q, 17, and 20q in smaller numbers of cases, suggesting that complex karyotypic abnormalities are present in the tumor

Xp11 Translocation Carcinoma

- Renal carcinomas associated with Xp11.2 translocation comprise a spectrum of tumors with unique light microscopic features and specific translocations involving the *TFE3* transcription factor gene (transcription factor binding to IGHM enhancer 3), located at chromosome Xp11.2 (Fig. 9.6a, b)
- Xp11 translocation carcinoma is thought to be the most common subtype of renal cell carcinoma in children, more frequently seen in children and young adults
  - Makes up a small percentage of renal tumors in adults (estimated 1% or less), in whom other subtypes of renal cell

carcinoma are seen with much greater frequency
    - ♦ However, gene fusion may be undetected in a subset of adult cases, which shows extensive morphologic similarity with clear cell renal cell carcinoma or papillary renal cell carcinoma
    - ♦ Overall numbers of cases in adults may, therefore, be greater than those in children
- The *TFE3* gene is a member of the microphthalmia transcription factor/transcription factor E (MITF–TFE) family, which also includes *TFEB*, *TFEC*, and *MiTF* (involved in melanocyte development)
    - ◦ Such genes have been implicated in various tumors, such as melanoma, clear cell sarcoma, alveolar soft part sarcoma, and some perivascular epithelioid cell neoplasms (PEComa)
    - ◦ Pigmented Xp11 translocation carcinomas with melanin have also been described, so-called "melanotic Xp11 translocation renal cancer" (Fig. 9.6c, d)
- A variety of fusion partners have been implicated in the translocations with *TFE3*, including the following:
    - ◦ *ASPL* gene—t(X;17)(p11.2;q25)
        - ♦ *ASPL–TFE3* gene fusion (unbalanced) is seen also in alveolar soft part sarcoma
            - ▪ *ASPL–TFE3* fusion protein appears to transactivate the *MET* promoter, increasing *MET* mRNA expression with associated high levels of MET protein by IHC and Western blot methods
    - ◦ *PRCC* gene—t(X;1)(p11.2;q21)
    - ◦ *PSF* gene—t(X;1)(p11.2;p34)
    - ◦ *NONO* gene—inv(X)(p11;q12)
    - ◦ *CLTC* gene—t(X;17)(p11.2;q23)
    - ◦ Other fusions with undetermined gene partners, including the following:
        - ♦ t(X;3)(p11.2;q23)
        - ♦ t(X;19)(p11.2;q13.1)
        - ♦ t(X;10)(p11.2;q23)
- Evidence of *TFE3* gene fusion can be demonstrated by immunohistochemical nuclear labeling for the TFE3 protein (Fig. 9.6b, d)
    - ◦ Cathepsin K has been reported to be another sensitive and specific marker by immunohistochemistry

- Light microscopy
    - Tumors may show variable admixtures of cells with clear cytoplasm and/or eosinophilic cytoplasm, as well as papillary and nested/solid architecture, sometimes with psammoma bodies
    - In contrast to other types of renal cell carcinoma, markers of epithelial differentiation are less frequently expressed by immunohistochemistry, such as epithelial membrane antigen (EMA) or cytokeratin
        - ◦ Similarly, decreased expression of vimentin can be seen
    - Tumors associated with *ASPL–TFE3* gene fusion tend to exhibit a characteristic light microscopic appearance including voluminous clear cytoplasm with nested and papillary architecture
    - In contrast, tumors with *PRCC–TFE3* fusion tend to demonstrate more compact architecture with less abundant clear cytoplasm
- Molecular differential diagnosis
    - Clear cell renal cell carcinoma
        - ◦ A significant component of clear cell cytology may raise the differential diagnosis between clear cell carcinoma and Xp11 translocation carcinoma
        - ◦ Underexpression of typical markers of clear cell carcinoma by immunohistochemistry, such as cytokeratin, EMA, and vimentin, coupled with nuclear expression of TFE3 protein may aid in resolving the differential diagnosis
        - ◦ FISH studies utilizing a breakapart probe for the *TFE3* gene have been proposed as a useful molecular tool for verification
    - Papillary renal cell carcinoma

- Papillary architecture, psammoma bodies, and variable amounts of eosinophilic cytoplasm may also raise consideration for papillary carcinoma
- Similarly, molecular studies including TFE3 evaluation and common chromosomal abnormalities for papillary carcinoma (7, 17, Y) may be helpful, combined with expression of TFE3 protein by immunohistochemistry and other typical markers of papillary carcinoma

- Implications of molecular genetic alterations on therapy
  - Due to transactivation of the *MET* promoter by the *ASPL–TFE3* fusion protein, therapy directed against the MET tyrosine kinase has been proposed as a potential therapeutic strategy
  - Elevated expression of phosphorylated S6 in Xp11 translocation renal cell carcinoma suggests the mTOR pathway as another potential therapeutic target

Renal Cell Carcinoma Associated with t(6;11)

- Renal cell carcinomas associated with t(6;11) (p21.1;q12–13) have also been recently described (also called *TFEB*-associated renal neoplasm)
  - Seen preferentially in young patients, although cases in the sixth decade or later have also been reported
  - Many tumors have shown generally indolent behavior, although metastases and aggressive behavior with death of disease have been reported in a smaller subset of cases
  - Light microscopy
    - Morphologic features may show significant overlap with other subtypes of renal cell carcinoma, such as clear cell carcinoma or Xp11 translocation carcinoma, including nested or solid growth with eosinophilic, granular, or clear cytoplasm
    - A characteristic second population of smaller cells with dense nuclear chromatin and less abundant cytoplasm, centered around collections of hyaline (basement membrane) material, is variably prominent
    - Coexpression of HMB45 and Melan A may bring epithelioid angiomyolipoma into the differential diagnosis
- At the molecular genetic level, tumors show fusion of the 5′ aspect of the *MALAT1 gene* (also known has Alpha) at 11q12, with the *TFEB* gene at 6p21
  - Significant upregulation of TFEB protein levels results from promoter substitution, likely leading to changes in expression of downstream genes
  - Similar to the TFE3 protein expression seen in Xp11 translocation carcinoma, immunohistochemical staining for nuclear expression of the TFEB protein may be helpful in establishing the diagnosis of renal cell carcinoma associated with t(6;11)
    - As the *MALAT1* gene lacks introns, both RT-PCR and DNA PCR have been utilized in confirmation of the gene fusion
    - Cathepsin K has been reported to have specificity in labeling these tumors (in addition to *TFE3*-positive Xp11 translocation carcinomas)
  - A few tumors have also been found to have losses of chromosomes 1 and 22
- Molecular differential diagnosis
  - A number of entities may be considered in the differential diagnosis of carcinomas associated with t(6;11), such as clear cell renal cell carcinoma, angiomyolipoma (and epithelioid angiomyolipoma), and Xp11 translocation carcinoma (particularly tumors associated with *ASPL–TFE3* fusion)
  - Molecular studies may be useful in resolving this differential diagnosis, combined with the unique light microscopic features, and immunohistochemical expression of melanocytic markers and TFEB protein

Tubulocystic Carcinoma

- Tubulocystic carcinoma is an uncommon renal neoplasm, characterized microscopically by tubular and cystic architecture, lined by a single layer of cells with eosinophilic cytoplasm, prominent nucleoli, and hobnail cells
- Relatively little is known about the molecular genetics of these unusual lesions
  - Gene expression profiling has demonstrated a unique molecular signature for tubulocystic carcinoma
  - The variable presence of chromosomal gains of 7 and 17 with loss of Y has led some authors to consider that the lesion bears a close relationship with papillary renal cell carcinoma (*see* section "Papillary Renal Cell Carcinoma")
    - Along these lines, the gene expression profile has been found to be most closely related to papillary carcinoma by some investigators
  - Alternative hypotheses have proposed that tubulocystic carcinoma represents a low-grade collecting duct carcinoma; however, gene expression profiles show significant differences between the two entities

Acquired Cystic Disease-Associated Renal Cell Carcinoma

- Patients with endstage renal disease and acquired cystic kidney disease are prone to development of various types of renal cell carcinoma, including clear cell carcinoma, papillary carcinoma, and clear cell papillary renal cell carcinoma
  - However, acquired cystic disease-associated renal cell carcinoma appears to represent a unique tumor subtype in this setting with distinctive histologic and possibly molecular features
- Light microscopy
  - Unique features in these tumors include abundant eosinophilic cytoplasm, variable solid, cribriform, tubulocystic, and papillary architecture
  - Deposits of calcium oxalate crystals are a characteristic and distinctive feature
- At the molecular genetic level, FISH studies have revealed the following:
  - Gains of chromosomes 1, 2, and 6, with or without gains of 10, or normal complements of these chromosomes (in one case)
  - Mixed trisomy and monosomy for chromosomes 3 and 16, with additional monosomy of chromosome 9 in the setting of sarcomatoid change
- Another case revealed gains of chromosomes 3, 7, 16, and X, and loss of Y by cytogenetics
- These molecular genetic characteristics suggest distinction of these unusual tumors from other well-known subtypes of renal cell carcinoma
- Further investigation of the molecular genetic characteristics may better characterize the pathogenesis of these neoplasms and the ability to establish the diagnosis by molecular genetic methods

Collecting Duct Carcinoma

- Collecting duct carcinoma (or carcinoma of the collecting ducts of Bellini) is a rare but highly aggressive renal neoplasm
  - Believed to originate from cells of the distal nephron/collecting duct epithelium
- Light microscopy
  - Tumors are characterized by trabecular, papillary, solid, and glandular architecture, with extensively infiltrating growth
- At the molecular genetic level, cytogenetic studies have found monosomy of chromosomes 1, 6, 14, 15, and 22
  - Frequent allelic loss has been identified on chromosomal arms 1q, 6p, 8p, 13q, and 21q
    - LOH on 1q is observed in two-thirds of cases, and LOH at 6p, 8p, 13q, and 21q have also been reported
    - Deletion of 1q32.1–32.2 was identified in 57–69% of collecting duct carcinomas, suggesting that this region may contain a tumor suppressor gene involved in carcinogenesis

    - As loss of chromosome arm 8p in clear cell renal cell carcinoma has been associated with high stage and aggressive behavior, this region may be important in the aggressive behavior of collecting duct carcinoma
        - Generally, losses of chromosome 3p are not seen in collecting duct carcinoma, although *VHL* allelic loss has been reported in some cases
    - Allelic loss of 9p, seen frequently in urothelial carcinoma, shows variable results in collecting duct carcinoma, ranging from absent to 50% of cases
- Loss of tumor suppressor gene *RB* (retinoblastoma) has also been reported to be present in a significant number of cases
- Expression of MET by immunohistochemistry represents an area of similarity between collecting duct carcinoma, urothelial carcinoma, and papillary carcinoma
- Recent studies have found loss of INI1 expression by immunohistochemistry in 15% of collecting duct carcinoma, suggesting possible alterations of the *INI1* gene (*SNF5/BAF47/SMARCB1*), although only a small number of cases have shown this finding
- Gene expression profile studies comparing collecting duct carcinoma with tubulocystic carcinoma reveal differential expression of selected genes, including *VIM*, *TP53*, and *AMACR*, supporting the interpretation that the two entities are distinct
- Molecular differential diagnosis
  - Urothelial carcinoma
    - Urothelial carcinoma may have considerable overlap with collecting duct carcinoma at the microscopic level
    - In general, gains of chromosomes 3, 7, and 17 and loss of 9p21 (as tested with the UroVysion probe set) are seen preferentially in urothelial carcinoma rather than collecting duct carcinoma; however, some overlap in the genetic characteristics of the two tumors is possible (such as variable allelic loss at 9p)
    - Collecting duct carcinomas show complex losses of 1, 6, 14, 15, and 22
  - Medullary carcinoma
    - Differentiation of medullary carcinoma from collecting duct carcinoma may be challenging; some authors have suggested that the two entities are related
    - Loss of expression of INI1 has been identified in medullary carcinoma and rarely in collecting duct carcinoma, possibly representing an area of distinction
  - Papillary renal cell carcinoma
    - Particularly in high-grade cases of papillary renal cell carcinoma, differentiation from collecting duct carcinoma may be aided by molecular studies for trisomy of chromosomes 7, 17, and Y

Renal Medullary Carcinoma

- Renal medullary carcinoma is a rare, aggressive malignancy, prone to affect individuals with sickle cell trait, although patients without hemoglobinopathies have also been reported
- Light microscopy
  - Histologic features of renal medullary carcinoma include tubular or cribriform architecture with marked desmoplasia and an acute inflammatory reaction
  - Cells are often eosinophilic, with prominent nucleoli
  - Sarcomatoid features are sometimes present
  - Overlap in appearance with collecting duct carcinoma raises the possibility that the two entities are related
- Loss of expression of INI1 protein by immunohistochemistry has recently been identified in renal medullary carcinoma, representing a point of similarity to rhabdoid tumor of the kidney and distinction from other renal tumors (such as urothelial carcinoma and other subtypes of renal cell carcinoma)
  - INI1 expression is also retained in renal cell carcinomas with a rhabdoid morphology
- Otherwise, molecular genetic abnormalities in renal medullary carcinoma are heterogeneous, with individual cases found to variably exhibit the following:
  - Loss of chromosome 22 by CGH

  - t(9;22) and t(10;16) with *BCR–ABL* gene rearrangement by FISH
  - Fusion between the genes for cytoskeletal protein vinculin (*VCL*) and *ALK*
  - Double (germline and somatic) mutations for the *FH* gene
    - Also involved in the HLRCC syndrome
  - Double somatic mutations in the *VHL* gene
    - Involved in the VHL syndrome and sporadic clear cell renal cell carcinoma
  - *VHL* gene promoter hypermethylation (epigenetic silencing)
  - Increased expression of HIF1α by immunohistochemistry
- In combination, these findings have led to the hypothesis that renal medullary carcinoma may be related to hypoxic conditions (as in sickle cell hemoglobinopathy) or mutations that affect the hypoxia-sensing pathways and contribute to HIF1α signaling (*VHL*, *FH*, and others)
- Implications of molecular genetic alterations on therapy
  - Studies utilizing whole genome expression analysis revealed increases of topoisomerase II (TopoII) with deregulation of DNA remodeling and repair
    - Utilization of TopoII-inhibiting agents shows potential for treatment of metastatic renal medullary carcinoma

Unclassified Renal Cell Carcinoma

- Unclassified renal cell carcinoma, as the name suggests, is a diagnostic category for tumors which fail to fit well into one of the known subtypes of renal cell carcinoma
  - Features that may contribute to this inability to definitively classify a tumor include the following:
    - A mixture of microscopic features of two or more distinct subtypes of renal neoplasm (mixed papillary and solid/tubular architecture or overlapping cytologic features of more than one subtype)
    - Oncocytic or granular, eosinophilic cytoplasm (which may be seen as a common endpoint in several renal cell carcinoma subtypes, as well as oncocytoma)
    - Spindle cell component (such as a sarcomatoid carcinoma without overtly identifiable features of a particular epithelial subtype)
    - Other unusual features in a tumor that appears to be of primary renal origin
  - Not surprisingly, molecular diagnostic studies may aid in resolving the differential diagnosis for such cases
    - Virtual karyotyping with single nucleotide polymorphism (SNP) microarrays has been proposed as a diagnostically practical method, often able to successfully categorize otherwise challenging cases
    - Other methods, such as FISH, for the characteristic chromosomal abnormalities of common renal neoplasms may be helpful (such as chromosome 3p, 7, 17, Y, and others)

Other Tumors

Epithelioid Angiomyolipoma and Other Renal PEComas

- In addition to angiomyolipoma, other members of the PEComa family of tumors affecting the kidney include epithelioid angiomyolipoma and a number of other variants, such as the following:
  - Microscopic angiomyolipoma and intraglomerular angiomyolipoma
  - Angiomyolipoma with epithelial cysts
  - Oncocytoma-like angiomyolipoma
  - Lymphangioleiomyomatosis of the renal sinus
- PEComas are considered to originate from the perivascular epithelioid cell (PEC), a unique cell type without a known normal counterpart
  - Notable for coexpression of markers of myogenic and melanocytic differentiation, suggesting a neural crest origin or acquisition of melanocytic expression through translocation or mutational event

- Epithelioid angiomyolipoma and other PEComas may be seen sporadically and also in the context of tuberous sclerosis (*see* section "Tuberous Sclerosis")
  - LOH of the *TSC2* gene has been reported in some cases of sporadic epithelioid angiomyolipoma, with variable frequency
  - Sporadic cases of renal angiomyolipoma and PEComa of other organs show activation of the mTOR pathway, with expression of phospho-S6 kinase and phospho-S6
    - Other genetic abnormalities besides disruption of the *TSC* genes may be involved in the pathogenesis of PEComas
  - Losses of chromosomes 1p, 17p, 18p, and 19 and gains of 2q, 3q, 5q, 12q, and X have been identified in renal angiomyolipoma with a similar distribution of abnormalities in PEComa

Adult Nephroblastoma (Wilms Tumor)

- Occurrence of nephroblastoma or Wilms tumor in adult patients is unusual (approximately 3% of cases), showing similar light microscopic features to those of pediatric patients
  - Recent studies using high-resolution genomic analysis revealed more pronounced genetic complexity than seen in pediatric cases, suggesting its distinct biological status compared to pediatric tumors
    - Uniparental disomies of the majority of chromosomes
    - Microdeletions of genes involved in tumor formation (*LRP1B*, *FHIT*, and *WWOX*) and organogenesis (*NEGR1* and *ZFPM2*)
  - In contrast, allelic loss patterns have revealed similar abnormalities by RFLP in both adult and pediatric patients

Carcinoid Tumor/Neuroendocrine Carcinoma

- Carcinoid tumor and neuroendocrine carcinoma (small cell carcinoma) of the kidney are uncommon neoplasms with similar morphologic features to neuroendocrine tumors seen in other organs
  - Histogenesis of neuroendocrine tumors in the kidney is not completely clear; hypotheses have included the following:
    - Neuroendocrine differentiation of a primitive totipotential cell line
    - Metastasis from an occult primary tumor elsewhere
    - Misplaced progenitor cells or teratomatous cells
      - Carcinoid tumor has been reported in a number of cases in association with horseshoe kidney or renal teratoma
      - Tumors have been found to generally lack reactivity for PAX2 and PAX8 by immunohistochemistry, in contrast to other tumors of renal origin
    - In small cell carcinoma, frequent association with other urothelial carcinoma components supports a multipotent urothelial stem cell as a potential origin rather than intrinsic urinary tract neuroendocrine cells
  - Relatively few studies have investigated renal carcinoid tumors at the molecular genetic level
    - FISH for translocation involving the *EWSR1* gene has been suggested as a helpful marker in distinguishing renal carcinoid tumor from primitive neuroectodermal tumor (PNET)
    - In individual cases, tumors have been found to exhibit chromosome 3p abnormalities, numerical/structural aberrations of chromosome 13, or a normal karyotype

Primitive Neuroectodermal Tumor

- PNET may sometimes arise primarily within the kidney (peripheral PNET or Ewing sarcoma)
  - The majority of tumors show the translocation t(11;22)(q24;q12) with a fusion transcript

between the *EWS* gene (22q12) and the *ETS*-related oncogene, *FLI1* (11q24)
    - 85–95% of cases, depending on method
  - Variant translocations with *EWS* include other *ETS*-related oncogenes: (*ERG* at 21q22), (*E1AF* at 7p22), (*FEV* at 2q33), and (17q12)
- Molecular differential diagnosis
  - Differential diagnosis can be challenging, including the following:
    - Intrarenal neuroblastoma
    - Carcinoid tumor
    - Neuroendocrine carcinoma/small cell carcinoma
    - Lymphoma
    - Wilms tumor (particularly blastemal predominant cases)
  - Although immunohistochemical staining has largely been considered useful in resolving difficult cases (positive for CD99, FLI1, and negative for WT1), molecular methods are recommended in many cases, as considerable overlap may be present
    - RT-PCR for the *EWS–FLI1* fusion transcript and/or breakapart FISH probe for *EWSR1* may be used to confirm the diagnosis of renal PNET

Urothelial Carcinoma

- Urothelial carcinoma (transitional cell carcinoma) in the upper urinary tract largely shows similar histologic features to tumors arising in the bladder
- In some cases, urothelial carcinoma may extensively infiltrate the kidney, raising a challenging clinicopathologic differential diagnosis with other malignancies, such as high-grade papillary renal cell carcinoma, medullary carcinoma, collecting duct carcinoma, and metastatic carcinoma to the kidney
  - Urothelial carcinoma frequently exhibits gains of chromosomes 3, 7, and 17 and loss of 9p21
    - FISH probes for abnormalities of these chromosomes (UroVysion) may support a diagnosis of urothelial carcinoma
    - However, other tumors involving the urinary tract may also show positivity with the probe set
      - Primary bladder tumors: squamous cell carcinoma, adenocarcinoma, and urothelial carcinoma with squamous differentiation
      - Primary renal tumors: clear cell, papillary, chromophobe, and sarcomatoid renal cell carcinomas
      - Secondary tumors: adenocarcinoma of colonic, prostatic, and cervical origin
- Other characteristic genetic abnormalities in urothelial carcinoma include the following:
  - Mutation of the *FGFR3* gene in superficial papillary neoplasms, associated with frequent recurrence and less frequent progression to invasion
  - Mutation of *TP53* in high-grade, invasive urothelial carcinomas
- Familial cancer syndromes
  - In the setting of hereditary nonpolyposis colorectal cancer (HNPCC) syndrome (Lynch syndrome), patients are predisposed to various tumors other than colorectal neoplasms
  - Other sites of involvement include endometrium, ovary, small bowel, stomach, hepatobiliary, skin, brain, and urinary tract (particularly the upper urinary tract)
  - The HNPCC syndrome involves the mutation of mismatch repair genes, including *MLH1*, *MSH2*, *MSH6*, and *PMS2*
    - Defective DNA mismatch repair leads to more rapid accumulation of errors in microsatellite regions
- Molecular differential diagnosis
  - Collecting duct carcinoma
    - Complex losses of chromosomes 1, 6, 14, 15, and 22 with absence of allelic loss at 9p may support a diagnosis of collecting duct carcinoma, though some overlap may exist with urothelial carcinoma
  - Medullary carcinoma
    - Loss of expression of INI1 by immunohistochemistry is seen in renal

medullary carcinoma, a feature that may be of diagnostic utility in differentiating tumors from urothelial carcinoma
  - Papillary renal cell carcinoma
    - Uncommonly, papillary renal cell carcinoma may show a high-grade, infiltrative tubular growth pattern with areas that may mimic urothelial carcinoma, particularly in the setting of biopsy specimens
    - Presence of the characteristic numerical abnormalities of chromosomes 7, 17, or Y may be helpful in supporting a diagnosis of papillary carcinoma

Benign Neoplasms

Angiomyolipoma

- Angiomyolipoma is a benign neoplasm and the most common tumor of the PEComa family, composed histologically of variable amounts of smooth muscle, adipose tissue, and blood vessels
  - Tumors show positivity for both markers of smooth muscle and melanocytic origin
- Represents a primary manifestation of the TSC, seen in 60–80% of patients (*see also* section "Tuberous Sclerosis")
  - Also found sporadically in approximately 1 in 300 individuals without TSC
    - Approximately 80% of patients with angiomyolipoma do not have TSC
    - Usually unifocal, compared to bilateral and multifocal involvement in TSC patients
- Proposed to arise from a renal mesenchymal precursor cell
  - RT-PCR detection of mRNA for gp100 (the antigenic target of HMB45) can be detected in low levels in proximal and distal tubules of the normal kidney
- Losses of chromosomes 1p, 17p, 18p, and 19 and gains of 2q, 3q, 5q, 12q, and X have been identified in renal angiomyolipoma, similar to the abnormalities seen in PEComa
- Similar to tuberous sclerosis, loss of *TSC2* has been identified in sporadic angiomyolipoma; however, frequency has been variable, ranging from 10% to 50%
  - By immunohistochemical methods, tumors show activation of the mTOR pathway with expression of phospho-S6 kinase and phospho-S6
- Implications of molecular genetic alterations on therapy
  - Targeted therapy with mTOR pathway inhibitors (rapamycin/sirolimus, everolimus) have shown potential for controlling angiomyolipoma growth
  - However, concern for resumption of tumor growth after cessation of therapy has been raised

Oncocytoma

- Renal oncocytoma is a benign neoplasm, characterized by a nested or trabecular architecture, composed of "oncocytic" cells with abundant, granular eosinophilic cytoplasm and round, generally uniform nuclei
  - Tremendous overlap may exist with chromophobe carcinoma, particularly the eosinophilic variant, leading to great potential utility for molecular diagnostic studies
- Loss of chromosomes 1, Y, and X, rearrangement/translocation of 11q12–13, and loss of 14q have been reported in oncocytoma
  - Many tumors have normal complements of chromosomes (Fig. 9.1g, h); however, abnormalities of chromosome 1 are the most common nondisomic finding in sporadic and familial cases
    - Similar findings have been demonstrated utilizing cytogenetics, FISH, CGH, and SNP-based oligoarray methods
  - Loss of a tumor suppressor gene residing on chromosome 1p has been proposed as an early genetic event in the development of oncocytoma

  - Another subset of tumors has been found to have rearrangements or translocations involving chromosome 11, with a breakpoint at 11q12–13
    - t(5;11) oncocytomas demonstrated a breakpoint flanked by the markers D11S443/D11S146 and the *CCND/BCL1* locus
      - Translocations have included the following:
        - t(5;11)(q35;q13)
        - t(9;11)(p23;q12)
        - t(9;11)(p23;q13)
        - Other chromosomal partners, including 1, 6, 7, and 8
    - FISH reveals close proximity of *CCND1* (*PRAD1*, *BCL1*) to the 11q13.3 breakpoint
    - In combination with cyclin D1 overexpression by immunohistochemistry, these findings suggest a role for cyclin D1 in oncocytomas with the 11q translocation
    - An abundance of mitochondria is a key feature in oncocytoma, imparting the granular/oncocytic cytoplasmic characteristics
      - 11q13 includes a number of genes for mitochondrial proteins, including *UCP2*, *UCP3*, *NDUFC2*, and *SDHD*
      - Mitochondrial protein 2D electrophoresis in oncocytoma reveals downregulation of NDUFS3 from complex I of the respiratory chain and upregulation of COX5A, COX5B, and ATP5H from complex IV and V
- Molecular differential diagnosis
  - Due to the prominent morphologic overlap with the eosinophilic variant of chromophobe carcinoma, a number of studies have been directed at identification of markers that are useful in distinguishing the two tumors
    - LOH was found in chromosomes 1, 2, 6, 10, 13, 17, and 21 at frequencies of 90%, 90%, 96%, 86%, 85%, 90%, and 72%, respectively, in chromophobe carcinoma, but not in oncocytoma

Renal Cell Neoplasms of Oncocytosis

- Renal oncocytosis is characterized by oncocytic changes within renal tubules, accompanied by the presence of multiple oncocytic tumors resembling oncocytoma or chromophobe carcinoma
- FISH studies for abnormalities of chromosomes 1, 2, 6, 10, and 17 have revealed a distinctive pattern of abnormalities in these tumors, including gains of all five chromosomes, gains of only 2 and 10, or no gains/losses of any of the chromosomes
  - In contrast, multiple oncocytomas studied as controls uniformly showed no abnormalities of 2, 6, 10, or 17, with a subset of cases showing loss of chromosome 1
- These findings have led to the hypothesis that renal cell neoplasms of oncocytosis are not closely related to either oncocytoma or chromophobe carcinoma

Papillary Adenoma

- Papillary adenoma is a common benign tubular proliferation, often found incidentally in kidneys resected for other lesions or at autopsy
  - Light microscopically, papillary adenomas are characterized by morphologic features similar to papillary renal cell carcinoma, including papillary or tubular architecture and calcification; however, size is by definition less than 5 mm
- The molecular relationship between papillary adenomas and papillary carcinomas is incompletely understood
  - Similar gains of chromosomes 7 and 17, and loss of Y are often present
  - Progression to papillary carcinoma through gains of additional chromosomes, such as 12, 16, and 20 has been postulated
  - However, FISH studies have demonstrated gains of chromosomes 12, 16, and 20 in small papillary adenomas
    - Frequencies of gains of chromosomes 7, 17, 16, 12, and 20, and loss of the Y

chromosomes were similar in both papillary adenomas and papillary renal cell carcinomas
  - As such, chromosomal alterations do not appear to be reliable for differentiating papillary adenoma from papillary carcinomas

Cystic Nephroma

- Cystic nephroma is a rare benign renal neoplasm seen predominantly in women, composed of epithelial and stromal components
  - Microscopically, the lesion is characterized by variably sized cystic spaces, lined by flattened, cuboidal, or hobnail cells with a spindle cell stroma, sometimes imparting an ovarian stroma-like appearance
- Molecular genetic characteristics of cystic nephroma are not completely understood
  - Recent studies using gene expression profiling have found a similar profile in cystic nephroma and mixed epithelial and stromal tumor (MEST) of the kidney, distinct from other renal neoplasms, including: urothelial carcinoma, chromophobe carcinoma, oncocytoma, clear cell carcinoma, papillary carcinoma, and normal kidney tissue
- Overlap in characteristics with MEST has led some authors to suggest the term "renal epithelial and stromal tumor (REST)" as a unifying name for the two lesions
- Familial syndromes
  - Germline mutation of the *DICER1* gene (located at chromosome 14q31) has been identified in the setting of familial cases of cystic nephroma
  - Patients with germline *DICER1* mutation have also been found to develop the following:
    - Pleuropulmonary blastoma (pleuropulmonary blastoma familial tumor and dysplasia syndrome)
    - Ovarian sex cord-stromal tumors (including Sertoli–Leydig tumor)
    - Wilms tumor
    - Intraocular medulloepithelioma
    - Medulloblastoma/PNET
    - Germ cell tumor
    - Rhabdomyosarcoma
    - Multinodular goiter

Mixed Epithelial and Stromal Tumor

- MEST of the kidney is a biphasic neoplasm, composed of a spindled stromal component and variable epithelial component
- Studies investigating the molecular genetic features are limited
  - Recent investigation into clonality found the same pattern of nonrandom X chromosome inactivation in the epithelial and stromal components for the majority of cases, supporting the theory that both components are neoplastic and arise from a common origin
  - Studies directed at differentiating MEST from congenital mesoblastic nephroma have found the two lesions to be distinct, with MEST lacking the typical genetic features of mesoblastic nephroma
    - t(12;15)(p13;q25) and resulting *ETV6–NTRK3* gene fusion (cellular variant)
    - Abnormalities of chromosomes 8, 11, and 17 by FISH
  - One study found t(1;19)(p22; p13.1) in a tumor from a male patient
- Gene expression profiling studies
  - Tumors show a similar gene expression profile to cystic nephroma, distinct from urothelial carcinoma, chromophobe carcinoma, oncocytoma, clear cell carcinoma, papillary carcinoma, and normal kidney tissue
  - Insulin-like growth factor 2 (*IGF2*) showed the greatest degree of differential expression in MEST compared to normal kidney tissue and other renal neoplasms (32-fold higher)
  - Carbonic anhydrase II (*CA2*) showed 16-fold lower expression in MEST
- A case of malignant MEST with rhabdoid features has been described, lacking the *SYT–SSX1* or *SYT–SSX2* fusion transcripts seen in synovial sarcoma

  - Molecular studies may be helpful in ruling out the diagnosis of synovial sarcoma, in which epithelial cysts may be embedded in the spindle cell stroma

Juxtaglomerular Cell Tumor

- Juxtaglomerular cell tumor is a rare renal neoplasm, thought to arise from the specialized smooth muscle of the juxtaglomerular apparatus
  - Tumors are associated with production of renin and, therefore, uncontrolled hypertension and hypokalemia
  - Light microscopic features include sheets of polygonal or spindled cells, round nuclei, and abundant eosinophilic/granular cytoplasm, and distinct cell borders; less commonly, epithelial channels and/or papillary architecture may be present
- In a study comparing the lesion with endocrine tumors of the pancreas, the juxtaglomerular cell tumors showed differential expression of a number of proteins by immunohistochemistry
  - Nuclear accumulation of cyclin D1, p21, and p27 was present, with the absence of cyclin D3, p53, p16(INK4a), and MDM2
  - BCL2 protein was strongly expressed and RB was moderately expressed
- Multiple methodologies have revealed loss of chromosome 9 as a frequent event, as well as loss of chromosome 11 (or 11q)
- Other combinations of abnormalities have been identified as potential pathogenetic events, including the following:
  - Additional loss or monosomy of chromosomes X, 6, 15, and 21
  - Gain of chromosomes 3, 4, 10, 13, 17, 18
  - Upregulation of genes: *BM1, KIT, PIP4K2A, TLX1, TNIP3*

Metanephric Adenoma

- Metanephric adenoma is a rare neoplasm, with generally benign behavior
- Tumors are highly cellular, composed of tightly packed acini, and branching tubular structures, lined by cells with scant cytoplasm and small, uniform nuclei
  - Psammoma bodies and papillary structures may be present, leading to significant resemblance to papillary renal cell carcinoma (particularly the solid variant)
- Analysis by a variety of molecular genetic methods has demonstrated normal karyotypes and diploid histograms by flow cytometric DNA content analysis in many cases; however, other studies have demonstrated the following:
  - Frequent gains of chromosome 19 in a study of nine tumors (19p more frequently than 19q)
    - Other cases in the study revealed a mixture of multiple chromosomal imbalances, normal karyotypes, or abnormalities of 11q
  - Deletion of chromosome 2p, with alteration of 2p13, suggesting the site of a tumor suppressor gene
    - Allelic imbalances were detected for chromosomes 2p, 7, 8p, 12q, 16q, and 20
  - Balanced pericentric inversion involving the short and long arms of chromosome 9, inv(9)(p12q13)
  - Balanced translocation t(9;15)(p24;q24) and balanced paracentric inversion inv(12)(q13q15)
  - Dual t(1;22)(q22;q13) and t(15;16)(q21;p13) translocations
- Molecular differential diagnosis
  - Papillary renal cell carcinoma
    - Chromosome panels specific for papillary renal cell carcinoma (7, 17, and Y) may be helpful in discrimination from metanephric adenoma
    - Although several studies have found these abnormalities to be lacking in metanephric adenoma, one study revealed trisomy of 7 and 17 with loss of Y in several cases, raising a challenging differential diagnosis with the solid variant of papillary renal cell carcinoma

- Wilms tumor
  - Common chromosomal abnormalities seen in Wilms tumor have been for the most part absent in metanephric adenoma (such as gain of 1q, 7q, and 12 and loss of 11p and 16q)

Renomedullary Interstitial Cell Tumor (Medullary Fibroma)

- Renomedullary interstitial cell tumor (formerly known as medullary fibroma) is a frequent incidental finding at autopsy or examination of the kidney for other reasons, generally comprising a small (1–5 mm) medullary nodule
  - Light microscopic features include small stellate to polygonal cells in a background of loose stromal material, sometimes with deposits of amyloid
    - Ultrastructural features more in keeping with medullary interstitial cells rather than fibroblasts led to the proposal of the designation renomedullary interstitial cell tumor rather than medullary fibroma
    - Otherwise, little is known regarding the molecular genetic characteristics of these tumors

Pediatric Neoplasms

Nephroblastoma (Wilms Tumor)

- Nephroblastoma or Wilms tumor is the most common pediatric renal malignancy and a relatively common solid tumor of childhood, composed of a triphasic population of blastemal, tubular, and stromal components (Fig. 9.7)
  - Believed to originate from nephrogenic rests
- A number of inherited tumor syndromes confer an increased risk of development of nephroblastoma (though germline mutations are the source of only approximately 5–15% of cases)

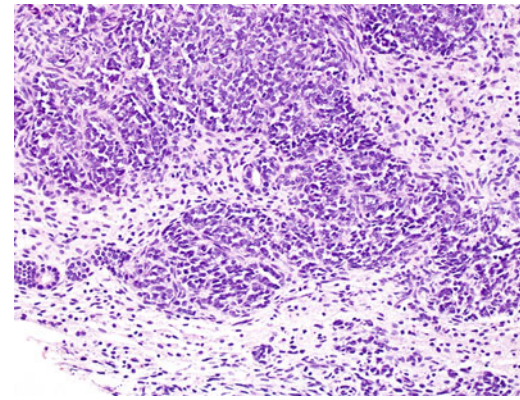

Fig. 9.7 Nephroblastoma (Wilms tumor) in a pediatric patient, showing a triphasic population of blastemal, tubular, and stromal components

  - Beckwith–Wiedemann syndrome
    - Associated with abnormality of 11p15.5 and abnormality of the *WT2* gene
    - The majority of children with the Beckwith–Wiedemann syndrome do not develop nephroblastoma; however, their risk is markedly increased compared to the general population
  - Wilms [tumor], aniridia, genitourinary [abnormalities], and [mental] retardation syndrome (WAGR)
    - Contiguous gene syndrome with larger deletions of 11p13 affecting adjacent genes, including *WT1* and *PAX6*
    - Risk for development of nephroblastoma is significant, though much lower in aniridia patients who do not have involvement of the *WT1* gene
  - Denys–Drash syndrome
    - Associated with nephropathy and gonadal dysgenesis
    - Approximately 90% risk for development of nephroblastoma, associated with point mutation of *WT1* gene
  - Frasier syndrome
    - Development of nephroblastoma is uncommon
- The majority of nephroblastoma cases are sporadic
  - 10–20% of patients with sporadic nephroblastoma have heterozygous or homozygous mutation of *WT1*

- Molecular genetic alterations frequently include loss of genetic material of chromosome 11p, the location of the *WT1* and *WT2* genes
  - *WT1* on chromosome 11p13
    - Encodes a transcription factor of the zinc finger family involved in the survival and differentiation of renal stem cells
  - Other abnormalities include gains of chromosomes 1q, 7q, and 12 and loss of 16q
    - LOH for 1p and 16q has been associated with relapse
  - Other genetic loci associated with the development of nephroblastoma have included the following:
    - *WT2* on chromosome 11p15.5
    - *WT3* on chromosome 16q
    - *WT4* (*FWT1*) on chromosome 17q12–q21
    - *WT5* on chromosome 7p15–p11.2
    - *FWT2* on chromosome 19q
    - *CTNNB1* (β-catenin)
      - Located at 3p21, mutations are seen in approximately 15% of tumors
      - Involved in Wnt signaling pathway
    - *WTX* (Wilms tumor on the X, *FAM123B*)
      - Tumor suppressor gene located at Xq11.1
      - Involving the single X allele in male patients or the active X in female patients
    - *TP53*
      - Mutations of *TP53* (17p13.1) have been associated with the presence of unfavorable, (anaplastic) histology, metastasis, and relapse
    - Other genes, including *FBXW7*, *BRCA2*, *HACE1*, and *GPC3*

Clear Cell Sarcoma

- Clear cell sarcoma of the kidney is a rare pediatric renal malignancy, composed of epithelioid and/or spindled tumor cells, arranged in nests and cords
  - A background of myxoid stromal material and fine, vesicular nuclear chromatin impart a clear appearance
  - A propensity for bone metastases led to the original name "bone metastasizing renal tumor of childhood"
- Unlike some of the other pediatric renal tumors, molecular genetic characteristics of clear cell sarcoma are not well understood
  - Association with a tumor predisposition syndrome is not a characteristic feature
- Occasional studies have identified tumors with molecular genetic abnormalities, including the following:
  - t(10;17) with a breakpoint at the *TP53* locus on chromosome 17p13
    - However, most tumors have lacked abnormality of *TP53*, with the exception of rare cases showing positivity by immunohistochemistry in the setting of anaplasia (similar to nephroblastoma)
    - Such translocations have included the following:
      - t(10;17)(q11;p12)
      - t(10;17)(q22;p13) recently reported to involved the *FAM22* and *YWHAE* genes
      - t(10;17)(q22;p13), del(14)(q24.1q31.1)
  - Other tumors have shown the following:
    - A complex karyotype including deletion of 14q23, loss of chromosome 11p, t(2;22)(q21;q11), loss of imprinting for *IGF2*, gain of 1q, loss of 10q, loss of terminal 4p, loss of chromosome 19, and gain of 19p
  - A significant number of cases have shown normal karyotypes or normal CGH profiles

Rhabdoid Tumor

- Rhabdoid tumor of the kidney is a highly aggressive pediatric renal neoplasm, characterized by sheets of tumor cells that overrun the normal architecture of the kidney

  - Vesicular chromatin, prominent nucleoli, and hyaline cytoplasmic inclusions are frequently seen
  - Similar tumors have been described in a variety of anatomic sites, designated "malignant extrarenal rhabdoid tumor," "malignant rhabdoid tumor of soft tissue," or "atypical teratoid/rhabdoid tumor" (in the central nervous system)
- Tumors are associated with a very poor prognosis
- The characteristic molecular genetic abnormality of rhabdoid tumor is mutation or deletion involving the *INI1* tumor suppressor gene on chromosome 22q11.2
  - A subset of cases may have reduction of INI1 protein expression due to epigenetic mechanisms
  - Germline mutations of *INI1* are seen in a subset of patients, presenting at an earlier age of 6 months, associated with a worse prognosis
    - Germline *INI1* mutation has also been described to predispose to schwannomatosis and meningioma, though interestingly schwannoma and rhabdoid tumor rarely occur together, perhaps due to variable penetrance
- Molecular differential diagnosis
  - Other kidney tumors may show rhabdoid features; however, loss of expression of INI1 protein by immunohistochemistry is a helpful diagnostic feature of rhabdoid tumor
  - Expression of INI1 is preserved in other tumors, such as nephroblastoma, mesoblastic nephroma, PNET, desmoplastic small round cell tumor, rhabdomyosarcoma, and renal cell carcinoma
  - Molecular genetic studies for the characteristic genetic abnormalities of other tumors may be helpful in challenging cases
    - Synovial sarcoma
      - t(X;18)(p11;q11) involving *SYT* gene on chromosome 18 and *SSX1*, *SSX2*, or *SSX4* on the X chromosome
    - Desmoplastic small round cell tumor
      - t(11;22)(p13;q12) resulting in *EWSR1–WT1*, or, alternatively, *EWSR1–FLI1* or *EWSR1-ERG*

Congenital Mesoblastic Nephroma

- Congenital mesoblastic nephroma is the most common congenital renal neoplasm, generally occurring in the first year of life, though making up only 2–4% of pediatric renal tumors
- Two forms are recognized, with similar histopathologic and molecular genetic characteristics to infantile fibromatosis and infantile fibrosarcoma
  - Classic congenital mesoblastic nephroma
    - Characterized by interlacing fascicles of fibroblastic cells with thin to fusiform nuclei, eosinophilic cytoplasm, collagen deposition, and low mitotic activity
    - Frequently, the tumor intermingles with the adjacent structures' renal parenchyma
    - Molecular genetic characteristics
      - Classic congenital mesoblastic nephroma is characterized by a diploid karyotype
      - In contrast to cellular congenital mesoblastic nephroma, the t(12;15)(p13;q25) is absent
  - Cellular congenital mesoblastic nephroma
    - Is remarkable for greater cellularity, decreased cytoplasmic volume, vesicular nuclear chromatin, and a pushing border
    - A high mitotic rate and areas of necrosis may be seen
    - Molecular genetic characteristics
      - Tumors have shown t(12;15)(p13;q25), associated with fusion of the *ETV6* and *NTRK3* genes
        - This fusion is also seen in infantile fibrosarcoma, supporting the morphologic similarity between the two lesions
      - Aneuploidy of chromosomes 8, 11, and 17 are also frequently seen

Metanephric Stromal Tumor

- Metanephric stromal tumor is a rare, benign renal neoplasm seen predominantly in children

- Light microscopic features include stellate/spindled tumor cells, with thin, hyperchromatic nuclei
  - Tumors are believed to interact with various renal elements, resulting in "onion skin" concentric rings around blood vessels (angioplasia), entrapment of renal tubules, and sometimes juxtaglomerular cell hyperplasia or heterologous elements (glial/cartilaginous)
- Molecular genetic characteristics of metanephric stromal tumor are incompletely understood
  - A recent case demonstrated partial triplication of the segment between bands 17q22 and 17q24.3 and duplication of the segment between bands 17q24.3 and 17q25.3 by cytogenetic analysis and FISH studies
  - An additional case of metanephric stromal tumor was recently reported in a patient with neurofibromatosis I
    - Some such patients also develop juxtaglomerular cell hyperplasia, renal artery aneurysms, and renovascular angiodysplasia, similar to the features seen in metanephric stromal tumor, leading to the hypothesis that neurofibromatosis and possibly nephroblastoma are linked to metanephric stromal tumor

Neuroblastoma

- Rarely neuroblastoma may present as a true intrarenal mass, raising the differential diagnosis radiographically of other pediatric renal neoplasms
  - Invasion of the kidney by adjacent neuroblastoma, in contrast, is more common
- Molecular genetic characteristics of neuroblastoma in general include amplification of *MYCN* in a subset of cases
  - A recent study of eight cases of primary intrarenal neuroblastoma revealed absence of *MYCN* amplification in all of the cases (six) with available information
    - However, other scattered cases have shown positive *MYCN* amplification

Renal Cell Carcinoma Associated with Neuroblastoma

- Cases of renal cell carcinoma arising in patients with long-term survival from neuroblastoma have also been described
- Although therapy may play a role in the development of these tumors, occasional patients develop renal cell carcinoma without treatment or simultaneously with the neuroblastoma
- Histopathologic and molecular genetic features are variable
  - Light microscopy
    - Tumors have shown a variety of morphologic features, including solid or papillary architecture, abundant eosinophilic cytoplasm, or alternatively features of clear cell renal cell carcinoma
  - Molecular genetic characteristics
    - At the molecular genetic level, tumors have shown multiple deletions by cytogenetic methods, or allelic imbalances of 20q13
    - Otherwise, the molecular genetic alterations of these unique neoplasms are not well understood

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Molecular Pathology of Prostate Cancer

10

Bora Gurel, Riley E. Alexander, Liang Cheng, and Angelo M. De Marzo

Contents

Introduction

- Prostate cancer (prostatic adenocarcinoma) is the most common noncutaneous malignancy in men, occurring primarily in elderly men
- Nearly all tumors are of the acinar adenocarcinoma subtype and originate from the normal prostatic epithelium
- Prostatic adenocarcinomas may arise from the peripheral zone of the prostate (Fig. 10.1) or less commonly from the transition zone (the site of most benign prostatic hyperplasia)
- Although not fully elucidated, the etiologic agents contributing to the development and progression of prostate cancer are numerous and include hereditary factors, dietary habits, hormonal alterations, and inflammation/infections (Fig. 10.2)
- The use of prostate specific antigen (PSA) serum testing has revolutionized detection and monitoring of prostate cancer
- Most cases are now diagnosed by transrectal needle biopsy conducted after detection of elevated serum PSA
- Clinically, the prognosis and treatment decisions are largely based on the histologic grade reported as the Gleason score
- Clinical progression is difficult to predict in low-grade cases and some may forego therapy for increased surveillance—so-called "active surveillance"
- For clinically localized prostate cancer, radical prostatectomy and radiation remain the mainstay of treatment with adjuvant radiotherapy and androgen deprivation therapy used in more aggressive cases

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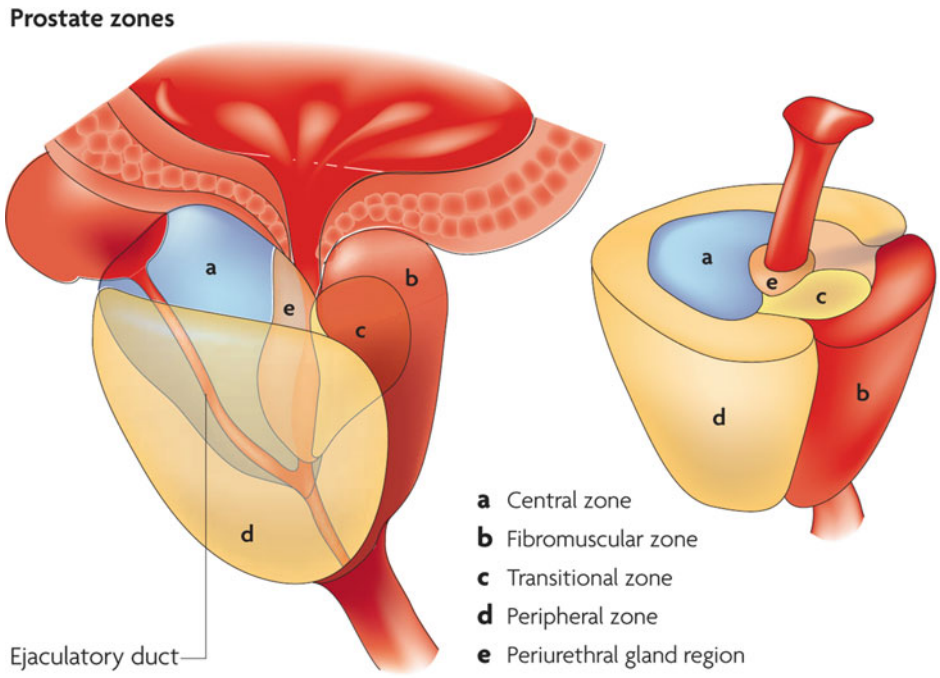
L. Cheng and J.N. Eble (eds.), *Molecular Surgical Pathology*,
DOI 10.1007/978-1-4614-4900-3_10,



	Prostate zone		
	Peripheral	Transition	Central
Focal atrophy			
Acute inflammation			
Chronic inflammation			
Benign prostatic hyperplasia			
High-grade PIN			
Carcinoma			

High prevalence
Medium-high prevalence
Low prevalence
None

Fig. 10.1 Zonal predisposition to prostate disease. Most cancer lesions occur in the peripheral zone of the gland, fewer occur in the transition zone, and almost none arise in the central zone. Most benign prostate hyperplasia (BPH) lesions develop in the transition zone, which might enlarge considerably beyond what is shown. The inflammation found in the transition zone is associated with BPH nodules and atrophy, and the latter is often present in and around the BPH nodules. Acute inflammation can be prominent in both the peripheral and transition zones, but is quite variable. The inflammation in the peripheral zone occurs in association with atrophy in most cases. Although carcinoma might involve the central zone, small carcinoma lesions are virtually never found here in isolation, strongly suggesting that prostatic intraepithelial neoplasia (PIN) lesions do not readily progress to carcinoma in this zone. Both small and large carcinomas in the peripheral zone are often found in association with high-grade PIN, whereas carcinoma in the transition zone tends to be of lower grade and is more often associated with atypical adenomatous hyperplasia or adenosis, and less often associated with high-grade PIN. The various patterns of prostate atrophy, some of which frequently merge directly with PIN and at times with small carcinoma lesions, are also much more prevalent in the peripheral zone, with fewer occurring in the transition zone and very few occurring in the central zone. Upper drawings are adapted from an image on Understanding Prostate Cancer website. *PIN* prostatic intraepithelial neoplasia. (From De Marzo 2007 Nat Rev Cancer, with permission.)

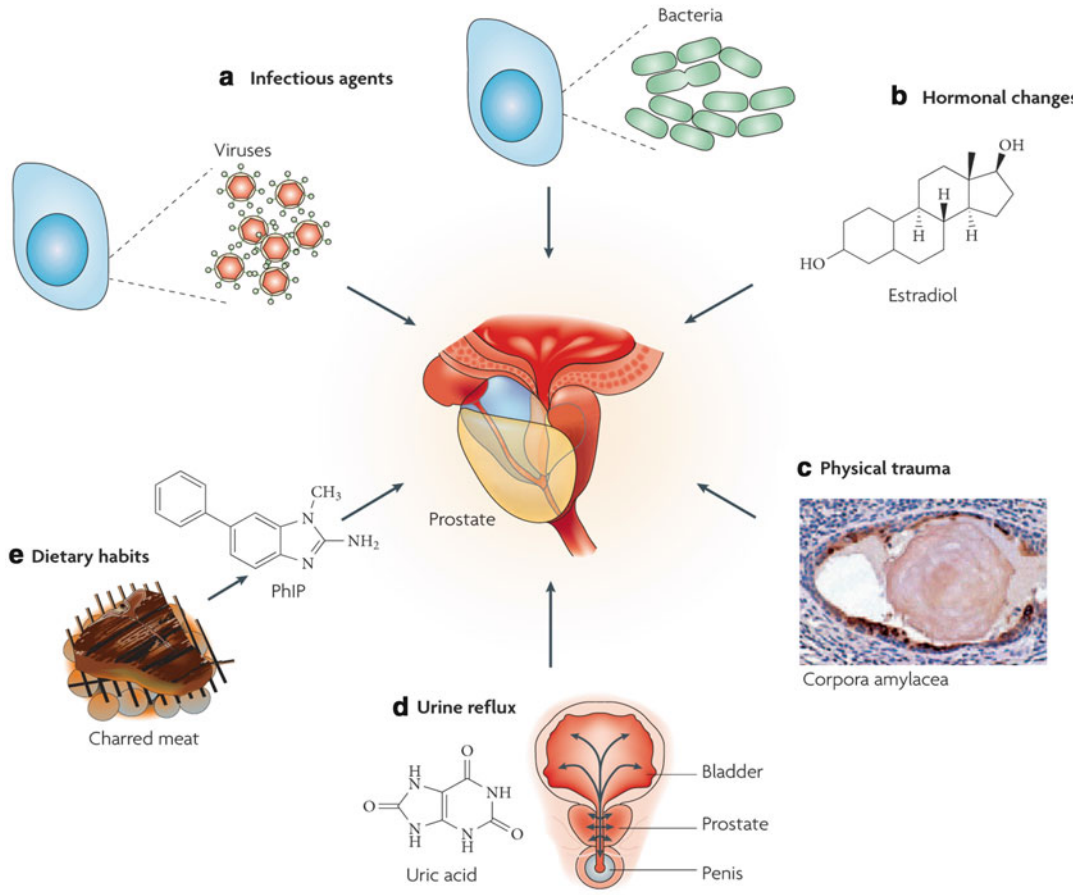


Fig. 10.2 Possible causes of prostate inflammation. (**a**) Infection. Chronic bacterial prostatitis is a rare recurring infection in which pathogenic bacteria are cultured from prostatic fluid. Viruses, fungi, mycobacteria, and parasites can also infect the prostate and incite inflammation. The figure represents two prostate cells infected either by bacteria or by viruses. (**b**) Hormones. Hormonal alterations such as oestrogen exposure at crucial developmental junctures can result in architectural alterations in the prostate that produce an inflammatory response. (**c**) Physical trauma. Corpora amylacea can traumatize the prostate on a microscopic level. The figure shows a corpora within a prostatic acinus in which its edges appear to be eroding the epithelium, resulting in an increase in expression of the stress enzyme cyclooxygenase 2 (PTGS2), represented by brown immunostaining. Prostate cell nuclei are visible in violet following hematoxylin staining. (**d**) Urine reflux. Urine that travels up back toward the bladder ("retrograde" movement) can penetrate the ducts and acini of the prostate. Some compounds, such as crystalline uric acid, can directly activate innate inflammatory cells. Although these compounds would not be expected to traverse the prostate epithelium, if the epithelium was already damaged this would facilitate the leakage of these compounds into the stromal space where they would readily activate inflammatory cells. (**e**) Dietary habits. Ingested carcinogens (e.g., 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP), which derives from charred meat) can reach the prostate through the bloodstream or by urine reflux and cause DNA damage and mutations, and result in an influx of inflammatory cells. (From De Marzo 2007 Nat Rev Cancer, with permission.)

Molecular Features of Prostate Cancer

- Advances in molecular diagnostic methods have led to a rapidly growing understanding of the molecular alterations present within prostate cancer
- As in other cancers, there appears to be a stepwise progression of molecular alterations during the development of prostate cancer, and the most well-recognized precursor to invasive prostate cancer is high-grade prostatic intraepithelial neoplasia (PIN)

- These techniques have yielded the identification of specific somatic genes and germline mutations that are involved in prostate cancer
- Recent whole-genome sequencing efforts on a limited number of prostate cancers revealed a relatively infrequent point mutational burden and a frequent occurrence of complex genome rearrangements
- In addition, the importance of epigenetic mechanisms in tumorigenesis has been established
- Despite these advances, translation of these findings into clinically relevant and useful applications is still in its early stages
- The discovery of the *TMPRSS2–ERG* rearrangement has been a significant development in the molecular understanding of prostate cancer

TMPRSS2–ETS Gene Fusions

- These rearrangements involve fusion of the androgen-regulated gene, *TMPRSS2*, with a member of one of the *ETS* family of transcription factors, *ERG* being the most common; both genes occurring on chromosome 21
- Gene fusions occur through two predominant mechanisms: interstitial deletion on chromosome 21 or translocations resulting in insertions
- The fusion of the genes leads to the androgen-mediated overexpression of the particular *ETS* transcription factor via *TMPRSS2*, which leads to a number of changes, most prominently altered cellular differentiation/reduced androgen signaling as well as invasion
- These fusions are highly prevalent in prostate cancer, occurring in 40–70% of cases
  - The gene fusion is much more common in peripheral zone adenocarcinomas than in transition zone adenocarcinomas
  - Recent studies have shown significant variation of the prevalence among different ethnicities
    - The prevalence is consistently seen to be around 50% in Caucasians and as low as 17% in Asians
    - The prevalence is also reported to be lower in African-Americans than in Caucasians
- Presence of the gene fusion has been found to be an early event in the development of tumors with *TMPRSS2–ERG* rearrangements
- Diagnostic implications
  - *TMPRSS2–ERG* fusions have an essentially 100% specificity for prostate cancer and more rarely high-grade PIN
  - Antibodies to the ERG protein product have been developed for immunohistochemical purposes and have proven useful as an aid to diagnosis in cases suspicious for but not diagnostic of cancer by H&E alone and routine immunohistochemical stains
- Detection of *TMPRSS2–ERG* gene fusion transcripts when added to multiplex RNA assays in urine and post-prostate massage specimens have been shown to increase sensitivity and specificity for predicting a positive repeat biopsy in men with initial negative biopsies
- Prognostic implications
  - The prognostic implications of *TMPRSS2–ERG* gene fusions remain controversial
  - Initial studies that were focused on levels of *ERG* mRNA suggested that ERG overexpression (and hence gene fusion) portends an improved prognosis or does not differentially affect prognosis
  - A study of patients diagnosed by transurethral resection who were not treated until symptomatic disease progression (e.g., watchful waiting cohort) showed that the presence of the fusion was related to poor outcome
  - Other studies using FISH or immunohistochemistry (IHC) on radical prostatectomy specimens to determine *TMPRSS2–ERG* gene fusion status have mostly indicated no association of the presence of the fusion gene per se
  - Early evidence has proposed differential prognosis for the mechanism of gene fusion, i.e., deletion being worse than insertion
  - A number of different mRNA transcripts can be produced for the gene fusion and the ratio of different transcripts to each other has been shown to be related to prognosis
  - Animal studies and preliminary human studies suggest that in combination with

PTEN loss, the presence of an *ERG* fusion gene may impart a worse prognosis
  - Overall, much more work needs to be done to determine the true implications of *ETS* family member gene fusions in prostate cancer
- Predictive implications
  - Preliminary studies have shown an increased response to adjuvant androgen deprivation therapy in patients with both *TMPRSS2–ERG* and the presence of *TOP2A* overexpression
  - Development of targeted therapy to *TMPRSS2–ERG*– positive patients is underway, but has yet to introduce a clinically viable treatment as of yet

Other Somatic Alterations in Prostate Cancer

- See Table 10.1

Genetic Alterations in Prostate Cancer

- Telomere shortening
  - Seen in most cases of high-grade PIN and adenocarcinoma
  - Such shortening can lead to chromosome instability
  - Occurs in many other preneoplastic and neoplastic conditions
- Many chromosomal deletions
  - Chromosome 8p
    - Deletions and loss of heterozygosity on the short arm of chromosome 8 (8p) are seen in prostate cancer
    - The most well-studied tumor suppressor gene in this area is *NKX3.1*
    - *NKX3.1* codes a prostate-restricted homeobox protein involved in developmental regulation and reducing free radical effects
    - Loss of *NKX3.1* generally involves one allele only
    - Since its expression is maintained (albeit at reduced levels compared to normal luminal cells) in carcinomas, and is not seen in other tumor types, it may eventually be used as a specific marker for prostate cancer
  - *PTEN*
    - A tumor suppressor gene located on 10q23
    - Found to be deleted in 20–40% of prostate cancer
    - Loss of *PTEN* is associated with high Gleason score and advanced stage (Fig. 10.3)
    - *PTEN* is a lipid and protein phosphatase whose best known function is to dephosphorylate PIP3, which counterbalances PI3 kinase—a protein involved in the PI3K–AKT pathway involved in cell proliferation and survival
    - Loss of *PTEN* is associated with shorter time to metastasis and decreased survival (Fig. 10.4)
  - *CDKN1B*
    - *CDKN1B* encodes p27, a cyclin-dependent kinase inhibitor that shows infrequent mutations and/or deletions, but is commonly decreased at the protein level in PIN and adenocarcinoma lesions
    - One mechanism by which p27 is also downregulated is by the PI3K–AKT signaling pathway
    - Loss of p27 is associated with a poor prognosis in prostate cancer
- Other tumor suppressor gene deletions
  - Deletions of tumor suppressor genes common to other cancers are also seen in prostate cancer
    - *TP53* (uncommon in primary tumors but is mutated in upwards of 50% of castrate-resistant prostate cancer)
    - *RB1* (loss is also more common in advanced disease)
- Oncogenes
  - *AR*
    - The androgen receptor (encoded by *AR*) is highly expressed in normal prostatic luminal cells and is associated with cellular differentiation, and binding to its main ligand, DHT, is required for luminal cell survival

Table 10.1 Common somatic genetic and epigenetic changes in prostate cancer

Gene and gene type	Location	Notes
Tumor-suppressor genes		
CDKN1B	12p13.1-p12	Encodes the cyclin-dependent kinase inhibitor p27. One allele is frequently deleted in primary tumors
NKX3.1	8p21.2	Encodes prostate-restricted homeobox protein that can suppress the growth of prostate epithelial cells. One allele is frequently deleted in primary tumors
PTEN	10q23.31	Encodes phosphatase and tensin homolog, which suppresses cell proliferation and increases apoptosis. One allele is frequently lost in primary tumors. Some mutations are found in primary tumors and more in metastatic lesions
TP53	17p13.1	Has many tumor-suppressor functions, including cell-cycle arrest in response to DNA damage, senescence in response to telomere dysfunction, and the induction of apoptosis. Mutations are uncommon early, but occur in about 50% of advanced or hormone-refractory prostate cancers
Oncogenes		
MYC	8q24	A transcription factor that regulates many target genes involved in cell proliferation, senescence, apoptosis, and cell metabolism. Overexpression can directly transform cells. mRNA levels are commonly increased in all disease stages through unknown mechanism(s). Low-level amplification of the *MYC* locus is common in advanced disease
ERG	21q22.3	Proposed new oncogene for prostate cancer. Fusion transcripts with the 5′ portion of androgen-regulated gene (*TMPRSS22*) arise from deletion or chromosomal rearrangements commonly found in all disease stages
ETV1–4	7p21.3, 19q13.12, 1q21-q23, 17q21.31	Encodes *ETS*-like transcription factors 1–4, which are proposed to be new oncogenes for prostate cancer. Fusion transcripts with the 5′ portion of androgen-regulated gene (*TMPRSS22*) arise from chromosomal rearrangements commonly found in all disease stages
AR	Xq11-12	Encodes the androgen receptor. Protein is expressed in most prostate cancers, and the locus is amplified or mutated in advanced disease and hormone refractory cancers
Activation of the enzyme telomerase		Maintains telomere function and contributes to cell immortalization. Activated in most prostate cancers, mechanism of activation may be through *MYC* activation
Caretaker genes		
GSTP1	11q13	Encodes the enzyme that catalyzes the conjugation of reduced glutathione to electrophilic substrates. Functions to detoxify carcinogens. It is inactivated in more than 90% of cancers by somatic hypermethylation of the CpG island within the upstream regulatory region
Telomere dysfunction	Chromosome termini	Contributes to chromosomal instability. Shortened telomeres are found in more than 90% of prostatic intraepithelial neoplasia (PIN) lesions and prostate cancer lesions
Centrosome abnormalities	N/A	Contributes to chromosomal instability. Centrosomes are structurally and numerically abnormal in most prostate carcinomas
Other somatic changes		
PTGS2, APC, MDR1, EDNRB, RASSF1α, RARβ2	Various	The hypermethylation of CpG islands within upstream regulatory regions occurs in most primary tumors and metastatic lesions. The functional significance of these changes is not yet known

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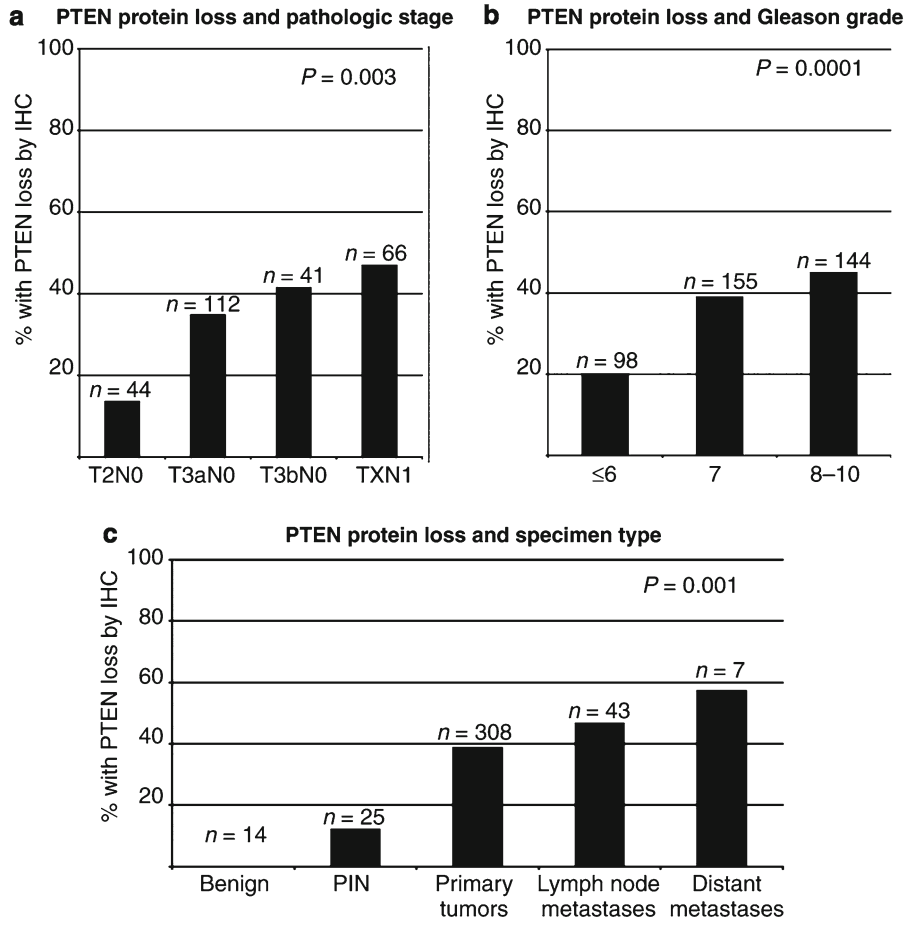

Fig. 10.3 Phosphatase and tensin homolog (PTEN) protein loss by immunohistochemistry is highly correlated with prostate cancer pathologic stage and grade. (**a**) PTEN protein is more frequent in higher pathologic stage tumors ($P=0.003$ by Pearson x2 test). (**b**) PTEN protein loss is more common in higher Gleason grade tumors ($P=0.0001$ by Pearson x2 test). (**c**) PTEN protein loss is least common in benign prostate tissues and PIN and most common in metastatic prostate tumors ($P=0.001$ by Pearson x2 test). (From Lotan 2011 Clin Cancer Res., with permission.)

- The protein product is expressed in most prostatic adenocarcinomas, and its inhibition by castration, medical castration, or by *AR* antagonists is a key well-known treatment for advanced prostate cancer
- Activating mutations are found in "castrate-resistant" prostate cancer—those cancers that no longer respond to castrate levels of testosterone and DHT (Fig. 10.5)
- *AR* gene amplification along with high levels of *AR* mRNA and protein expression is seen in many of these tumors
- These mutations are thought to increase the sensitivity to androgen levels, which at times are now shown to be produced endogenously by the tumor
- These findings regarding *AR* support the concept of oncogene addiction in prostate cancer
  - The need for androgen effectors on proliferation and prevention of cell death is inherent to prostatic cells, normal or cancer

– *MYC*
- *MYC* is located at 8q24 and low-level amplification is associated with high Gleason score, advanced stage, and disease progression in a subset of prostate cancers

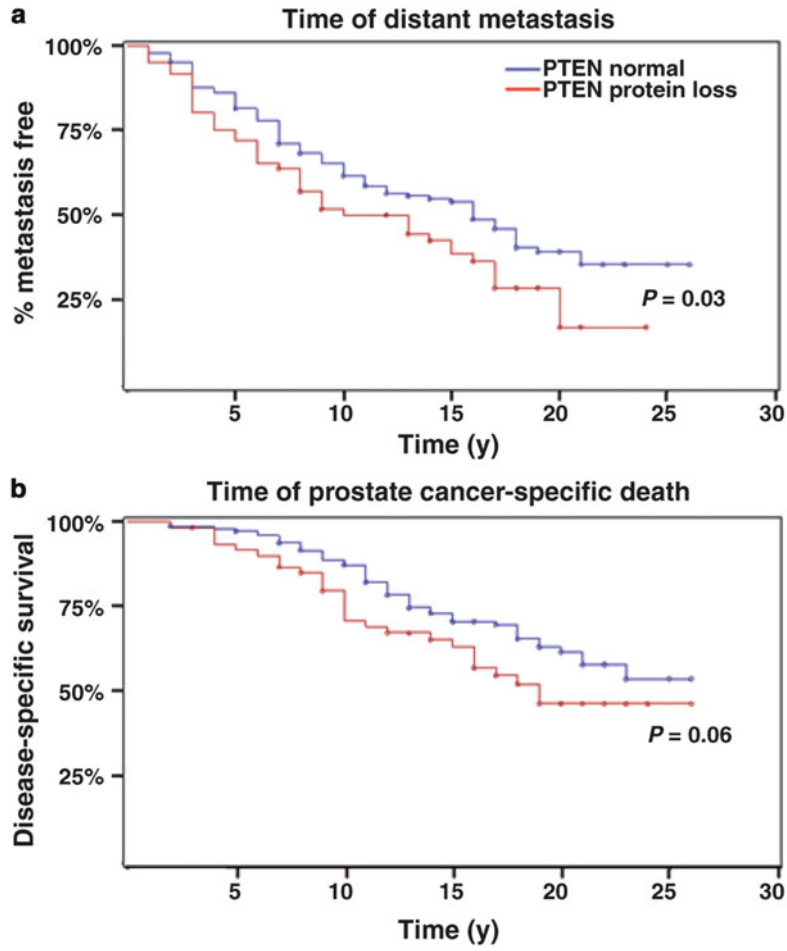

Fig. 10.4 Phosphatase and tensin homolog (PTEN) protein loss by immunochemistry (IHC) is associated with poor clinical outcomes in a surgical cohort of high-risk prostate cancer patients. (**a**) The Kaplan–Meier curve shows a significant decrease in metastasis-free survival for patients with PTEN protein loss by IHC ($P = 0.03$). (**b**) The Kaplan–Meier curve for disease-specific survival shows a nonsignificant decrease in prostate cancer-specific survival in patients with PTEN protein loss ($P = 0.06$). (From Lotan 2011 Clin Cancer Res., with permission.)

- Recent evidence suggests that overexpression of *MYC* mRNA and protein, decoupled from 8q24 gain, arises as an early event in prostate cancer
- *MYC* expression may be enhanced by *TMPRSS2–ERG* gene fusions

– *SPOP*
- *SPOP* encodes the substrate-recognition component of a Cullin3-based E3-ubiquitin ligase
- A missense mutation has been shown to be present in 6–13% of prostate cancers and is associated with increased invasion
- The mutation has been seen to be mutually exclusive with *ERG* rearrangements, indicating a possible distinct class of prostate cancer

– *EZH2*
- *EZH2* histone lysine methyltransferase involved in chromatin remodeling as part of the *PRC2* polycomb repressive complex
- It is overexpressed in all phases of prostate cancer including the precursor lesion, high-grade PIN
- *EZH2* promotes proliferation, invasion, and tumorigenicity of prostate cancer cells

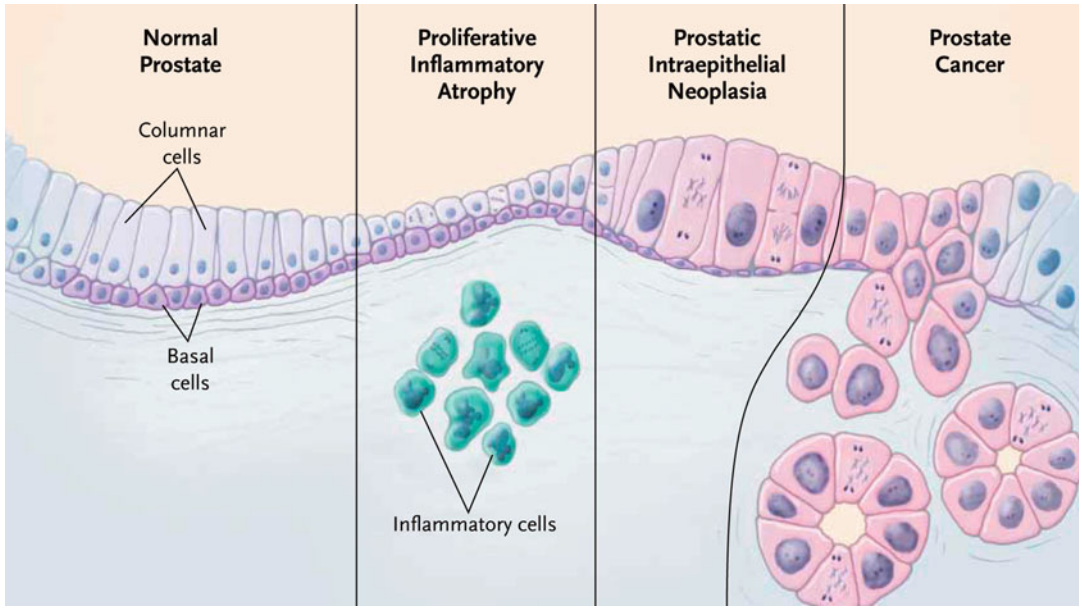

Fig. 10.5 Proliferative inflammatory atrophy as a precursor to prostatic intraepithelial neoplasia and prostate cancer. (From Nelson 2003 N Engl J Med., with permission.)

- Upregulation of *EZH2* in prostate cancer can result from the following:
  - Gene amplification, by deletion of its negative regulator mir-101
  - Transcriptional regulation by *ETS* gene family members
  - Transcriptional regulation directly by *MYC*
  - Downregulation of other negative regulators mir-26a and mir-26b, which are themselves negatively regulated by *MYC*

– Chromosomal gain
  - *PSCA* is a gene on chromosome 8q
    - It encodes prostate stem cell antigen and is a potential therapy target
  - Other genes implicated in chromosomal gain are *MYC*, *AR* and *EIF3S3,* and *EZH2*

Epigenetic Alterations in Prostate Cancer

- CpG hypermethylation
  – Involves the methylation of deoxycytidine residues within CpG dinucleotides, usually in the upstream regulatory regions of specific genes
  – The most well understood gene affected is *GSTP1*
    - *GSTP1* encodes a protein that is part of a family of enzymes that counteract damage from reactive chemical species via a glutathione-mediated mechanism
    - CpG hypermethylation results in silencing of the gene and increased sensitivity to genetic damage from oxidative stress
    - This somatic genome alteration has been found in approximately 90% of all prostate cancers and can be detected in blood, urine, and prostate fluid
    - CpG hypermethylation of *GSTP1* is present in >90% of prostatic adenocarcinomas, ~70% of PIN, and between 4% and 6% of prostate atrophy lesions but is not present in normal-appearing prostatic epithelium
  – Other genes known to be affected by CpG hypermethylation
    - *APC*
    - *RASSF1a*
    - *ENDRB*
    - *PTGS2*
    - *MDR1*
    - *PITX2*

Germline Alterations in Prostate Cancer

- *RNASEL*
  - *RNASEL* encodes a latent endoribonuclease that is thought to degrade viral and cellular RNA
  - Disabling mutations have been shown to be found in a higher percentage of men with prostate cancer than those without, a difference as high as 6.9% to 2.9%, respectively, in certain populations
  - More conclusive work is needed to identify its true role in prostate cancer
- *MSR1*
  - *MSR1*, macrophage-scavenger receptor 1 gene located at 8p22, mutations have been shown in families with a high incidence of prostate cancer
  - Mutant alleles have been found in as high as 3% of men affected with prostate cancer compared to 0.4% in a nonaffected control population
- *AR*
  - The role of *AR* in prostate cancer is well established; however, most mutations are somatic in nature
  - Studies have shown that in populations with a higher incidence of prostate cancer (African–Americans), *AR* is found to have shorter polymorphic polyglutamine repeats
  - This is in contrast to populations with a low incidence of prostate cancer (Asians) who seem to have long polymorphic polyglutamine repeats
  - This has led to speculation that the length of these repeats affects prostate cancer susceptibility—large studies have provided conflicting evidence to this theory
- *CYP17*
  - *CYP17* encodes cytochrome P-450c17-alpha, which is involved in steroid synthesis
  - Preliminary evidence has shown a linkage between a mutant allele and prostate cancer, but has lacked sufficient supporting evidence
- *SRD5A2*
  - *SRD5A2* encodes an isoenzyme of 5-alpha-reductase, the key enzyme involved in the conversion of testosterone to dihydrotestosterone and the target of drugs like finasteride
  - Two variants have been associated with both an increased risk of prostate cancer and a poor prognosis
  - The role that germline mutations in *SRD5A2* play at a population level is unclear
- *HOXB13*
  - Encodes a homeodomain gene that is expressed in adult tissues in a prostate and distal GI-tract-specific manner
  - A recent large study identified variants associated with prostate cancer risk in both familial and sporadic prostate cancer
- *BRCA1/2*
  - Tumor suppressor genes, in which inherited mutations are associated with high penetrance of breast cancer and a somewhat less, although significant, risk of ovarian cancer
  - A recent study estimating the prevalence of germline *BRCA2* mutations in the United Kingdom resulted in an estimated 8.6-fold increased risk of prostate cancer by age 65, which corresponds to an absolute risk of 15% by age 65

Clinical Implications

Diagnostic Utility

- Current methods of diagnosis rely on PSA testing to screen for appropriate candidates for needle biopsy
- The low specificity of PSA leads to overutilization of needle biopsy; the less-than-perfect sensitivity of needle biopsy leads to cases of missed diagnoses of prostate cancer
- Ideally, the use of specific molecular markers will help accomplish the following (Fig. 10.6):
  - Early detection of prostate cancer with a greater specificity than PSA to reduce overutilization of needle biopsy and make more prudent use of rebiopsy in patients with negative results on first-time needle biopsy

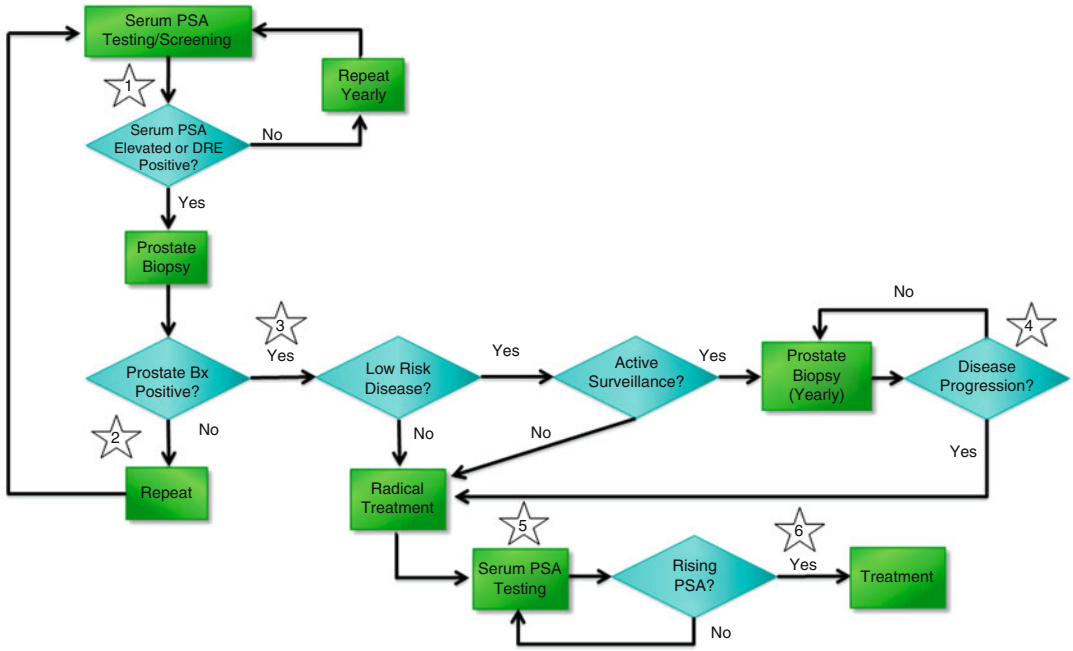

Fig. 10.6 Current state of "Standard of Care" practice for prostate cancer disease states. Starred numbers represent areas where molecular testing is currently employed or where significant value could be added by molecular testing.

  - Aid in determining which men diagnosed with prostate cancer require immediate treatment
  - Effective monitoring of disease progression in those patients with known prostate cancer, particularly those electing active surveillance
  - More accurate prediction and detection of disease recurrence after treatment
  - Allow for the use of markers to assess the efficacy of nonsurgical treatments in advanced prostate cancer
- The lower prevalence of mutated protein products in prostate cancer makes immunohistochemical methods more challenging (Fig. 10.7)
  - *NKX3.1* is expressed in most cases of prostate cancer and in very few other malignancies and is under investigation as part of a panel of immunohistochemical markers for prostate cancer in cases of metastatic tumors of unknown origin or in cases in which one is attempting to distinguish high-grade prostate cancer from bladder cancer
  - FISH targeting *ERG* rearrangement has a high specificity for prostate cancer
  - Immunohistochemical methods to detect *ERG* rearrangement have been developed that have shown similar high specificity to that of FISH
  - Both methods targeting *ERG* rearrangement suffer from the high number of ERG-negative carcinomas, with the prevalence in Western countries shown to be closer to the 50% mark of the 40–70% range stated earlier
  - Alpha methylacyl CoA racemase (AMACR) is used in cases, along with basal cell markers, to help distinguish small foci of atypical glands from adenocarcinoma
- Multifocal prostate cancer
  - The increased sophistication of molecular diagnostic techniques has allowed for the molecular distinction of separately arising lesions within the prostate
  - These findings have confirmed the tumor heterogeneity in prostate cancer even within the same patient many times

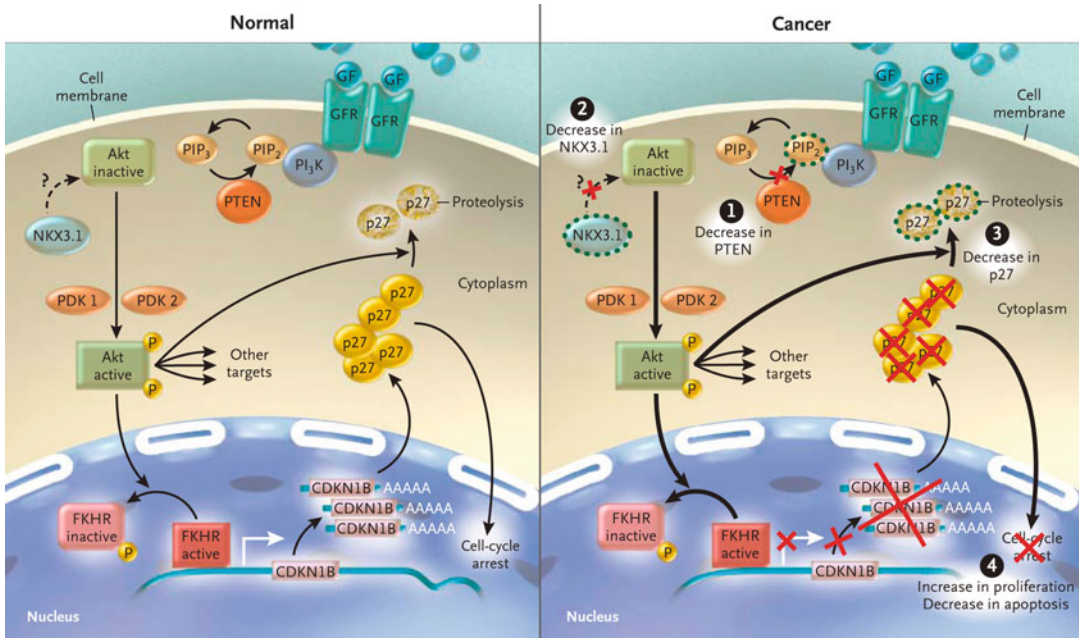

Fig. 10.7 Molecular events in the pathogenesis of prostate cancer. In the normal prostate, NKX3.1, PTEN, and p27 regulate the growth and survival of prostate cells. Inadequate levels of PTEN (*1*) and NKX3.1 (*2*) lead to a reduction in p27 levels (*3*) by a variety of mechanisms and to increased proliferation and decreased apoptosis (*4*). *GF* denotes growth factor; *GFR* growth factor receptor; *PIP3* phosphatidylinositol 3,4,5-triphosphate; *PIP2* phosphatidylinositol 4,5-diphosphate; *PI3K* phosphatidylinositide 3-OH kinase; *PTEN* phosphatase and tensin homolog; *AKT* protein kinase B; *PDK1* 3-phosphoinositide-dependent protein kinase-1; *PDK2* 3-phosphoinositide-dependent protein kinase-2; *FKHR* forkhead transcription factor. A *red X* indicates blocked processes and molecules that have not been produced, a *dotted outline* reduced levels of molecules, and an A the poly-A tail of messenger RNA. The *question mark* and *dotted arrow* in the *left-hand panel* represent the suspicion, not yet proven, that NKX3.1 interacts directly with AKT. (From Nelson 2003 N Engl J Med., with permission.)

  - Most early methods used FISH to achieve this goal
  - *TMPRSS2–ERG* provides a promising single target for a more rapid assessment of multifocality in prostate cancer
    - It has a prevalence of between 40 and 70%; which means it will be present in many lesions, but not all, so the likelihood of two separately arising lesions having differing rearrangement status is high
  - Utilization of these molecular methods allows for distinction of the specific tumor type in metastases

Applications to Urine and Post-prostate Massage Urine Specimens

- DNA methods
  - Methylation-specific PCR (MSP)
    - *GSTP1* promoter hypermethylation
      - Detected in 94% of prostate tumor tissue
      - Sensitivity of 73–75% with a specificity of 98% in urine specimens
      - A recent clinical grade test has been developed that combines GSTP1 with RAR-beta and APC, which shows promise for predicting a positive biopsy in men with elevated PSA
      - Disadvantages of using MSP-based assays include the fact that DNA must be first treated with sodium bisulfate. This is very harsh and damages DNA, which is already in low quantities in urine
  - Combination of methylated-DNA precipitation and methylation-sensitive restriction enzymes (COMPARE-MS)
    - Does not rely on bisulfate treatment

    - Ability to multiplex a number of genes
    - Methods based on this approach may show advantages when applied to urine
- RNA methods
  - Prostate cancer 3 (PCA3) Expressed exclusively in prostate tissue with a much higher level in prostate cancer
    - Encodes an RNA product of unknown function that allows for targeted detection
    - Using this method yields a sensitivity of 58–67%, a specificity of 66–89%, and a negative predictive value of 84–90%
    - A clinical grade test has recently been approved by the United States Federal Drug Administration
    - Indicated to aid in the decision for repeat biopsy in men 50 years of age or older who have had one or more previous negative prostate biopsies and for whom a repeat biopsy would be recommended by a urologist based on the current standard of care

Prognostic Utility

- A number of biomarkers have been shown to add value to prediction of outcome
  - Ploidy status
  - Immunohistochemical staining for Ki67, BCL2, p53, and $p27^{Kip1}$
  - FISH analysis for chromosome 8q24 amplification
  - FISH (chromosome 10q) or IHC for PTEN
- None of these are currently routinely employed in clinical practice
- Epigenetic alterations
  - Hypermethylation of *PITX2* has been shown in a number of studies to correlate with prostate cancer biochemical recurrence after initial prostatectomy
  - Those with *PITX2* hypermethylation have been shown to experience a fourfold increase in PSA relapses after radical prostatectomy
  - The prognostic utility has not been validated in biopsy specimens
- PSA
  - While there is no universal agreement, the use of serum PSA velocity and/or doubling time is a promising currently available biomarker
  - Has very strong predictive powers for disease progression after primary treatment and is easily obtainable
- *TOP2A*
  - *TOP2A* is a gene involved in cell proliferation and DNA chromosomal formation and codes for a protein product of the same name
  - Increased expression of TOP2A in prostate cancer has been associated with higher Gleason score and hormone insensitivity
  - Overexpression of *TOP2A* has been strongly associated with decreased time to systemic progression and may prove to be an important prognostic indicator
  - Since this marker is tightly linked to cellular proliferation, it is not clear whether it adds different information to that obtained by measuring Ki67

Predictive Utility

- *PTEN*
  - *PTEN* deletions lead to increased signaling of the PI3K–AKT pathway
  - Detection of low PTEN levels may predict response to AKT inhibition
  - Clinical trials are underway on inhibitors of this pathway and may provide targeted therapy in this subset of patients, especially in combination with newly emerging antiandrogens and CYP17 inhibitors (which reduce androgen levels)
- SPINK1
  - SPINK1 is a protein found overexpressed in some prostate cancer with a high homology to epidermal growth factor receptor (EGFR)
  - Preclinical work is underway to develop monoclonal antibodies to SPINK1 as has been done with EGFR in colon cancer
- *TOP2A*
  - The gene, which encodes a topoisomerase, is a target for many currently commercially available drugs and may prove to be a predictive marker in prostate cancer
  - Clinical trials with current topoisomerase inhibitors in the setting of prostate cancer need to be performed to confirm their utility in this setting

- Multifocal cancer
  - As mentioned earlier, molecular techniques have confirmed that prostate cancer is commonly a multifocal disease with varying genetic alterations
  - This implies that directed monotherapies will likely not be effective against all tumors within a prostate. Should successful targeted therapies toward molecular alterations come to fruition, identification of multifocal disease will become significantly more important before treatment initiation to predict efficacy

Summary of Keypoints

- Despite the prevalence of prostate cancer, the molecular understanding of the disease is still early in its development
- The genetic and epigenetic events contributing to prostate cancer are diverse and include many mutations common to other tumors, but few distinct to prostate cancer
- Of the discovered molecular alterations in prostate cancer, it is most likely that an interrelationship of multiple somatic genome alterations are responsible for the tumorigenesis and progression of prostate cancer
- The loss or suppression of tumor suppressor genes is common in prostate cancer
  - Chromosome 8p is commonly affected with loss of one allele of *NKX3.1*, the most well studied and specific to the prostate
  - *PTEN* loss occurs in 20–40% of prostate cancers and leads to activation of the PI3K–AKT pathway
  - *CDKN1B* loss leads to decreased p27, which also leads to increased cellular proliferation
- Oncogenes also contribute to the tumorigenesis of prostate cancer
  - Mutations in *AR* are present in advanced "castrate resistant" tumors and the method of action is still poorly understood
  - Amplifications of *MYC* are associated with high Gleason score and advanced disease
- Gene fusions of *TMPRSS2* with members of the *ETS* family of transcription factors are among the most important recent discoveries in prostate cancer
  - Gene fusions are seen in 40% to 70% of cases; *TMPRSS2–ERG* is the most common rearrangement seen
  - This gene fusion is highly specific for prostate cancer and research is underway to evaluate its potential as both a diagnostic adjunct and target for therapy
- Epigenetic alterations are very common in prostate cancer
  - The most common epigenetic alteration seen is CpG hypermethylation
  - Hypermethylation of *GSTP1* is seen in >90% of prostate cancer; it is not specific for prostate cancer, but has never been reported in normal tissue, so may prove useful in distinguishing cancer from normal tissue
- Germline mutations of many genes have been implicated in increased susceptibility to prostate cancer; however, most are poorly understood
- With the greater understanding of molecular alterations in prostate cancer, it is hopeful that the following can be used to increase the diagnostic accuracy of the disease
  - *NKX3.1* is expressed in most cases of prostate cancer and in very few other malignancies, and immunohistochemical markers to the protein product are in development
  - *TMPRSS2-ERG* has a high specificity for prostate cancer and may be helpful in difficult cases, and urine detection methods are in development
  - *GSTP1* hypermethylation is highly prevalent in prostate cancer and highly specific for malignancy; urine detection methods are in development as a screening mechanism
  - *PCA3* is a noncoding RNA highly overexpressed in prostate cancer, and urine detection has recently been approved to aid in determining which patients with elevated serum PSA require rebiopsy
  - Ideally, with greater understanding of these molecular alterations, the diagnostic algorithm of prostate cancer will be significantly

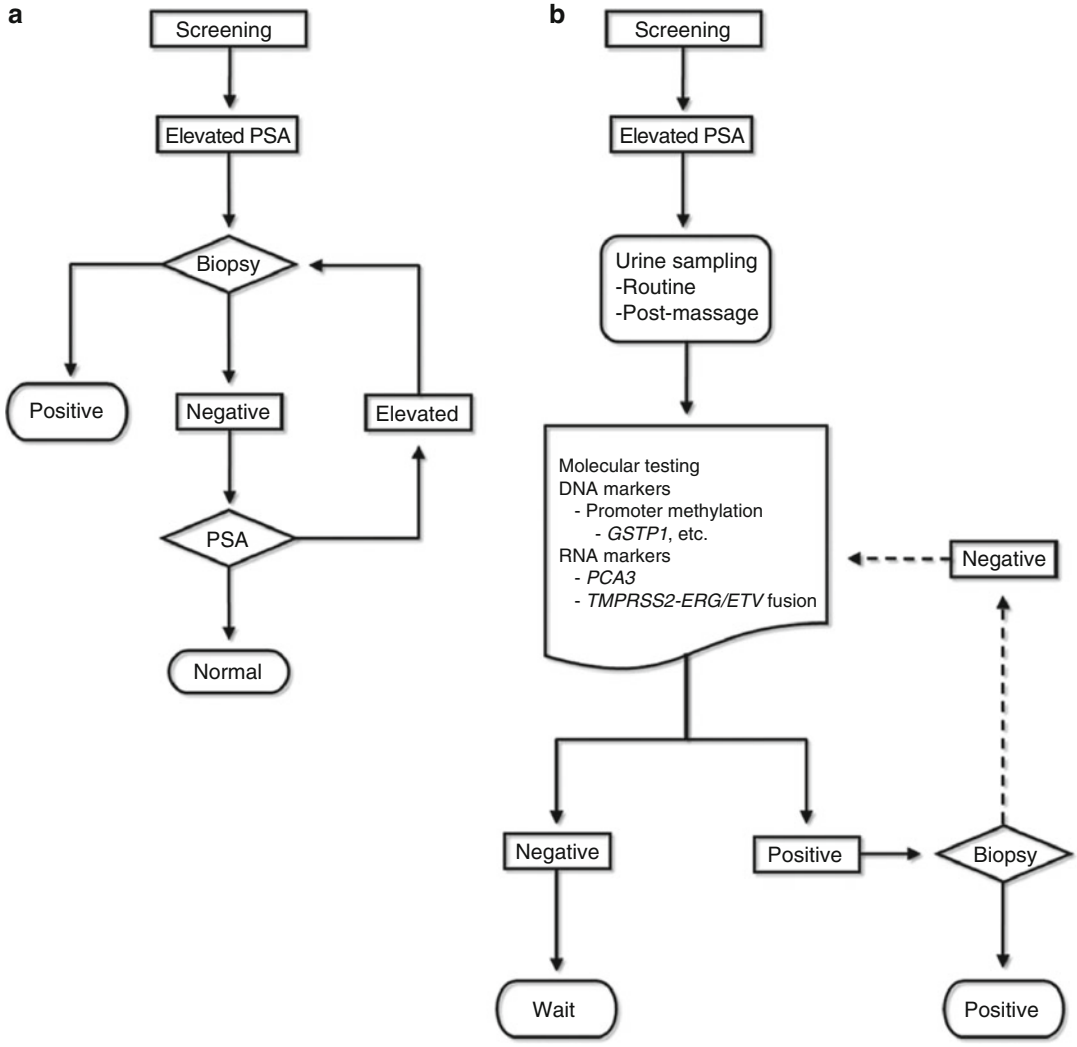

Fig. 10.8 (**a**) Current guidelines in the management of patients with elevated prostate-specific antigen (PSA) at the time of screening. (**b**) The inclusion of molecular testing for prostate cancer markers may help in predicting a positive prostate biopsy, therefore, reducing the number of patients that would otherwise enter an "elevated PSA, negative biopsy" loop (*dashed line*). It is also likely that molecular-based urine testing may supplant, or be used in combination with, serum PSA testing for screening for prostate cancer in general. (From Gurel 2008 Adv Anat Pathol., with permission.)

altered to both increase diagnostic accuracy and decrease overutilization of invasive procedures (Fig. 10.8)

- The prognostic capabilities of the molecular alterations in prostate cancer are not currently well understood
  - *TMPRSS2-ERG* rearrangement has gained much interest and some studies have shown that the presence of the rearrangement is strongly correlated with a poor prognosis in patients meeting a "watchful waiting" criteria
  - Others have refuted these findings and claim an improved prognosis in patients with *TMPRSS2-ERG* rearrangements—the prognostic capability of the fusion is still controversial
  - Hypermethylation of *PITX2* has been shown to have a strong correlation with prostate cancer progression

  - Loss of *PTEN* and increased cellular proliferation are associated with prostate cancer progression
- Limited targeted therapies are currently available to patients with the described molecular alterations
  - *PTEN* is one of the most promising targets—decreased expression of PTEN may signal efficacy in AKT inhibition, especially in combination with therapies targeting the AR pathway
  - Overexpression of SPINK1 occurs in a subset of prostate cancers and it is postulated that SPINK1 can be inhibited with the same success as EGFR has been in colon and lung cancer

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Molecular Pathology of Urinary Bladder Cancer

11

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Contents

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Oncogenic Pathways in Urothelial Carcinoma of the Urinary Bladder

- Nonmuscle invasive (NMI; superficial) and muscle invasive (MI) urothelial carcinoma (UC) of the bladder display two distinct clinical phenotypes in regard to biologic behavior and prognosis
  - Two distinct broad pathogenic pathways in bladder cancer development
    - The majority of invasive UCs are thought to originate through progression from dysplasia to flat carcinoma in situ (CIS) and high-grade noninvasive lesions
    - Superficial urothelial lesions are thought to originate from benign urothelium through a process of urothelial hyperplasia
  - 10–15% of the entire pool of noninvasive lesions ultimately progress to MI-UC disease
  - Genetic instability facilitates the accumulation of genetic alterations required for progression to MI-UC
- Clinically, a significant proportion of NMI-UC (pTa and pT1) will recur following transurethral

L. Cheng and J.N. Eble (eds.), *Molecular Surgical Pathology*,
DOI 10.1007/978-1-4614-4900-3_11,

resection (TURB) with only a minority of cases enduring progression to high-grade carcinoma that will ultimately progress to MI-UC

- Three primary genetic alterations are associated with the pathogenesis pathway of NMI-UC: tyrosine kinase receptor FGFR3, HRAS, and PIK3CA
  - RAS-MAPK and PI3K-AKT pathway alterations are responsible for promoting cell growth in urothelial neoplasia
  - Activating mutations in *RAS* leads to activation of mitogen-activated protein kinases (MAPK) and PI3K pathways
  - Activating mutations in upstream tyrosine kinase receptor *FGFR3* seems to be mutually exclusive with *RAS* mutations given that both signal through a common downstream pathway in urothelial oncogenesis
  - *PIK3CA* and *FGFR3* mutations generally cooccur, suggesting a potential synergistic additive oncogenic effect of *PIK3CA* mutations
- The pathogenic pathway for MI-UC primarily involves alterations in tumor suppressor genes (TSG) involved in cell cycle control including *TP53, p16,* and *RB* (see Figs. 11.1 and 11.2)
  - Progression of the subset of NMI-UC into higher-grade MI-UC disease is similarly based on alterations in *TP53* and *RB* TSG (Fig. 11.1)

Prognostic Biomarkers in Bladder Cancer

- Established clinicopathologic prognostic parameters for NMI-UC
  - pT stage
  - WHO/ISUP grade
  - Tumor size
  - Tumor multifocality
  - Presence of CIS
  - Frequency and rate of prior recurrences
- Prognostic parameters to accurately predict progression in patients with NMI-UC tumors are actively sought to identify patients in need of vigilant surveillance and aggressive treatment

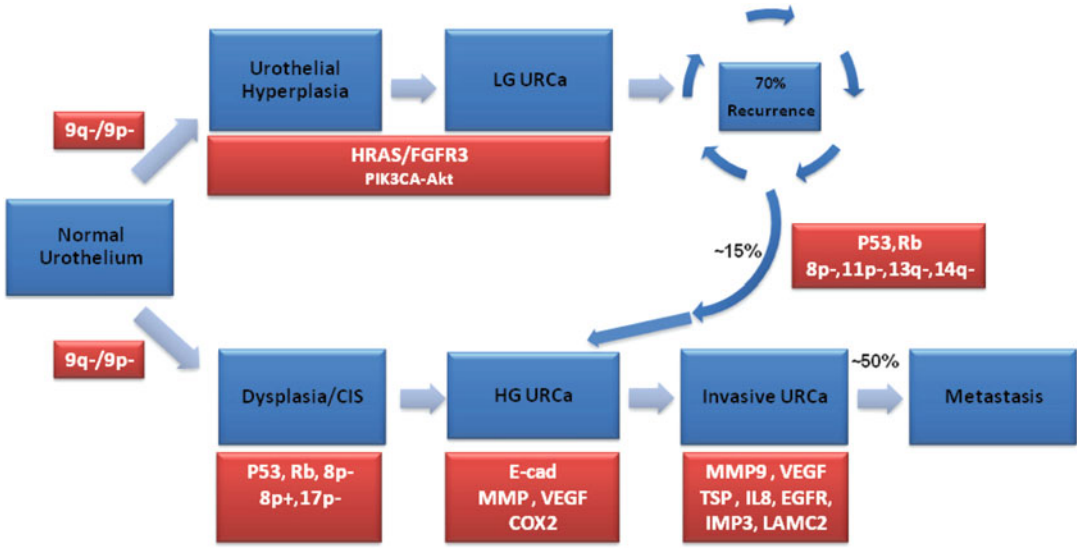

Fig. 11.1 Divergent molecular pathways of oncogenesis in NMI and MI urothelial carcinoma of urinary bladder; genetic alterations are depicted in key stages of disease progression. *URCa* urothelial carcinoma of urinary bladder; *LG* noninvasive low grade; *HG-URCa* noninvasive high grade. (From Netto GJ and Cheng L. Emerging critical role of molecular testing in diagnostic genitourinary pathology. Arch Pathol Lab Med. 2012;36:372–390; with permission.)

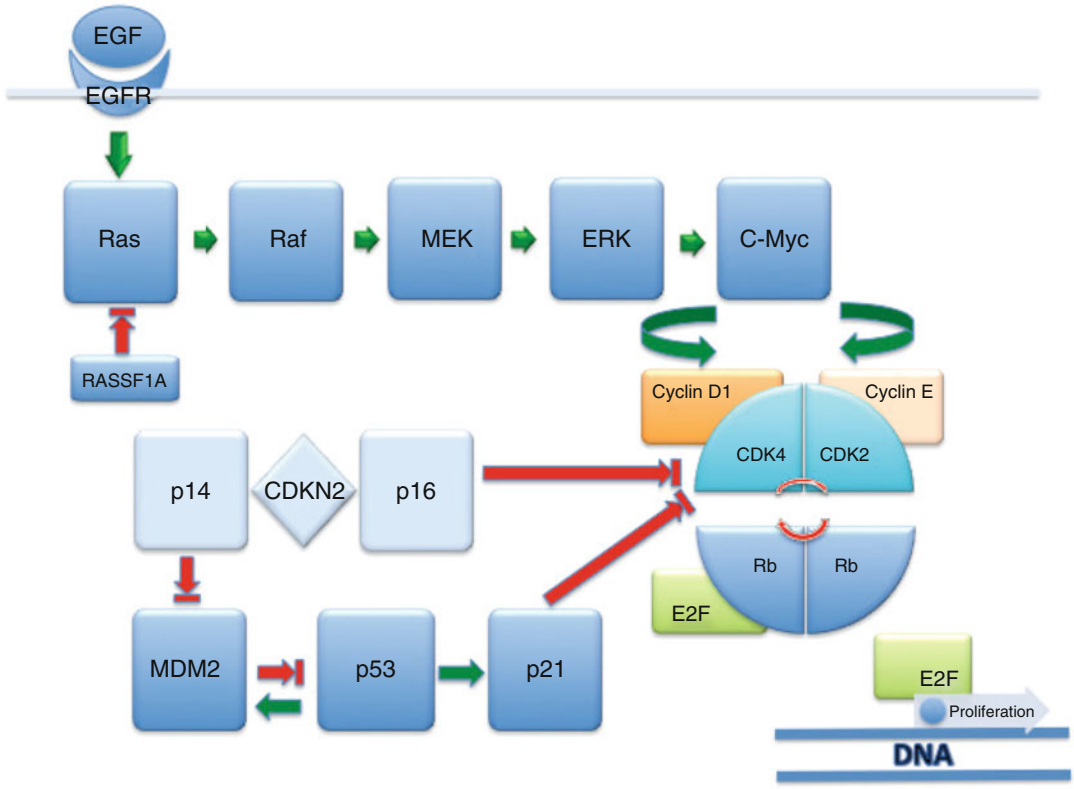

Fig. 11.2 Receptor tyrosin kinase (EGFR/RAS/MEK/ERK) and cell-cycle regulator (p14, p16, p53, p21, Cyclin D1, Cyclin E, and Rb) pathways in urothelial carcinoma; *green* and *red arrows* represent stimulation and inhibition, respectively. (From Netto GJ and Cheng L. Emerging critical role of molecular testing in diagnostic genitourinary pathology. Arch Pathol Lab Med. 2012;36:372–390; with permission.)

  - The financial burden and quality of life for patients under surveillance can be significant
- Bladder cancer is the most expensive single solid tumor in the United States
  - Estimated annual cost to our healthcare system, $3 billion (US)
  - Poor outcome of MI-UC disease (60% or less overall survival rate)
  - Markers that can improve prognostication are needed
- The translational field of molecular prognostication, theranostics, and targeted therapy in bladder cancer has sharply gained momentum with our understanding of molecular pathways involved in urothelial oncogenesis
- A rigorous validation process ought to precede the incorporation of such molecular biomarkers in clinical management
  - Initial retrospective discovery studies need to be confirmed and validated in large independent cohorts
  - The subsequent crucial step is validating the robustness of the proposed biomarker in a well-controlled multi-institutional randomized prospective study
- Prospective study should support an additive role for the inclusion of the new biomarker over existing management algorithm(s)
  - The lack of the above crucial steps in biomarkers development has hindered the streamlining of clinical utilization of several promising markers

Chromosomal Numerical Alterations, Early Culprit of Genetic Instability in Bladder Cancer

- Chromosome 9 alterations are the earliest genetic alterations in both of the above-described divergent pathways of bladder cancer development
  - Responsible for providing the necessary milieu of genetic instability that in turn allows for the accumulation of subsequent genetic defects
- Several additional structural/numerical somatic chromosomal alterations are also a common occurrence in bladder cancer
  - Gains of chromosomes 3q, 7p, and 17q and deletion of 9p21 (p16 locus) are of special interest, given their potential diagnostic and prognostic value
- A multitarget interphase FISH-based urine cytogenetic assay was developed based on the above numerical chromosomal alterations and is now commercially available and commonly used in clinical management
  - Initially FDA approved for surveillance of recurrence in previously diagnosed bladder cancer patients and subsequently gained approval for screening in high-risk (smoking exposure) patients with hematuria
  - Appears to enhance the sensitivity of routine urine cytology analysis and can be used in combination with routine cytology as a reflex testing in cases with atypical cytology
  - Sensitivity range of 69–87% and specificity range of 89–96% have been reported
  - More sensitive than routine cytology
- An additional advantage could be the anticipatory positive category of patients identified, patients where FISH assay detects molecular alteration of bladder cancer in urine cells several months prior to cancer detection by cystoscopy or routine cytology
  - Two-thirds of patients categorized as "anticipatory positive" develop bladder cancer
  - Potential early detection and allocation of vigorous frequent followup cystoscopy in at-risk patients
- Recent studies have pointed to the potential prognostic role for multitarget FISH analysis (Fig. 11.3)
  - Low-risk FISH-positive patients, defined as 9p21 loss/Ch3 abnormalities, have a higher rate of recurrence compared to FISH-negative patients
    - The recurrence rate is even greater in patients with a high-risk positive FISH (Ch7/Ch17 abnormality)
  - Using bladder washings and formalin-fixed, paraffin-embedded (FFPE) transurethral biopsy samples, loss of 9p21 predicts recurrence but not progression in NMI-UC
  - Urine cytology and FISH in post-Bacillus Calmette Guérin (BCG) bladder washings predict failure to BCG therapy in patients with NMI disease
- Such promising prognostic role for multitarget FISH awaits prospective randomized trial before clinical integration into practice algorithm as well as clear guidelines for interpretation and test performance parameters in term of interobserver reproducibility

Receptor Tyrosine Kinase Alterations

- Recent studies have pointed to the potential prognostic value of evaluating the expression of receptor tyrosine kinases (RTK) such as FGFR3, EGFR, and other ERB family members (HER2 and ERBB3) in NMI-UC and MI-UC diseases
- *FGFR3* mutations commonly occur in NMI-UC; theoretically, they can be used alone or combined with *RAS* and *PIK3CA* oncogenes as markers of early recurrence during surveillance
  - Sensitive PCR assays have been developed for detecting *FGFR3* mutations in voided urine
    - Positive urine sample associated with concomitant or future recurrence in 81% of NMI-UC cases
    - A predictive value of 90% was achieved in patients with consecutive *FGFR3*-positive urine samples

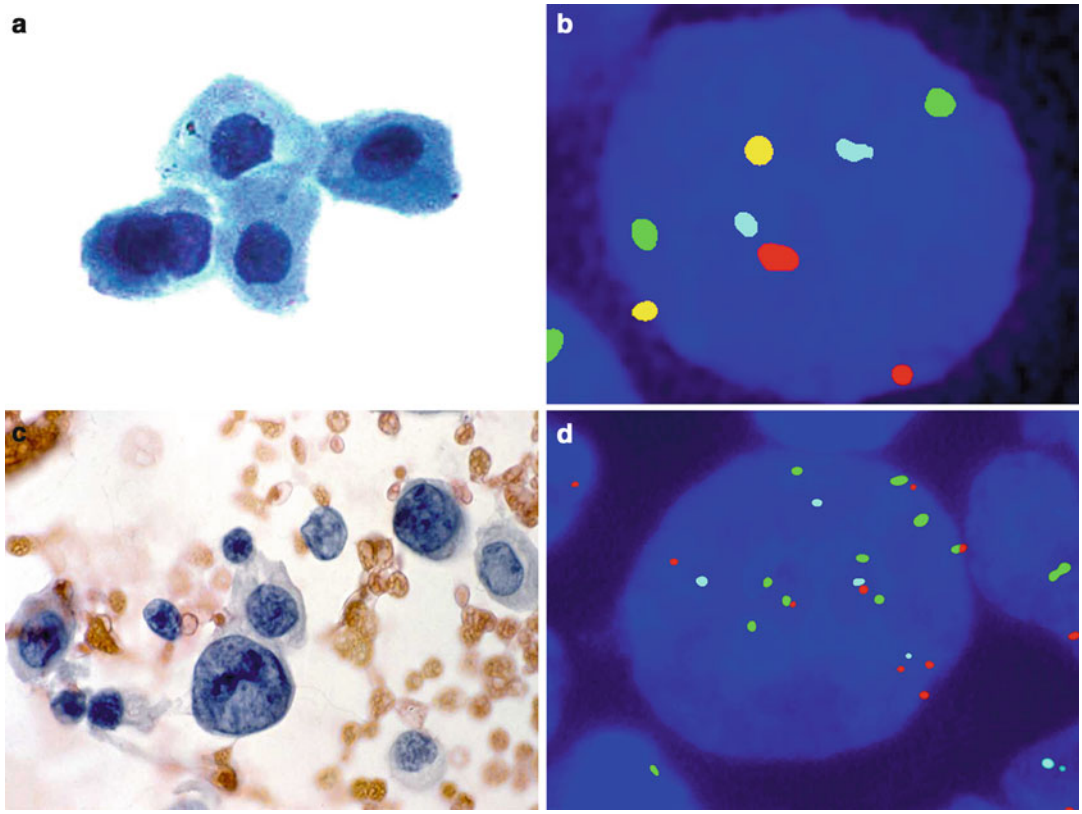

Fig. 11.3 Detection of urothelial carcinoma by the UroVysion FISH analysis. Normal urothelial cells (**a**) showed two signals from each probe for CEP3 (*red*), CEP7 (*green*), CEP17 (*aqua*), and 9p21 (*gold*) (**b**). Malignant urothelial cells (**c**) demonstrated gaining of chromosomes as indicated by 7 *red* (CEP3), 9 *green* (CEP7), 4 *aqua* (CEP17), and loss of 9p21 as indicated by the absence of *yellow* signals (9p21) (**d**). (From Cheng L et al. Bladder cancer: Translating molecular genetic insights into clinical practice. Hum Pathol 2011;42:455–481; with permission.)

    - Detection of *FGFR3* mutations in 53% of patients
    - Superior to cytology (78% vs. 0%) in detecting post-TURB recurrence in NMI-UC harboring *FGFR3* mutations in primary tumors
- A multiplex PCR assay has been developed for mutational analysis detecting the most frequent mutation hot spots of *HRAS, KRAS, NRAS, FGFR3,* and *PIK3CA* in FFPE TURB samples
  - Evidence of at least one mutation in up to 88% of low-grade NMI-UC samples was demonstrated
  - Revealed *FGFR3* mutations to be more common among low malignant potential neoplasms (LMPN; 77%) and TaG1/TaG2 tumors (61%/58%) than among TaG3 (34%) and T1G3 tumors (17%)
  - Mutations were associated with increased risk of recurrence in NMI-UC
- A molecular grade parameter (mG) based on a combination of *FGFR3* gene mutation status and MIB1 index as an alternative to pathologic grade in NMI-UC has been proposed
- Van Rhijn et al. (*Eur Urol*, 2010) elegantly validated their previously proposed mG parameter and compared it to the European Organization for Research and Treatment of Cancer (EORTC) NMI-UC risk calculator (weighted score of six variables including WHO 1973 grade, stage, presence of CIS, multiplicity, size, and prior recurrence rate)

  - mG was more reproducible than the pathologic grade (89% vs. 41–74%)
  - *FGFR3* mutations significantly correlated with favorable disease parameters, whereas increased MIB1 was frequently seen with pT1, high grade, and high EORTC risk scores
  - EORTC risk score remained significant for recurrence and progression
  - mG maintained independent significance for progression and disease-specific survival
  - The addition of mG for progression increased the predictive accuracy from 74.9% to 81.7%
- Several studies have suggested a negative prognostic role for HER2 amplification/overexpression in MI-UC
- HER2-positive MI-UC patients have twice the increased risk for recurrence and cancer-specific mortality as seen on multivariable analyses adjusted for pathological stage, grade, lymphovascular invasion, lymph node metastasis, and adjuvant chemotherapy

p53, Cell Cycle Regulators, and Proliferation Activity Index

- p53 alterations are a strong independent predictor of disease progression in bladder cancer (NMI-UC, MI-UC, as well as CIS)
- p53 is predictive of increased sensitivity to chemotherapeutic agents that lead to DNA damage
  - Recent studies have further supported the prognostic role of p53 in pT1–pT2 patients following cystectomy showing an independent role for p53 alteration in predicting disease-free survival (DFS) and disease-specific survival (DSS)
- Among other G1-S phase cell cycle regulators, cyclin D3, cyclin D1, p16, p21, and p27 have also been evaluated as prognosticators in NMI-UC
  - Cyclin D3 and cyclin D1 overexpression has been found to predict progression in pTa and pT1 tumors
    - These findings are in contrast to subsequent findings, emphasizing the need for further validation in large multi-institutional cohorts of patients
- A synergistic prognostic role for combining p53 evaluation with other cell cycle control elements such as pRB, cyclin E1, p21, and p27 is emerging in both NMI-UC and MI-UC
  - NMI-UC patients with TURB demonstrating synchronous immunohistochemical alterations in all four tested markers (p53, p21, pRB, and p27) are at significantly lower likelihood of sustaining DFS compared to patients with only three markers
  - The negative predictive effect was decreased with decreasing number of altered markers (3 vs. 2 vs. 1)
  - Combining p53, p27, and Ki67 assessment in pT1 radical cystectomy specimens improved the prediction of DFS and DSS
- A similar synergistic prognostic role for the assessment of immunoexpression of multiple molecular markers (p53, pRB, and p21) has been demonstrated in patients undergoing cystectomy for MI-UC
  - The superiority of multimarker approach compared to prior single-marker approach merits further assessment
  - Such multimarker approach of prognostication could soon be integrated in the standard of care in bladder cancer management
- Tumor proliferation index measured immunohistochemically by either Ki67 or MIB1 has been consistently shown to be a prognosticator in bladder cancer
  - Tumor proliferation index (MIB1) in NMI-UC plays a prognostic role as one of the elements of the mG parameter
  - The independent prognostic role of proliferation index measured by Ki67 has also been shown
  - Ki67 index in NMI-UC TURB biopsy is predictive of DFS and DSS
- A similar role for proliferation index assessment as prognosticator is established in MI-UC

  - Building on initial findings of significance in an organ-confined subset of MI-UC, a bladder consortium multi-institutional trial confirmed the role of proliferation index, measured in cystectomy specimens
  - Ki67 improved prediction of both DFS and DSS when added to standard prediction models, supporting a role for proliferation index assessment in stratifying patients for perioperative systemic chemotherapy
  - Ki67 assessment is a step closer to clinical applicability in MI-UC

Gene Expression and Genomic Analysis in Urothelial Carcinoma

- Several recent gene expression studies have highlighted sets of differentially expressed genes that may play a role in diagnosis and in predicting recurrence and progression in bladder cancer
  - Oligonucleotide arrays were used to analyze transcript profiles of NMI-UC and MI-UC
    - Predictive algorithms were 89% accurate for tumor staging using genes differentially expressed in NMI vs. MI tumors
    - Accuracies of 82% (entire cohort) and 90% (MI-UC) were obtained for predicting overall survival
    - A genetic profile consisting of 174 probes was able to identify patients with positive lymph nodes and poor survival
  - Two independent Global Test runs confirmed the robust association of the suggested profile with lymph node metastases and overall survival simultaneously
  - One of the top-ranked genes coding for a soluble protein, synuclein, was selected from the gene expression profile to evaluate association with its prognostic significance
  - Immunohistochemical analyses on tissue arrays confirmed the significant association of synuclein with tumor staging and clinical outcome independent of clinicopathologic parameters
- An attempt has been made to identify genes predictive for recurrence and progression in Ta using a quantitative pathway-specific approach in a set of 24 key genes by real-time PCR in tumor biopsies at initial presentation
  - CCND3 expression was found to be highly sensitive and specific for recurrence (97% and 63%, respectively)
  - HRAS, E2F1, BIRC5/surviving, and VEGFR2 were predictive for progression by univariate analysis
  - The combination of HRAS, VEGFR2, and VEGF expression status predicted progression with an impressive 81% sensitivity and 94% specificity on multivariable analysis
- Combined molecular and histopathologic classification of bladder cancer may prove more powerful in predicting outcome and stratifying treatment
  - A combined gene expression analysis, whole-genome array-CGH analysis, and mutational analysis of *FGFR3, PIK3CA, KRAS, HRAS, NRAS, TP53, CDKN2A*, and *TSC1* identified two intrinsic molecular signatures (MS1 and MS2)
  - Genomic instability was the most distinguishing genomic feature of MS2 signature, independent of *TP53/MDM2* alterations
  - Genetic signatures classified UCs into low-grade and high-grade tumors as well as NMI-UC and MI-UC with high precision and sensitivity
  - A gene expression signature that independently predicts metastasis and DSF was also defined, supporting the role of molecular grading as a complement to standard pathologic grading
- Gene expression analysis by TaqMan Arrays found a 12 + 2 gene expression signature demonstrating 98% sensitivity and 99% specificity in discriminating between bladder cancer and control and 79% sensitivity and 92% specificity in predicting tumor aggressiveness (NMI-UC vs. MI-UC)

- Mutational screen of a set of six genes (*FGFR3, PIK3CA, TP53, HRAS, NRAS,* and *KRAS*) and quantitatively assessed promoter methylation status of 11 additional genes (*APC, ARF, DBC1, INK4A, RARB, RASSF1A, SFRP1, SFRP2, SFRP4, SFRP5,* and *WIF1*) in NMI-UC tumor biopsies and corresponding urine samples detected oncogenic mutations and hypermethylation events
  - The total panel of markers provided a sensitivity of 93% and 70% in biopsies and urine samples, respectively
  - *FGFR3* mutations in combination with three methylation markers (*APC, RASSF1A,* and *SFRP2*) provided a sensitivity of 90% in tumors and 62% in urine with 100% specificity
- Selecting patients for neoadjuvant chemotherapy on the basis of risk of node-positive disease has the potential to benefit high-risk patients while sparing other patients toxic effects and delay to cystectomy
  - Smith et al. (*Lancet Oncol*, 2011) recently reported a 20-gene expression model to predict the pathological node status in primary tumor tissue from three independent cohorts that clinically lacked evidence of nodal metastasis
  - The model was able to predict nodal status independent of standard clinicopathologic prognostic criteria
  - The validation step in an independent cohort is vital, given recent reports that gene expression tumor signatures are not portable across cohorts
- With the impending cost and turnaround time advantages of next-generation sequencing technology, the power of genomic approach in providing a noninvasive diagnostic and predictive tool should be actively pursued

Epigenetic Alterations

- Epigenetic analysis is also gaining momentum in bladder cancer as a noninvasive diagnostic tool for screening and surveillance
  - As a prognostic tool, epigenetic analysis has similarly shown promising potential in bladder cancer patients
- Hypermethylation analysis at 11 CpG promoter islands, performed by MSP-PCR
  - Promoter methylation was found in 86% of all tumors and the incidence was relatively higher in upper tract tumors compared to bladder cancer
  - Methylation was associated with advanced tumor stage and higher tumor progression and mortality rates
  - Methylation at the *RASSF1A* and *DAPK* gene promoters was associated with disease progression independent of tumor stage and grade on multivariate analysis
- Five loci associated with progression (*RASSF1a, CDH1 [E-cadherin], TNFSR25, EDNRB*, and *APC*) were found using quantitative methylation-specific PCR (MSP) at 17 candidate gene promoters
  - Multivariate analysis revealed that the overall degree of methylation was more significantly associated with subsequent progression and death than tumor stage
  - An epigenetic predictive model developed using artificial intelligence techniques identified likelihood and timing of progression with 97% specificity and 75% sensitivity
- The diagnostic role of promoter hypermethylation was evaluated by Lin et al. (*Clin Genet*, 2006) using MSP assay in four genes (*ECDH1, p16, p14,* and *RASSF1A*) in primary tumor DNA and urine sediment DNA
  - MSP detected hypermethylation in the urine of 80% of tested patients
  - Hypermethylation analysis *of CDH1, p14,* or *RASSF1A* in urine sediment DNA detected 85% of superficial and low-grade bladder cancer and 79% of high-grade and 75% of invasive bladder cancers
  - The study highlighted the great potential of such test in detecting NMI-UC
- Methylation-specific multiplex ligation-dependent probe amplification assay

(MS-MLAP) analysis of 25 TSGs thought to play a role in bladder cancer oncogenesis
  - The TSG included *PTEN, CD44, WT1, GSTP1, BRCA2, RB1, TP53, BRCA1, TP73, RARB, VHL, ESR1, PAX5A, CDKN2A,* and *PAX6*
  - *BRCA1, WT1,* and *RARB* were found to be the most frequently methylated TSGs with receiver operating characteristic curve analyses revealing significant diagnostic accuracies in two additional validation sets
- Assessment of promoter hypermethylation is giving additional insights on bladder cancer oncogenesis
  - Promoter hypermethylation of CpG Islands and "shores" controlling miRNA expression is one such example

Ploidy and Morphometric Analysis

- Several studies have pointed to the independent prognostic role of ploidy and S-phase analysis in NMI-UC
  - Ploidy analysis can be performed by flow cytometry or automated image cytometry (ICM)
  - Applicable to urine cytology specimens as well as biopsy supernatant and disaggregated TURB FFPE specimens
- Stage, DNA ploidy, tumor multiplicity, history of recurrence, tumor configuration, and type of adjuvant therapy independently predict recurrence
  - Recurrence at 3-months, grade, and DNA ploidy were the only predictors of progression to muscle invasion
- Ploidy status and S phase measured by ICM are strong independent predictors of recurrence and progression in pTa and pT1 patients
- Despite all the above encouraging data, ploidy analysis still awaits prospective randomized trials to bring ICM or flow cytometry technique into current standard management algorithms for NMI-UC

Additional Emerging Prognostic Biomarkers

- There are other biomarkers with encouraging but less robust data on their potential prognostic role in bladder cancer
  - Tumor microenvironment markers such as cell adhesion markers such as *CDH1* and *CDH2 (N-cadherin)* and angiogenesis modulators such as HIF1a, HIF2, VEGF, CA-IX, and *THBS1*
  - mTOR pathway markers
  - Other markers such as AURKA have also been investigated in this setting
- miRNA profile alterations will certainly be a new area of heavy investigation as a noninvasive diagnostic tool and as a prognostic tool in bladder cancer patients

New Targets of Therapy and Predictive Biomarkers in Urothelial Carcinoma

- RTK/HRAS/MAPK, mTOR, as well as angiogenesis pathway of the tumor microenvironment offer promising opportunities for new targeted treatments of bladder cancer (Fig. 11.2)
- Among receptor tyrosin kinases, HER2 has been targeted in a multicenter phase II trial reported in 2007 by Hussain et al. (*J Clin Oncol*, 2007)
  - Advanced bladder cancer patients with metastatic disease and evidence of tumor HER2 positivity were treated with a combination of carboplatin, paclitaxel, and gemcitabine with the humanized monoclonal anti-HER2 antibody trastuzumab
  - Approximately 70% of treated patients demonstrated partial (59%) or complete (11%) response with a median overall survival of 14.1 months
  - A higher response rate was associated in patients with 3+ HER2 expression by immunohistochemistry (IHC) or *HER2* gene amplification by FISH compared to those with 2+ HER2 expression and FISH-negative tumors
  - Interestingly, in contrast to the strongly correlated *HER2* gene amplification and

3+ IHC HER2 overexpression usually seen in breast cancer, the majority of HER2 overexpression in bladder UC is not associated with *HER2* gene amplification

- A second ongoing randomized phase II trial is evaluating the role of anti-EGFR recombinant humanized murine monoclonal antibody Cetuximab
  - Patients with metastatic, locally recurrent, or nonresectable disease are treated with standard GC (gemcitabine and carboplatin) chemotherapy with or without Cetuximab
  - By blocking EGF binding to the extracellular EGFR domain, Cetuximab inhibits downstream signal transduction pathway accounting for its antiproliferative activity in solid tumors
  - In bladder cancer, a potential added synergistic antiangiogenic effect could be also at play
  - A separate phase II Cancer and Leukemia Group B trial investigated the role of a small molecule inhibitor of EGFR (Gefitinb) in advanced bladder cancer patients
  - Gefitinib, in combination with GC, had no survival or time to progression advantage over GC alone
- A phase II single arm trial suggested a therapeutic advantage for Lapatinib (a tyrosine kinase inhibitor (TKI) targeting both EGFR and HER2) in EGFR-positive or HER2-positive bladder cancer tumors
  - A phase II/III randomized trial is looking at the role of maintenance with Lapatinib (vs. placebo) in patients with objective response to first-line chemotherapy and positive for either marker by IHC or FISH studies
- In an attempt to target bladder cancer dependence on angiogenesis, monoclonal antibodies and small molecule inhibitors of angiogenesis are under investigation in advanced disease
  - An initial phase II trial evaluating the role of Bevacizumab, a recombinant humanized monoclonal anti-VEGF antibody, in combination with GC as a first-line therapy in metastatic bladder cancer, revealed objective response in two-thirds of patients with 14% showing complete response, albeit with significant treatment-related toxicity
  - A CALBG phase III randomized trial for GC with and without Bevacizumab for metastatic UC is underway as well as other phase II trials for Bevacizumab in combination with other chemotherapeutic agents such as M-VAC (methotrexate, vinblastine, adriamycin, and cisplatin)
- The role of multitarget TKI in bladder cancer has been investigated with mixed results
  - Sorafenib (inhibits *RAF* kinase*, PDGFRB, VEGFR2,* and *VEGFR3*) phase II trials have failed to show significant objective response
  - Sunitinib (inhibits *VEGFR2* and *PDGFRB*) has shown a more promising effect in a recent phase II trial where clinical benefit was observed in almost one-third of patients
  - A subsequent randomized double-blind phase II trial is underway, investigating the efficacy of Sunitinib in delaying progression as a maintenance agent in patients with initial response to standard chemotherapy
  - Given the recent evidence suggesting the presence of mTOR pathway alterations in bladder cancer, a phase II trial evaluating the potential role of Everolimus, an inhibitor of mTOR pathway, in advanced bladder cancer is underway

Major Urothelial Carcinoma-Related Molecular Pathways

FGFR3 Pathway

- Over 70% of low-grade noninvasive papillary urothelial neoplasms harbor *FGFR3* mutations (Fig. 11.4), strongly implying that *FGFR3* activating mutation is one of the key genetic events underlying the genesis of low-grade urothelial tumors

  - *FGFR3* is a member of four highly conserved and structurally related tyrosine kinase receptor genes
  - Located at chromosome 4p16.3 and is composed of 19 exons spanning 16.5 kb
  - Encodes an 806-amino acid protein belonging to the fibroblast growth factor receptor family
  - The protein consists of an extracellular region, composed of three immunoglobulin-like domains, a single hydrophobic transmembrane segment, and a cytoplasmic tyrosine kinase domain
  - Extracellular portion interacts with fibroblast growth factors and initiates cascades of downstream signals, ultimately influencing cell growth, migration, differentiation, and angiogenesis
  - Alternative splicing between exons 8 and 9 creates two different variants of the juxtamembrane Ig-like domain, isoforms *FGFR3*b and *FGFR3*c
    - These alternative mRNA splicing forms are tissue specific
    - *FGFR3*b is the expression form of epithelial cells
    - *FGFR3*c is predominantly expressed in mesenchymal cells
- Signal transduction pathways for *FGFR3* receptors are shared by many RTK
  - Mutations between the IgII and IgIII domains (exon 7) are by far the most common, accounting for 50–80% of all mutations
  - Mutations affecting the transmembrane domain (exon 10) account for 15–40%
  - Mutations affecting tyrosine kinase 2 domain (exon 15) account for 5–10%
  - Mutations located at exons 5 and 10 often create a novel cysteine
    - May be responsible for receptor dimerization and tyrosine kinase phosphorylation in the absence of ligand, resulting in constitutive activation
- *FGFR3* activation triggers several downstream kinase pathways
  - Most important is the *RAS* cell cycle regulation pathway
    - Induces mitogenic signals
    - Plays a central role in the proliferation and renewal of epithelial cells
  - *FGFR3* and *RAS* mutations seem to be mutually exclusive
    - Both may be alternative paths to a similar phenotype
  - Alternatively, activated *FGFR3* activates the phosphatidylinositol 3-kinase (*PI3-K*)
    - Generates specific inositol lipids for regulation of cell growth, proliferation, survival, differentiation, and cytoskeletal changes
  - Activated *FGFR3* can also trigger the signal transducer and activator of transcription (*STAT*) pathway
    - Interacts with proline-rich tyrosine kinase 2 (*PYK2*), leading to further *STAT* pathway activation
- *FGFR3* mutations were discovered in individuals with thanatophoric dysplasia and hypochondroplasia
  - These mutations seem to mediate opposing signals
    - Acting as a negative regulator of growth in bone and as transforming oncogene in several epithelial tumor types
  - Expression of a mutated, constitutively activated *FGFR3* in bladder and cervical cancer has been reported
  - Experiments indicate that mutations of *FGFR3* gene can transform NIH3T3 cells when targeted to the kinase domain
  - Knockdown of the S294C mutation of *FGFR3*b in tumor cells inhibits cell proliferation and clonogenicity
- *FGFR3* appears to be the most frequently mutated oncogene in bladder cancer
  - Such mutations in UC are frequently found in exons 7, 10, and 15
  - Ranked by frequency, *FGFR3* mutations are found on exon 7 codon 249 (63%), exon 10 codon 375 (18%), exon 7 codon 248 (9%), and exon 10 codon 372 (6%)
  - Mutation is strongly associated with low recurrence rates in superficial papillary bladder cancers

  - van Rhijn et al. analyzed bladder cancer patients and found *FGFR3* mutation in 64% of pTaG1–2 bladder cancers
    - None of the patients with higher-stage tumors had *FGFR3* mutations
  - A 12-month followup study on patients with superficial bladder cancer revealed that 61% of the patients in the wild-type *FGFR3* group developed recurrence, compared with only 20% of patients in the mutant group
  - The per year recurrence rate was 4.7-fold lower for tumors with *FGFR3* mutation than for tumors with wild-type *FGFR3* gene
- It is postulated that mutation in *FGFR3* results in activation of the RAS-MAPK pathway
  - Such activation has not been proven in all *FGFR3* mutated tumors
  - Most *FGFR3* mutations are known to result in constitutive activation of the receptor
  - Evidence suggests that *FGFR3* mutations give a growth advantage to affected cells, but that cell cycle regulation and apoptosis mechanisms remain intact
  - This may explain the differences between indolent and aggressive urothelial cancers, the latter bearing *TP53* mutations that cause impaired apoptosis
  - The reported frequency of *FGFR3* mutation in pTa tumors is 70–80%
    - There are several specific missense mutations associated with these tumors
  - These mutations are significantly associated with low tumor grade, early stage, and low recurrence rate in tumors
  - Urothelial cancer bearing *FGFR3* mutation confers a better overall prognosis than cancer bearing *TP53* mutation
  - *TP53* mutations appear to indicate a worse prognosis and higher recurrence rate
  - *FGFR3* mutation is detectable in only 10–20% of invasive tumors
    - Suggest strongly that *FGFR3* activating mutation is one of the key genetic events in the genesis of low-grade noninvasive papillary bladder tumors
  - Activating *FGFR3* mutations are found most frequently in G1 tumors (80%)
  - Low-grade tumors show a strong correlation with *FGFR3* expression (Figs. 11.4 and 11.5)

TP53 Pathway

- Alterations of the *TP53* TSG play a crucial role in the carcinogenesis of many tumors, including bladder urothelial cancers
  - Located at the short arm of chromosome 17 (17p13.1), spans 19.2 kb, and is composed of 11 exons
  - Encodes a 393-amino acid protein (p53) regulating the cell cycle, DNA repair, and apoptosis
  - The N-terminus of p53 contains several functional domains
    - The activation domain 1 (amino acids 1–42) activates transcription of downstream factors
    - Activation domain 2 (amino acids 43–63) regulates apoptotic activity
    - The proline-rich domain (amino acids 80–94) is also important in apoptosis
    - The DNA-binding domain (amino acids 100–300) activates downstream transactivation of other genes
    - The nuclear localization signaling domain (amino acids 316–325) and the homo-oligomerization domain (amino acids 307–355) are essential for the structure and homing of p53
- *TP53* mutations in cancers are usually missense, loss-of-function mutations occurring in the DNA binding domain
  - These impair the binding of p53 to its target DNA, further affecting transcriptional activation of downstream genes
  - Mutant forms of p53 can also dimerize with wild-type p53, blocking its function
  - *TP53* mutations induce a series of downstream effects, including decreased expression of p21
  - An important downstream target of p53
    - Downregulated in the majority of

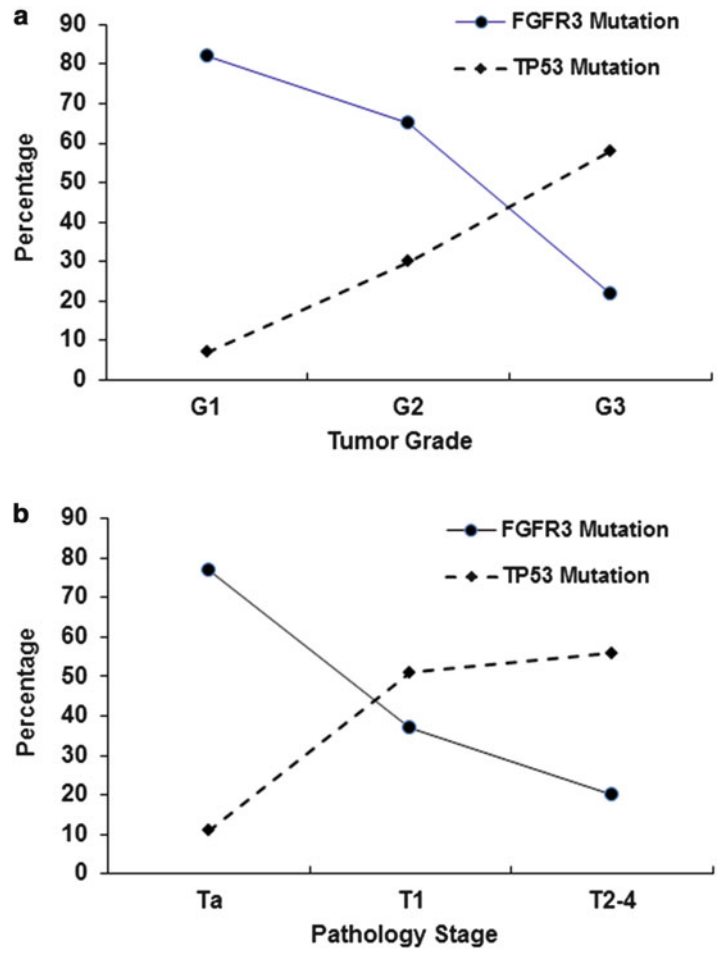

Fig. 11.4 *FGFR3* and *TP53* mutations according to tumor grade (**a**) and pathology stage (**b**) based on available literature. (From Cheng L et al. Bladder cancer: Translating molecular genetic insights into clinical practice. Hum Pathol 2011;42:455–481; with permission.)

urothelial carcinomas with *TP53* mutations (Fig. 11.4)

- Numerous studies have indicated that *TP53* mutations are strongly associated with high tumor grade, invasive behavior, risk of recurrence, and adverse clinical outcome
  - *TP53* mutations are generally mutually exclusive of *FGFR3* mutations
    - The rate of *TP53* mutation is twofold higher in high-grade urothelial cancers than in low-grade tumors
  - When assessing high-grade NMI tumors, *FGFR3* and *TP53* mutations are not mutually exclusive
    - Suggest that doubly mutant T1G3 tumors are either at the crossroads of the two main molecular pathways or
    - Represent progression from low-grade papillary tumors to high-grade tumors by acquisition of *TP53* mutations

Molecular Markers, Early Detection of Urothelial Carcinoma

- Urine cytology is currently the most widely used method for bladder cancer screening
  - Diagnostic cytologic criteria are largely based upon cell morphology
    - Accuracy is hampered by the element of subjectivity
  - Cytology is highly effective in detecting high-grade cancers

    - Sensitivity and specificity in detecting low-grade UC are poorer
  - Accumulated knowledge in the molecular processes involved in carcinogenesis has resulted in the development of many new markers for diagnosis, surveillance, and prognostication of UCs
  - UroVysion™, BTA-Stat™/BTA-TRAK™, NMP22, and ImmunoCyt/uCyt™ are currently available testing methods
    - Relatively widely used
    - Possessing either Food and Drug Administration (FDA) clearance or approval
  - Many others are under investigation and have shown promise
- UroVysion is a multicolor, multitargeted FISH assay
  - Uses chromosome enumeration probes for chromosomes 3, 7, and 17, and a locus-specific indicator probe for 9p21
  - Polysomy of one or more of these three chromosomes or deletion of the 9p21 locus was chosen for their ability to detect common abnormalities in urothelial neoplasia (Fig. 11.3)
  - May be helpful for followup of known bladder cancer, further evaluation of suspicious urine cytology findings, post-BCG followup, or as a general adjunct to urinary cytology
  - Overall sensitivity varies between 69% and 87%, but significantly lowers for low-grade and low-stage tumors
  - May be potentially useful as a grading tool
  - UroVysion patterns may predict the risk of recurrence and DFS of such patients
  - UroVysion is particularly attractive because it is an objective rather than a subjective assessment of urothelial cell abnormalities
  - Diagnosis of urothelial CIS may be especially challenging, as lesions are not always cystoscopically identifiable
    - More extensive investigation of CIS may reveal clinical circumstances for which this method is particularly useful
    - This technique is being proposed as an aid for resolution of histologically challenging biopsies as well as cytologic samples
- BTA-Stat (bladder tumor antigen) is a point-of-care (qualitative) immunoassay using two monoclonal antibodies to detect human complement factor H-related protein in the urine
  - Factor H, a soluble glycoprotein regulator of complement activation, appears to have an immuno-protective effect for tumor cells and is frequently released into urine by urothelial neoplasms
  - The counterpart to BTA-Stat is BTA-TRAK, a quantitative standard ELISA assay
  - Both tests improve in sensitivity for detection of high-grade lesions
  - Sensitivity results have varied in different studies
    - Sensitivity of the test improved with grade from 50% (grade 1) to 72% (grade 2) and 91% (grade 3)
  - As a screening modality, BTA-Stat is associated with false-positive results that are usually caused by inflammatory conditions in the urinary tract
- NMP22 is a nuclear matrix protein usually present in very low quantities in the urine of a normal individual, but with greatly elevated levels in the urine of patients with bladder cancer
  - NMP22 is a quantitative sandwich ELISA test using two antibodies recognizing two epitopes
  - The sensitivity and specificity of NMP22 are 55.7% and 85%, respectively, compared to 15.8% and 99.2% for cytology
  - A nomogram has been developed to better predict the probability of urothelial cancer recurrence and progression, using the NMP22 test
    - Reliability is uncertain due to the reported variability in the diagnostic performance of the test between different institutions
- ImmunoCyt test relies upon the visualization of tumor-associated antigens in urothelial carcinoma cells using a panel of fluorescent-labeled monoclonal antibodies including two

mucin-like proteins and carcinoembryonic antigen
  - ImmunoCyt showed a specificity of 79.3% for grade 1, 84.1% for grade 2, and 92.1% for grade 3 tumors
  - The combination of cytoscopy and ImmunoCyt testing provided 100% sensitivity in UC detection
    - Combining cystoscopy and cytology marginally improved upon the sensitivity of cystoscopy alone
  - The major advantage of ImmunoCyt over other tests is its sensitivity in detecting both low-grade and high-grade tumors
- Other markers are also being evaluated for their utility in detecting cancer cells in urine sediment, including epigenetic markers, telomerase, and survivin
- Microsatellite analysis of urine samples has been used for the surveillance of patients after treatment for UC
  - Data suggest that microsatellite alteration is a strong predictor for tumor recurrences
- Single-nucleotide polymorphism (SNP) analysis has shown that 100% of urine DNA samples from patients with bladder tumors had 24 or more SNP DNA alterations
  - This suggests that the HuSNP chip is a valuable tool for the detection of bladder cancer
- Efforts to develop DNA methylation markers in urine sediments for detection of bladder cancer are also underway
- Studies of the methylation status of Wnt-antagonist genes have shown significantly higher methylation levels of Wnt antagonists in bladder tumors than in normal bladder mucosa
  - The overall sensitivity was 77.2% and specificity was 66.7%

Molecular Predictors and Tumor Recurrence

- The incidence of tumor recurrence in bladder cancer patients ranges from 50% to 90%, and 25% of cancers that recur ultimately progress to invasive cancers
  - Close to 50% of UC patients with lymph node-negative invasive cancers suffer recurrence after radical surgery and die within 5 years of radical cystectomy
  - Current data suggest that UC recurrence may arise from cancer stem cells remaining in an affected field after gross tumor ablation
  - Most current therapies eliminate differentiated cells, which are more sensitive to therapy than cancer stem cells
  - Clonal expansion of fugitive cancer stem cells results in the establishment of recurrent neoplasms wherever the surviving cells find a favorable niche
- Clinical and pathological parameters are widely used to predict clinical outcome
  - These parameters have limited utility for predicting tumor recurrence in individual patients
  - Reliable parameters for individual tumor recurrence risk would be valuable when advising patients regarding surveillance measures and aggressiveness of therapy

FGFR3 Mutation and Low Risk of Recurrence

- Bladder carcinomas likely arise through at least two separate mechanisms
  - *FGFR3* mutation is strongly associated with low recurrence rates in superficial papillary bladder cancers
  - *FGFR3* mutations correlate with recurrence, progression, and cancer-associated mortality in NMI bladder tumors
  - *FGFR3* exon 7 and 10 mutations were analyzed by direct sequencing
  - *FGFR3* mutations are more common among LMPN and TaG1/TaG2 tumors than among TaG3 tumors and T1G3 tumors
  - In noninvasive tumors, mutations are paradoxically associated with increased risk of recurrence
  - *FGFR3* mutations characterize a subgroup of bladder cancers with good prognosis, but that patients with mutant TaG1 tumors may have a higher risk of recurrence (Fig. 11.6)

TP53 Mutation and High Risk of Recurrence

- Numerous studies have indicated that *TP53* mutations are strongly associated with high tumor grade, invasive behavior, risk of recurrence, and adverse clinical outcome
  - *TP53* mutations are typically mutually exclusive of *FGFR3* mutations
  - Studies of primary and recurrent urothelial cancers for *TP53* mutation at exons 5–8 found that *TP53* mutations occur predominantly in highly malignant, invasive tumors
  - High-grade primary tumors and their metachronous recurrences usually harbor the same *TP53* mutation, indicating a common clonal origin
- *TP53* genetic status evaluation found that the overall tumor recurrence rate was 76.0% in noninvasive UC cases
  - Progression-free survival is significantly shorter in patients with *TP53* mutations
  - Frequency of tumor progression was significantly higher in mutated tumors as compared to wild-type tumors
  - *TP53* mutation predicts patients at higher risk of tumor recurrence and progression (Figs. 11.4 and 11.6)

Ki67

- Ki67 is an established marker of cell proliferation that is present during the G1, S, G2, and M stages of the cell cycle, commonly detected using the MIB1 antibody
  - Investigators have reported a prognostic role for Ki67 index in advanced urothelial carcinoma of the urinary bladder
  - Ki67 positivity is statistically significantly associated with an increased probability of disease recurrence

Gene Expression Signature and Bladder Cancer Recurrence Predication

- It has been suggested that the origin and progression of bladder carcinoma result from accumulation of specific genetic or epigenetic alterations, often termed genetic signatures
  - Studies in bladder cancer have established links between specific expression patterns and clinical outcome
  - Gene expression profiles may prove helpful in selecting genes associated with invasion, progression, and tumor recurrence
- DNA microarray expression profiling may also identify genes underlying the clinical and pathological heterogeneity of bladder cancer
  - Gene expression profiling of human bladder cancers provides insights into the mechanisms underlying cancer progression and helps to categorize patients into distinct clinical subgroups

Biomarkers and Nomograms, Combined

- Stratification of risk is a key component of decision-making in clinical practice
  - A variety of factors influencing selection of treatment for a given patient
  - TNM staging system continues to be the standard determinant of UC after radical cystectomy
    - Heterogeneity of tumor biology and patient characteristics within each prognostic group results in significant variation of outcome within each staging category
    - Incorporation of molecular variables with the TNM classification might improve the risk prediction
- A nomogram is a graphical representation of a mathematical formula or algorithm that incorporates several predictors, modeled as continuous variables, to predict a particular endpoint
  - Provides more accurate predictions than models based on risk grouping
  - Generally surpasses clinical experts at predicting outcomes
- Molecular markers provide a promising approach for improving the predictive accuracy of current prognostic indices
  - Risk prediction may be more precise when several predictive variables are considered simultaneously

  - Multivariate nomograms, which facilitate the probability of event predictions at specific points after cystectomy, may provide incremental predictive accuracy
  - Calculation of the probability of the risk of UC recurrence, progression, or metastasis utilizes the knowledge of clinical, pathological characteristics, and the molecular alterations identified in the patient's tumor
  - Several postcystectomy nomograms have been developed
    - Assist in predicting the natural outcome of surgically treated bladder cancers
    - Assist in deciding upon the use of adjuvant therapy after radical cystectomy
- Adding a panel of five cell cycle regulators including p53, pRB, p21, p27, and cyclin E1 improved the predictive accuracy of competing-risk nomograms for predicting recurrence and survival after radical cystectomy in patients with pTa-pT3 node-negative UCs
  - The alteration of cell-cycle regulators were detectable in 82% of patients, and 20% of patients had three and 16% four or five altered biomarkers
  - Patients with three or more altered biomarkers had a four to ten times higher risk of bladder cancer recurrence and mortality after radical cystectomy
- Nomograms have been developed that accurately predicted disease recurrence and progression in patients with Ta, T1, or CIS UC
  - Nomograms incorporating traditional staging and predictive information with molecular features may be used to ultimately counsel patients regarding recurrence risks and aid in treatment selection
- When compared Neuro-fuzzy modeling, artificial neural networks, and traditional statistical methods, for the behavior of bladder cancer, both artificial intelligence methods predicted relapse with an accuracy ranging from 88% to 95%, superior to regular statistical methods

Molecular Staging

- Tumor staging is critical in predicting the disease course of an affected individual
  - American Joint Committee on Cancer TNM staging system is the most common tool to predict outcomes of bladder cancer patients
    - Offers general outcome estimates based on classic pathologic criteria
    - Predictive accuracy of TNM staging alone is limited
  - Nomograms combining molecular markers and classic pathologic criteria have been developed to improve prediction of clinical outcomes after cystectomy
- *FGFR3* and *TP53* mutations are frequently found in superficial papillary and invasive disease, respectively
  - *FGFR3* mutations are associated with low-stage tumors, whereas *TP53* mutations are associated with high-stage tumors
  - *FGFR3*mut/*TP53*wt is the most prevalent genotype in pTa tumors
    - *FGFR3*wt/*TP53*wt is the second most prevalent
  - *FGFR3*wt/*TP53*wt is the most frequent genotype in pT1 tumors, followed by *FGFR3*mut/*TP53*wt and *FGFR3*wt/*TP53*mut
  - *FGFR3*wt/*TP53*wt genotype accounted for 53% of cases of pT2–pT4 tumors
    - *FGFR33*wt/*TP53*mut was observed in 42% tumors
  - There is significant overlap with the TNM stages, and molecular staging subcategorized patients more accurately
  - Molecular staging reflects tumor behavior more closely and may be more useful than traditional staging methods
- Chromosomal instability could potentially be used as a staging parameter
  - Identification of genomic instability independently enhances the accuracy of molecular staging of UC
- Expression microarray analysis of divergent sets of bladder tumors could further classify tumors into more homogeneous and clinically relevant subgroups

  - Unsupervised hierarchical clustering successfully classified the samples into two subgroups containing superficial (pTa and pT1) vs. MI (pT2–pT4) tumors
  - Supervised classification had a 90.5% success rate, separating superficial from MI tumors based on expression of a gene panel
  - Tumors could also be classified into transitional vs. squamous subtypes (89% success rate) and good vs. bad prognosis (78% success rate)
- Polysomy 17 is related to the stage of UC
  - microRNAs (miRNAs) are thought to play roles in cancer development, differentiation, and progression
  - Specific groups of miRNAs are differentially expressed in various cancers and may influence tumor phenotype and behavior
  - The specific role of miRNAs in the metastatic process is largely unknown
- Expression and genome profiling of bladder cancers allow reasonably good correlations between molecular findings and pathologic stage
  - Stage pTa UCs are characterized by genetic stability, since they commonly lack *TP53* mutations
  - Chromosomal changes are predominantly limited to chromosome 9, whereas the majority of pT1 UCs show increased genetic instability with chromosomal changes in 17p, 13q, and 8p

Molecular Detection, Lymph Node Metastasis

- Approximately 25% of patients undergoing radical cystectomy with pelvic lymph node dissection are found to have lymph node metastases
  - Lymph node metastasis is predictive of poor clinical outcome
  - Molecular markers enable the detection of micrometastatic disease with high sensitivity and specificity, and potentially guide therapeutic decision-making
  - When molecular findings are taken into consideration, 25% of microscopically negative lymph nodes appear to harbor metastases

Molecular Detection, Circulating Cancer Cells

- Circulating tumor cells (CTC)
  - Originate from the primary tumor
  - Migrate to distant body sites through the blood stream
  - At times, establish new colonies at these sites, forming detectable metastases
- Presence of CTCs in whole blood before and during radical cystectomy is a parameter for determining the need for adjuvant or even perioperative chemotherapy
  - Results and conclusions gained from studies have been contradictory and inconclusive
  - It is notable that mobilization of tumor cells from the primary site is necessary but not sufficient to produce distant metastases
- Attempts at molecular detection of CTCs in the blood have been hampered by a paucity of molecules specific to urothelial cancer
  - Immunodetection, a technique that relies upon the use of antibodies specifically chosen to attach to bladder cancer cells, such as certain cytokeratins, uroplakin, or MUC7
  - Such detection methods may lack sufficient sensitivity and specificity for bladder cancer tumor cells
- CTC numbers may be useful indicators of the risk of metastasis
  - Detection of CTCs correlates with an increased risk of metastasis
- Use of PCR-based technologies to detect CTCs in the blood is a field of intense investigation
  - Several bladder cancer cell markers such as UPII, CK20, EGFR, and MUC7 have been analyzed for use as candidate detection molecules

  - The sensitivity of these techniques is well documented, but their specificity for diagnostic purposes remains debatable
  - Peripheral blood of patients was studied by nested RT-PCR assay for uroplakin (UP) Ia, Ib, II, and III and EGFR
  - The combination of UPIa/UPII detected 75% of the CTCs, with a specificity of 50%
  - In one study, MUC7 positivity was detected in all bladder cancer cell lines, in 38% peripheral blood samples from patients with Ta or T1 bladder cancer, and in 78% of patients with advanced-stage bladder cancer (≥T2)
  - These findings suggest the potential utility of these novel approaches

Molecular Grading

- The morphological heterogeneity of UC is influenced by molecular events involved in carcinogenesis
  - The morphological criteria used for UC grading have been continuously updated
  - Current bladder cancer classification of urothelial neoplasms is based on an attempt to reconcile molecular genetic and pathologic findings
  - Most of the WHO 2004 categories have been successfully validated by expression and genome profiling and by identification of distinctive genetic alterations
- Mutations in the *FGFR3* and *TP53* genes define two independent and distinct pathways in superficial papillary and invasive/flat UCs (Figs. 11.4, 11.5, and 11.6)
  - Tumors characterized by these two pathways present as heterogeneous groups with distinct phenotypes and genotypes, and with markedly different biological behaviors and clinical outcomes
  - *FGFR3* mutations are usually present in low-grade papillary carcinomas with limited genetic instability, whereas high-grade UCs are characterized by *TP53* mutation
  - *TP53* mutations have been found to be almost always mutually exclusive of *FGFR3* mutations
    - A fact that could potentially be exploited as a tool for the molecular grading of UC (Fig. 11.6)
- Data suggest that pathologic grading alone may not accurately predict tumor behavior
- Molecular grading may potentially discriminate high-risk cases from low-risk cases in patients with similar pathological grades
  - Combining morphological and molecular grading markers may allow better risk stratification for patients with UC
  - In one study of *FGFR3* mutation status and three molecular markers (MIB1 or Ki67, p53, and P27kip1), three molecular grades (mG) could be identified
    - mG1 has *FGFR3* mutation/normal MIB1 expression and portends a favorable prognosis
    - mG2 has either mutated *FGFR3* or elevated MIB1 and is associated with an outcome intermediate between mG1 and mG3 tumors
    - mG3 tumors with wild-type *FGFR3* and high expression of MIB1 were associated with a poor clinical outcome
  - The molecular variables are more reproducible than the pathologic grade
  - Molecular grading provides a new, simple, and highly reproducible tool for clinical decision-making in UC patients
- Differential gene expression profiles can also be used to stratify tumor grade
  - Microarray data show clear distinctions between low- vs. high-grade tumors in bladder washing samples of patients with low-grade and high-grade UCs
- Recent investigations suggest a regulatory role for miRNA in urothelial cancer
  - miRNA alterations occur in a tumor phenotype-specific manner
  - High-grade UC tumors are characterized by miRNA upregulation, including mRNA-21, which suppresses p53 function
  - Low-grade UCs are characterized by downregulation of miRNAs-99a/100, leading to

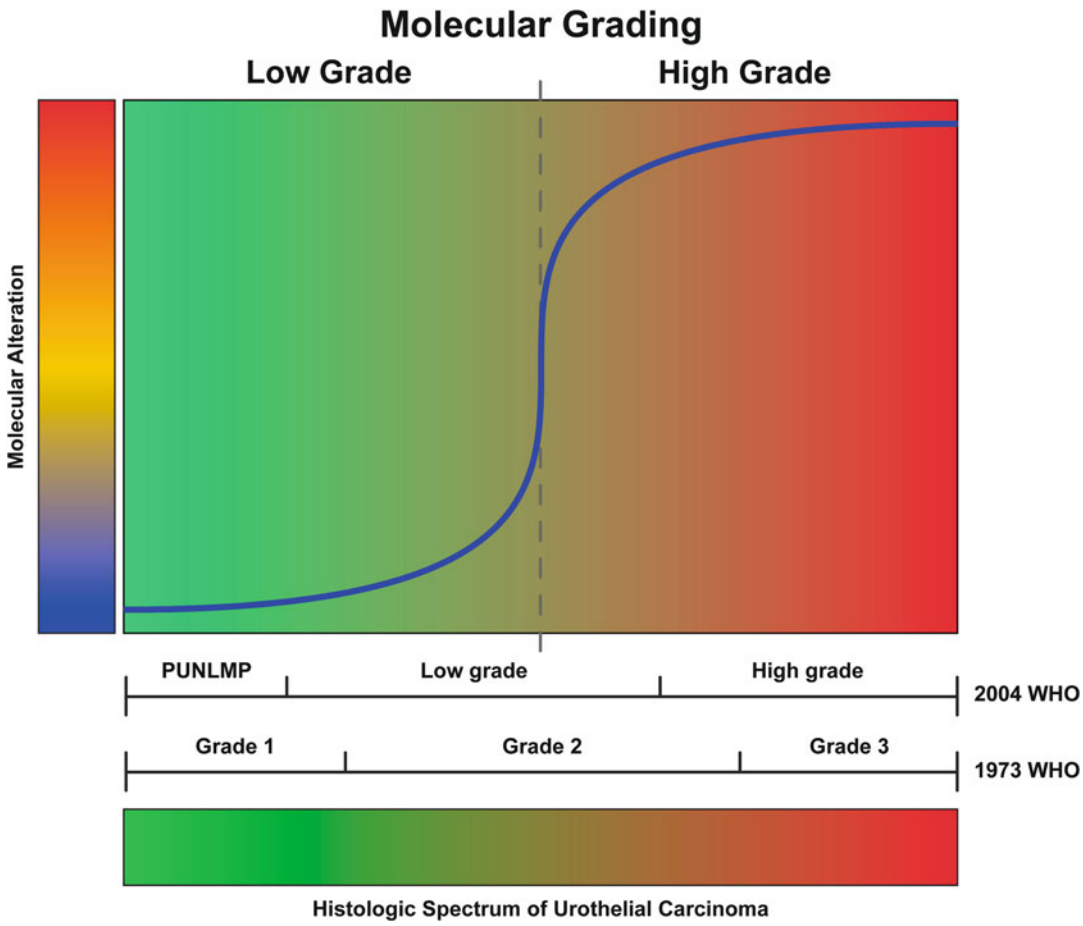

Fig. 11.5 Molecular grading of urothelial carcinomas. The grading of urothelial carcinomas encompasses a continuous spectrum, both molecularly and morphologically. Histological grading according to the severity of atypia is shown on the *horizontal bar* with progression from *green* to *red*; molecular grading reflects the severity of molecular alterations, and is shown in the *vertical bar*, gradient from *blue* to *red*. The *blue curve* represents the probability of significant molecular alterations in tumors exhibiting morphologic features commensurate with their degree of architectural and cytologic atypia (grade) at each point along the grading spectrum. The *blue curve*, as it proceeds from *green* to *red* zones, may more precisely define tumor behavior. (From Cheng L et al. Bladder cancer: Translating molecular genetic insights into clinical practice. Hum Pathol 2011;42:455–481; with permission.)

upregulation of *FGFR3* even before its mutation

- A retrospective study was performed to evaluate differences in chromosomal aberrations in recurrent UC
  - The number of chromosomal aberrations differed significantly between tumor grades, regardless of whether grading was done using WHO 1973 or WHO 2004 grading parameters
  - The most frequent gains of chromosomal material were found on 19p, 7q, 16, 19q, 89, 12q, and 20, and the most frequent losses of chromosomal material were detected on 9, 13q, 5q, 8p, 11p, and 18q
  - Chromosomal aberrations correlated well with both grading systems
  - High-grade tumors showed aberrations usually associated with higher-grade

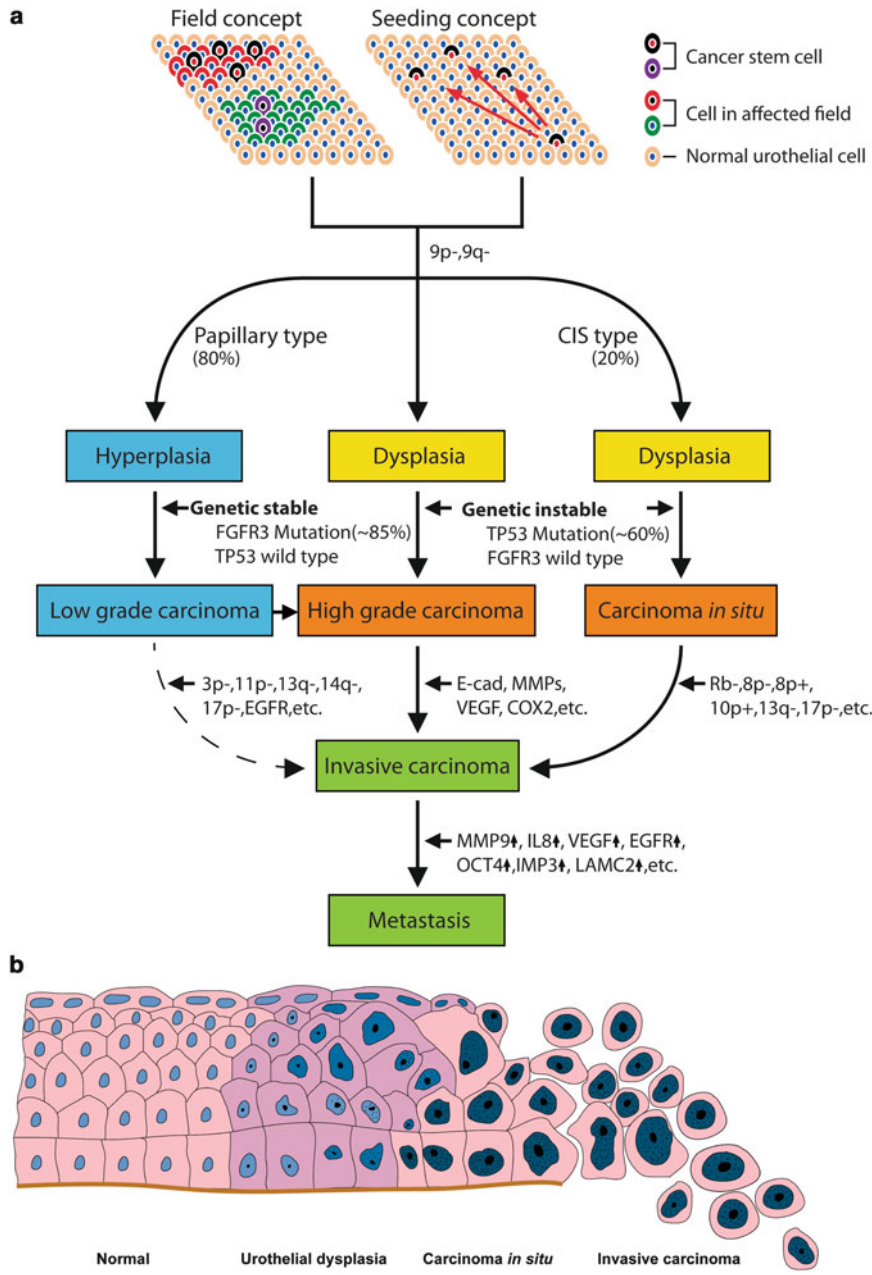

Fig. 11.6 Proposed pathways of urothelial carcinogenesis, progression, and metastasis. (**a**) It is proposed that urothelial carcinoma (UC) originates from cancer stem cells in the affected field or that tumor cells are distributed in the urothelium through a migration-seeding mechanism. Cancer stem cells acquire genetic alterations leading to tumor initiation by clonal expansion. Cancer stem cells are the source of tumor recurrences as well as synchronous or metachronous multifocal tumors. Two distinct pathways are involved in the initial tumorigenesis in cancer stem cells. Papillary neoplasms are thought to develop through intermediate steps of hyperplasia or dysplasia, whereas high-grade flat lesions are believed to evolve through dysplasia/CIS. Papillary tumors account about 80% of all UCs. They are characterized by activating mutations of *FGFR3* and wild-type *TP53*, and in general they are genetically stable. However, these tumors may possibly gain further genetic alterations leading to progression and metastasis (*dashed line*). High-grade flat lesions account about 20% of UCs and are characterized by loss-of-function mutations of *TP53* and intrinsic genetic instability. These two pathways provide a genetic framework for understanding urothelial cancers and their clinical behavior. (**b**) The spectrum of morphologic changes found in urothelial neoplasms largely reflects the molecular alterations these tumors have undergone during their genesis. (From Cheng L et al. Bladder cancer: Translating molecular genetic insights into clinical practice. Hum Pathol 2011;42:455–481; with permission.)

chromosomal alteration panels (1p+, 16p+, −2, and −5q) and poor clinical outcome
  - Polysomy of chromosome 17 by FISH was not seen in G1 tumors, but was seen in G2 tumors (28%) and G3 tumors (100%)
- Distinct molecular pathways of development for superficial papillary UC and those of flat invasive UC are well established
  - Molecular markers efficiently distinguish low-grade bladder tumors from high-grade tumors
  - Low-grade (G1–2) tumors possess few molecular alterations apart from deletions involving chromosome 9 and activating mutations of the *FGFR3*
  - Loss of 11p and inactivation of *TP53* are more commonly seen in tumors of higher grade

Conclusions

- Current approaches to the diagnosis and management of bladder cancer will continue to evolve based on our sharper understanding of the complex molecular mechanisms involved in bladder cancer development
- The current paradigm of clinicopathology-based prognostic approach to predict progression in NMI-UC will soon be supplemented by a molecular guided approach based on some of the markers listed in Table 11.1

Table 11.1 Established clinicopathologic and potential molecular prognostic parameters in NMI and MI urothelial carcinoma of bladder

Clinicopathologic Prognostic Parameters	
Nonmuscle invasive urothelial carcinoma	**Muscle invasive urothelial carcinoma**
WHO/ISUP grade	pTNM
pT stage	LVI
Presence of associated CIS/dysplasia	Resistance to neoadjuvant chemotherapy
Disease duration	
Time to and frequency of recurrences	***Divergent histology***
Multifocality	Micropapillary
Tumor size (>3 cm)	Osteoclast rich
Failure of prior BCG Rx	Undifferentiated/giant cell
Presence of LVI	Plasmacytoid
Depth of lamina propria invasion	

Emerging molecular prognostic markers	
Nonmuscle invasive urothelial carcinoma	**Muscle invasive urothelial carcinoma**
Proliferation index (Ki67, MIB1, S phase)	p53 inactivation/accumulation
FGFR3 mutation/overexpression (protective)	Alterations of Rb expression
mG (FGFR#/MIB1)	Loss of p21 expression
p53 inactivation/accumulation	Alteration of p16 expression
DNA ploidy status	Loss of E-cadherin
Multitarget FISH	
HRAS	***RTK***
ERBB3, ERBB4 overexpression (protective)	EGFR overexpression
Loss of E-cadherin	HER2 overexpression/amplification
Cell cycle control	***Angiogenesis markers***
Downregulation of Rb expression	VEGF overexpression
Downregulation of p21 expression	HIF 1A overexpression

(continued)

Table 11.1 (continued)

Emerging molecular prognostic markers	
Downregulation of p27 expression	TSP1 overexpression
Cyclin D3 overexpression	
Cyclin D1 overexpression	***mTOR-Akt pathway***
	mTOR
Multimarker immunoexpression analysis	Phos S6 expression (protective)
(p53, p27,ki67, Rb, p21)	
	Genomic and gene expression array panels
Angiogenesis markers	
VEGF overexpression	***Epigenetic alterations***
HIF 1A overexpression	RASSF1 promoter hypermethylation
TSP1 overexpression	CDH1 (E-cadherin) promoter hypermethylation
	EDNRB promoter hypermethylation
Genomic and gene expression array panels	
Epigenetic alterations	
RASSF1 promoter hypermethylation	
DAPK promoter hypermethylation	
APC promoter hypermethylation	
CDH1 (E-cadherin) promoter hypermethylation	
EDNRB promoter hypermethylation	

- Several new targeted therapy agents are under investigation in randomized clinical trials in combination with standard chemotherapy agents either as first-line treatment or on a maintenance base to prolong response in patients with advanced bladder cancer

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Molecular Pathology of Testicular Cancer

12

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Contents

Introduction to Normal Testis

The testis is a complex organ with multiple functions, including generation of [mature] germ cells (spermatogenesis) and hormone production (i.e., testosterone). The first is dependent on the second, although visa versa is not the case. In other words, testis might be completely functional regarding formation of hormones, in spite of a complete lack of germ cell formation and production, resulting in infertility. To allow these processes to occur at the proper time and place, various cell types and structures are required. Most of them are initiated during early embryogenesis, while they further develop at different time points, even up to adult life. The most obvious structures within the testis are the seminiferous tubules and the interstitial space. These compartments contain specific types of cells, dependent on age in various stages of maturation.

- Testicular functions, i.e., germ cell formation and hormone production, are regulated by a highly sophisticated network of specific cell types in distinct compartments (see Fig. 12.1a). In the interstitial compartment, i.e., the stromal space in between the seminiferous tubules, different cell types and (microscopic) structures are present, from which only the Leydig cells are testis specific. The Leydig cells produce androgens (testosterone) when stimulated by luteinizing hormone (LH) produced by the pituitary gland (see Fig. 12.1b). In addition, INSL3 is formed, required for the first phase of testicular descent. Other cells and microscopic structures of the interstitial compartment include vascular structures, fibroblasts, macrophages, and lymphocytes
- The intratubular compartment is separated from the interstitial space by a highly organized barrier composed of both cells (e.g., peritubular cells) and extracellular matrix components (basal lamina). Within the seminiferous tubule in principle two types of cells are present under physiological conditions. These are the Sertoli cells and the germ cells (see Fig. 12.1c, d). The Sertoli cells are nursing the germ cells, from the initial embryonic phase to the mature spermatozoa. In the adult

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L. Cheng and J.N. Eble (eds.), *Molecular Surgical Pathology*,
DOI 10.1007/978-1-4614-4900-3_12,

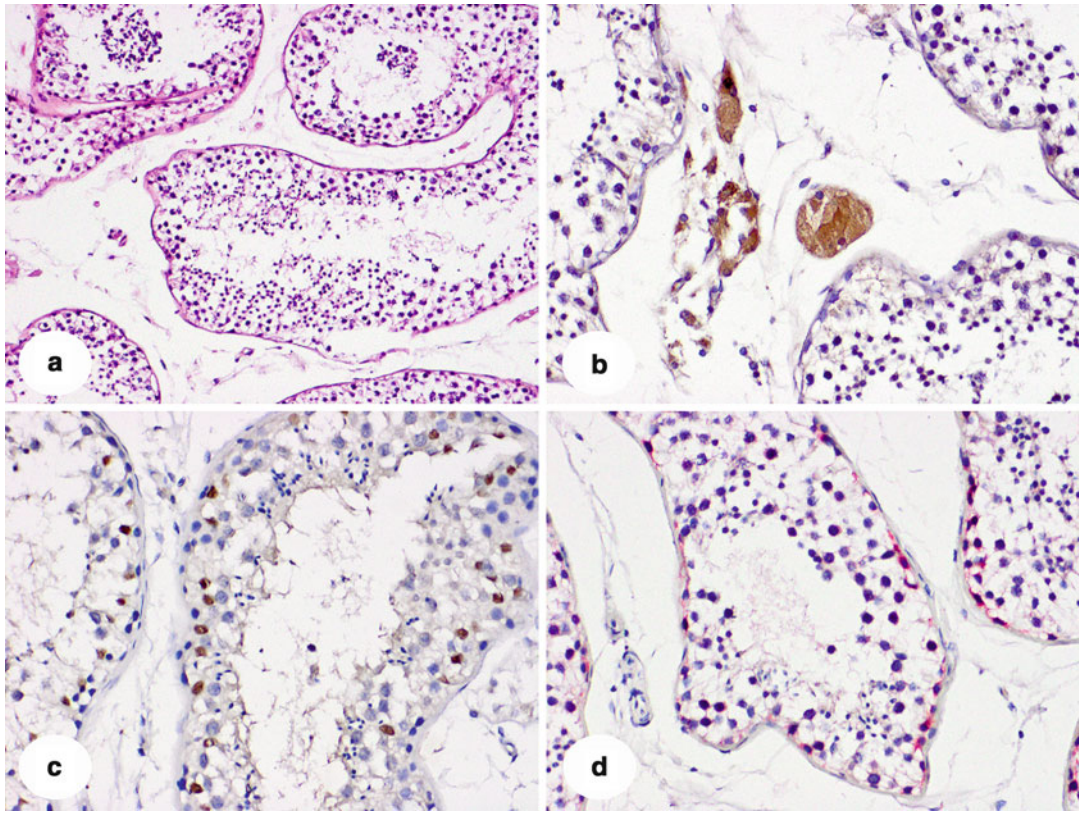

Fig. 12.1 Representative examples of normal adult testis histology, including: (**a**) hematoxylin and eosin (H&E), 100×; (**b**) LH-R, (**c**) SOX9, (**d**) TSPY, all 200×. The immunohistochemical markers used are informative to identify the germ cells in the stage of spermatogonia, sertoli cells, and Leydig cells, respectively

(postpubertal) testis, the germ cells present, i.e., spermatogonia, undergo a process of both mitosis and meiosis, including defined steps of further maturation. The spermatogonia are situated at the inner side of the basal lamina, under the tight junctions formed by the Sertoli cells. The developmental stages that follow are spermatocytes (undergoing meiosis) and spermatids, and finally spermatozoa. This process of mitosis and meiosis, followed by spermiogenesis, is highly dependent on production of androgens. Because of the epidemiological characteristics, this chapter will focus on germ cell tumors (GCTs), although at the end some characteristics of sex cord–stromal tumors will be mentioned

- The majority of GCTs do not arise from adult germ cells, as found after puberty, but from early (embryonic) germ cells blocked in their normal maturation during fetal development (see Fig. 12.2). The only exception is the rare type III GCT (spermatocytic seminoma) (see below). The prerequisite for development of a type I or type II GCTs is thus the escape from the strictly regulated maturation process from an embryonic germ cell to a (pre-) spermatogonium
  - Primordial germ cell (PGCs) arise from the proximal epiblast
  - PGCs retain an intrinsic, although suppressed capacity to pluripotency
  - PGCs move along the hindgut to the developing genital ridges (to develop into testes in an XY chromosomal constitution)
  - Migration of PGCs is regulated by the stem cell factor (SCF/KITLG)–c-KIT pathway
  - PGCs lose their biparental pattern of genomic imprinting (erasement)

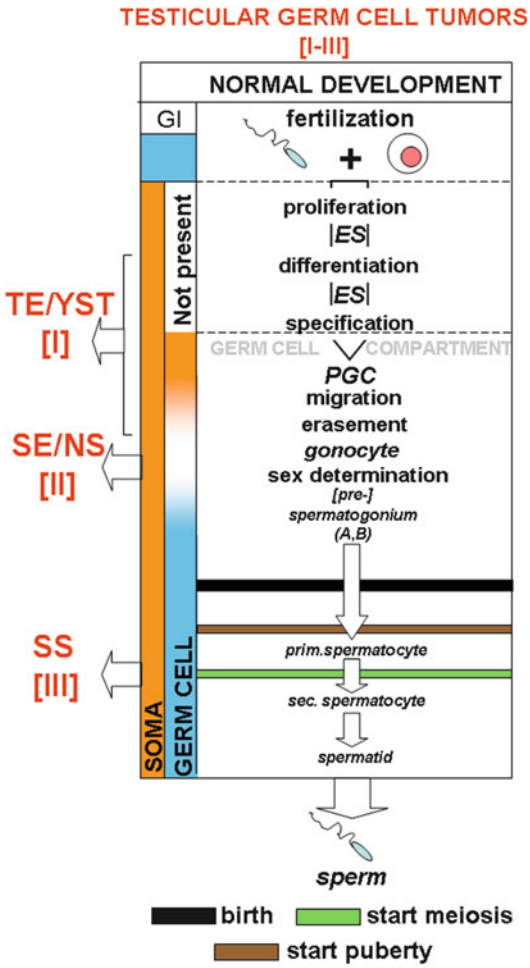

Fig. 12.2 Schematic representation of normal germ cell development (in *black*) and malignant germ cell development (in *red*). Embryogenesis starts with fertilization, and generation of pluripotent stem cells, referred to as embryonic stem cells (ES), which are responsible for formation of the various differentiation lineages (both somatic and extra-embryonic). All these cells have a biparental pattern of genomic imprinting (GI) (represented in *orange*), resulting from a pure male- (*blue*) and female- (*red*) mature germ cell. The primordial germ cell (PGC) erases this biparental pattern, which in the male differentiation lineage in fully becoming paternal, via the stages prespermatogonia (A,B), primary and secondary spermatocyte and spermatid leading to fully matured sperm. The types of germ cell tumors originate from different stages of stem cell/germ cell development, representing their developmental potential. Type I are the teratomas and yolk sac tumors found in neonates and infants, Type II are the seminomas and nonseminomas diagnosed in adolescents and young adults, and the Type III are the spermatocytic seminomas, predominantly occurring in elderly males. The timing of birth, start of meiosis, and puberty are indicated

- Germ cells entering the genital ridge are called gonocytes
- During the second and third trimester of pregnancy, gonocytes mature into prespermatogonia
- Prespermatogonia lose expression of embryonic germ cell markers
- Proper maturation is required for spermatogenesis
- Spermatogenesis is activated by androgens at puberty
- Gonocytes can be delayed or blocked in the maturation process in a suboptimal microenvironment (i.e., cryptorchidism)
- Presence of germ cells with embryonic (PGC/gonocyte-like) characteristics after the first year of life indicates a maturation defect
- Delayed/blocked gonocytes can survive in postnatal testis
- Blocked gonocytes can transform and progress to neoplasm(s)

Introduction to Germ Cell Tumors

Germ Cell Tumors: Classification of Germ Cell Tumors (Types I–III)

In principle, all cells of the testis can give rise to neoplasms. This results in the fact that in the testis an enormous variety of histological variants of tumors can be observed, significantly influenced by age, amongst others. Overall, it is of relevance to distinguish two main subgroups: GCTs and non-GCTs. Again within these categories, a large numbers of histological subgroups can be distinguished. Although officially incorrect, the GCTs of the testis are often referred to as testicular cancer, mainly based on epidemiological criteria; they are the most frequent type of neoplasm of this organ. Understanding the existence of the various types of neoplasms of the testis, and to get insight into their pathogenesis, requires knowledge about the normal anatomy and physiology of both the developing and mature testis. This resulted in novel information about the origin of especially the various types of GCTs, and

identification of informative diagnostic markers. The GCTs represent the most frequent neoplasm, followed by the sex cord–stromal tumors (Leydig cell and Sertoli cell tumors), and others (lymphoma, etc.). Based on morphological criteria, GCTs are subdivided into (classic) seminoma, spermatocytic seminoma, embryonal carcinoma, yolk sac tumor, teratoma, and choriocarcinoma. These can be either pure or (inter)mixed in composition.

- In contrast to the histological description of GCTs, on which all pathological classification systems are based, an alternative is presented. This has been appreciated by the World Health Organization as well as specialized pathologists in the field. According to developmental potential, cell of origin, age at clinical presentation, pattern of genomic imprinting, and molecular characteristics, GCTs can be classified into five entities (type I–V) (see Fig. 12.2), each with their own pathogenesis and pattern of (identified) risk factors. In this chapter, only the type I–III GCTs will be discussed, as they can be found in the testis. The type IV GCTs (dermoid cyst of the ovary) and type V GCTs (hydatidiform mole of placenta) are discussed elsewhere

Type I (Pediatric) Germ Cell Tumor

- Clinical features
  - Tumor of predominantly neonates and infants (incidence of testicular GCT is 1–2 cases per million person-years)
  - First peak of incidence in first 2 years (mean age, 20 months for teratoma), second peak at age of 10
  - Can be found in sacrococcygeal region, retroperitoneum, intracranial, and mediastinum
  - Mostly benign (in contrast to type II teratomas, see below)
  - Malignant progression can occur, leading to yolk sac tumor (malignant)
  - No risk factors have been identified so far and no increase in incidence in the general population have been reported
  - No familial predisposition seems to be significant
- Gross and microscopic features
  - Teratoma
    - Most common GCT in pediatric population
    - Heterogeneous, mixed cystic and solid mass with gray or brown cut surface
    - Mixture of differentiated (mature) somatic tissue, possibly containing (immature) neuroepithelial structures
    - Derivates of all three germinal (somatic) layers might be present (pluripotent)
    - Cartilage, and fetal mesenchymal tissue is often observed
    - Immature neural tissue might be intermixed in various quantities and include nests glands and tubules lined by immature embryonal-like cells with high mitotic activity
    - Malignant transformation to yolk sac tumor is possible
    - Microscopic foci of yolk sac tumor (<3 mm) do not affect prognosis
    - Patients with multiple large foci of yolk sac tumor might require additional chemotherapy (depending of the tumor stage)
    - Can histologically not be distinguished from type II teratoma (see below)
  - Yolk sac tumor
    - Can be primary malignancy in the testis
    - Malignant progression to yolk sac tumor can occur in teratoma (might be mixed)
    - Can histologically not be distinguished from type II yolk sac tumor (see below)
- Precursor lesions and cell of origin
  - Type I GCT, both teratoma and yolk sac tumor, arise from early immature embryonic stem or germ cells. This is supported by:
    - Pattern of genomic imprinting—similarly to embryonic stem cells or early PGCs (biparental or partially erased)
    - Mouse teratoma models histologically represent type I GCTs: Pten-knockout mouse, Ap2gamma-knockout mouse, Fhit-knockout mouse, p53-knockout mouse, Kit-ligand deficient mouse in the 129/Sv background
  - No precursor lesions of type I GCT are histologically identified yet

- Molecular features
  - Type I teratomas show normal chromosomal content (diploidy). In contrast, type I yolk sac tumors are aneuploid, with recurrent chromosomal changes including:
    - Loss of part(s) of 1p, 4, 6q
    - Gain of part(s) of 1q, 12p13, 20q, and 22
    - No genetic mutations have been found so far (teratoma and yolk sac tumor)

Type II (Adult) Germ Cell Tumors

- Clinical features
  - Incidence
    - Account for 60% of all malignancies diagnosed in Caucasian males between 20 and 40 years of age
    - Incidence of 6–11 per 100,000, although dependent of Ethnic background
    - Asian and Blacks significant lower incidence, not influenced by migration
    - Plateau in rise related to World War II
    - Highest incidence in the northern European countries (Denmark, Germany, Norway, Sweden)
    - Significant rise in incidence during last decades (3–6%)
    - Family predisposition involved
    - No high penetrance cancer susceptibility gene identified
    - Nonseminomas develop earlier than seminomas (median age, 25 vs. 35 years)
    - In immunocompromised patients (HIV) seminoma present clinically at the age of nonseminoma
    - Bilateral tumors occur in up to 5% of the patients (synchronous or metachronous)
    - Can also be found in retroperitoneal region, intracranial site and mediastinum
  - Known risk factors
    - Are associated with the aberrant germ cell maturation during the fetal development
    - Clinical predisposition is related to the testicular dysgenesis syndrome (TDS)
      - TDS includes a spectrum of disorders of the male reproductive system including cryptorchid testis, hypospadias, microlithiasis, sub- or infertility; widely accepted view on its pathogenesis is that environmental endocrine disrupting chemicals act on Leydig cells and/or testicular Sertoli cells, resulting in abnormal development of the testis
    - Specific types of disorder of sex development (DSD)
      - DSD (previously intersex) is defined as a congenital condition in which development of a chromosomal, gonadal, or anatomical sex is atypical. The risk for type II GCTs is specifically related to hypovirilization and gonadal dysgenesis, related to presence of part of the Y chromosome (likely TSPY as candidate)
    - Genome-wide association studies have implicated single nucleotide polymorphism (SNPs) related to SCF (KITLG) DMRT1, SPRY4, HTERT/CLPM1L, ATF7IP, and BAK1 genes as risk modifiers
    - Low and high birth weight suggested to be associated with increased risk
    - Most likely a combined action between genetic and environmental factors is the most important determinant in risk determination, referred to as GENVIRONMENT
  - Prognosis
    - Type II GCTs are malignant neoplasms, with a high tendency to metastasize to retroperitoneal lymph nodes and different other organs, influenced by histological composition. In spite of this, they overall show a good prognosis. In patients with metastasized disease, three prognostic groups are identified: good, intermediate, and poor
    - Overall 10-year survival rate over 90%
    - Radiotherapy is effective for metastatic seminoma
    - Worse prognosis in patients with disseminated choriocarcinoma
    - Late relapses (after 2 years) can occur in nonseminomas, often with worse prognosis

- ° Most patients with metastasized disease cured using cisplatin-based chemotherapy
  - ♦ Sensitivity to DNA-damaging agents (including irradiation and chemotherapy) supposed to be multifactorial related to the embryonic germ cell origin. Embryonal stem cells are sensitive due to lack of DNA repair mechanisms combined with no G_1 arrest checkpoint. In addition, low level for apoptosis induction of germ cells is involved in preventing transmission of mutated DNA to the next generation
- ° Retroperitoneal lymph node dissection might be indicated
- ° Long-term side effects of the chemo (radio)therapeutic treatment are common, including subfertility, fatigue, cardiovascular complications, metabolic syndrome, and, less frequently, secondary cancer

– Serum markers
- ° Three principal tumor serum markers for type II GCTs are available and used according to the guidelines for primary diagnosis, staging, monitoring of therapeutic response and followup
- ° Alpha-fetoprotein (AFP) (half-life of 4.5 days) is elevated in up to 70% of patients. Predominantly generated by the yolk sac component
- ° Beta subunit of the human choriogonadotropin (HCG) (half-life of 24–36 h) elevated in 50% of patients. Predominantly generated by the choriocarcinoma component
- ° Marijuana usage can result in false positive hCG finding
- ° Lactate dehydrogenase (LDH1), being less specific. Elevated in 40–60% of patients
- ° If these tumor markers do not decline expected based on half-life after treatment, residual disease is likely
  - ♦ Normal level of the markers does not prove absence of disease (only 40–50 and 30% of relapses in patients under active surveillance for clinical stage I disease and after systemic chemotherapy are associated with marker increases)

• Gross, microscopic, and immunohistochemical features by specific histological type
– Histologically, different variants of type II GCT can be identified. These are subdivided into seminoma and nonseminoma. According to the British Classification, a combined tumor is composed of both a seminoma and nonseminoma component, referred to as nonseminoma to the other classification systems. Nonseminoma defines a cancer with the following possible histological types: embryonal carcinoma, teratoma, yolk sac tumor, and choriocarcinoma (with or without a seminoma component). Overall, about 40% of type I GCTs are seminomas and 60% nonseminomas. Nonseminoma show mostly a mixture of different histological components
– The different histological components are described below in more detail
– Seminoma (see Fig. 12.3)
- ° Solid tumors with gray, white, or pink surface
- ° Microscopically, the tumor consists of sheets or lobules separated by fibrous septa with lymphoid infiltrate
- ° Round to polygonal tumor cells with clear or eosinophilic cytoplasm
- ° Nuclei are central and contain prominent nucleoli
- ° Epithelioid cells, Langhans giant cells, or sarcoid-like granulomas can be present
- ° Up to 25% contain syncytiotrophoblastic giant cells
- ° Atypical seminoma show a greater degree of polymorphism and a higher mitotic rate; however the clinical significance of this subtype is doubtful
- ° There is nuclear staining for markers of undifferentiated germ cells, including OCT3/4, SALL4, NANOG, AP2gamma (>90% of tumor cells are positive)
- ° OCT3/4 is nowadays the most sensitive and specific marker for seminoma

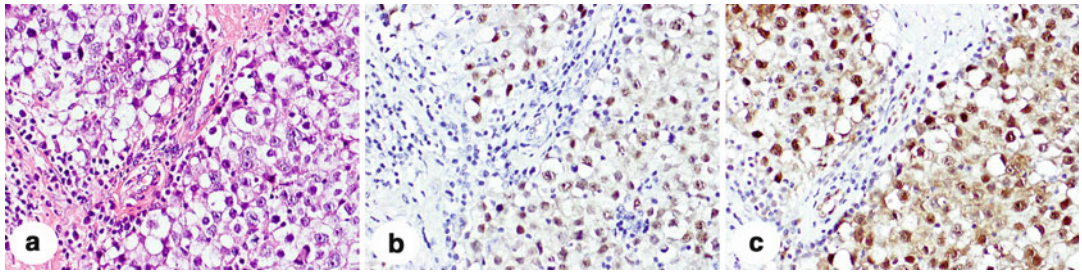

Fig. 12.3 Representative examples of an invasive seminoma, stained with (**a**) H&E, and the immunohistochemical markers (**b**) OCT3/4, and (**c**) SOX17

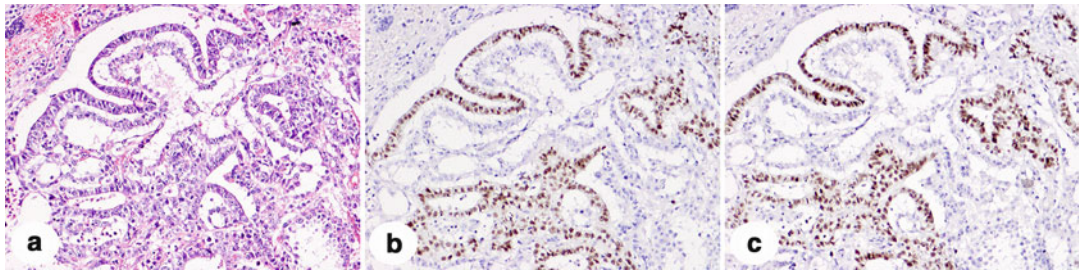

Fig. 12.4 Representative example of a mixed nonseminoma, containing an embryonal carcinoma and yolk sac tumor component, stained with (**a**) H&E, and the immunohistochemical markers (**b**) OCT3/4, and (**c**) SOX2

(as well as for CIS and embryonal carcinoma), always nuclear in localization
    - Variable membranous staining for markers of germ cell differentiation, including CD117 (c-KIT), D2-40, as well as for placental alkaline phosphatase (PLAP)
    - In contrast to embryonal carcinoma, (see below) no expression of EMA in seminoma
    - Immunohistochemistry for cytokeratins can be positive without clinical impact
    - SOX17 is positive in seminoma and can differentiate seminoma from embryonal carcinoma, which is SOX17 negative. However, normal PGCs and gonocytes as well as spermatogonia are positive as well
- Embryonal carcinoma (see Fig. 12.4)
    - Solid tumor with gray to pink appearance and foci of hemorrhage and necrosis
    - Growth pattern varies from solid to papillary and syncytial
    - Typical epithelial-like cells with large irregular nuclei
    - Mitotic figures are frequent
    - Syncytiotrophoblastic giant cells might be scattered
    - There is a nuclear (and cytoplasmic) staining for markers of undifferentiated germ cells, including OCT3/4, SALL4, NANOG (>90% of tumor cells are positive)
    - OCT3/4 also shows both a nuclear and cytoplasmic localization
    - Membranous staining for CD30, EMA, and PLAP
    - Markers differentially expressed in embryonal carcinoma vs. seminoma are SOX2, CD30 (exclusively positive in embryonal carcinoma), and EMA of SOX17 (positive in seminoma)
    - Vascular invasion is often the result of embryonal carcinoma
- Yolk sac tumor (see Fig. 12.4)
    - Solid soft tumors, gray-white to yellow surface
    - Necrosis may be present

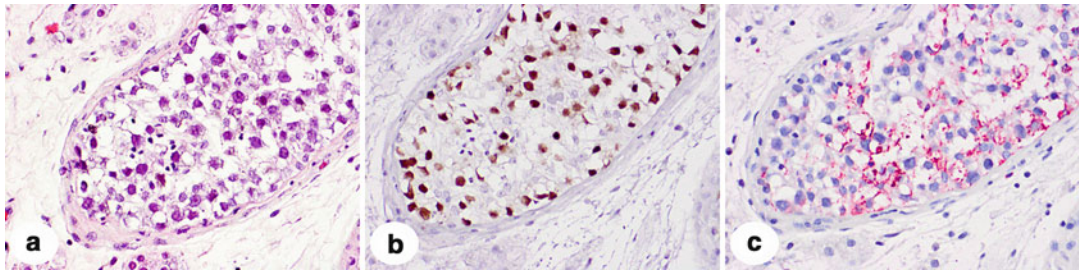

Fig. 12.5 Representative examples of a seminiferous tubules containing carcinoma in situ (CIS) cells and intratubular seminoma cells, stained with (**a**) H&E, and the immunohistochemical markers (**b**) OCT3/4, and (**c**) stem cell factor (KITLG)

    - There are numerous pattern of differentiation: microcystic, macrocystic, endodermal sinus, papillary, glandular, solid, polyvesicular, vitelline, hepatoid, myxoid, parietal pattern
    - Various pattern are usually admixed in one tumor
    - Foci of yolk sac tumor are frequently seen in nonseminomas
    - Pure yolk sac tumors are rare
    - AFP and glypican 3 are variably expressed in yolk sac tumors and can be informative to discriminate from other components and cancers
    - Combination of these markers can increase sensitivity for the detection
    - Low molecular weight cytokeratins are positive in yolk sac tumors
  - Choriocarcinoma
    - Tumor represents as nodules with hemorrhage
    - Composed of trophoblast-like cells and syncytial large cells
    - Frequently present in nonseminomatous
    - Rare in a pure form (<0,1% of all TGCT)
    - Pure choriocarcinoma is likely to present as a highly aggressive disease with hematogenous metastases
    - Syncytiotrophoblasts are positive for beta-HCG, inhibin (alpha subunit), and EMA
    - Cytokeratin is expressed in trophoblasts and syncytiotrophoblasts
  - Teratoma
    - Malignant tumor showing somatic differentiation with endoderm, ectoderm, and endoderm derivates
    - Teratomatous component is often present in nonseminomas
    - Various somatic malignancies might arise in the background of teratoma including sarcoma, PNET, carcinoma, and nephroblastoma
    - No specific immunoprofile, the differentiated areas of teratoma show immunophenotype which is in accordance with the underlying cell type
    - AFP can be expressed in intestinal or hepatoid areas
    - Intratesticular epidermoid cyst represents a rare benign teratoma in the adult and should not be mixed up with the type II malignant teratoma
      - This benign teratoma shows in contrast to the malignant type II teratoma no CIS (see below) in the adjacent testis. Careful examination of the seminiferous tubules should be done under usage of the immunohistochemistry for OCT3/4 (or another marker for CIS). Detection of CIS supports a malignant type II teratoma
- Precursor lesions and cell of origin (see Fig. 12.5)
  - The precursor of all type II GCTs of the testis is the so-called carcinoma in situ (CIS) of the testis, also referred to as intratubular germ cell neoplasia unclassified

(IGCNU) or testicular intraepithelial neoplasia (TIN). It is expected that all patients with CIS will eventually develop an invasive cancer (in the prospective study, 70% of the patients with CIS developed an invasive GCT within 7 years)
- CIS cells are located at the inner side of the basal lamina of the seminiferous tubule, most frequently in a single row in close connection with Sertoli cells, under their interconnecting tight junctions
- CIS is often present in the adjacent parenchyma of invasive type II GCTs, especially nonseminomas
- Activated immune system as found in seminomas can also eradicate CIS
- PGCs and CIS cells share the same pattern of genomic imprinting (erased), telomerase activity, and gene and protein expression profile
- CIS shows homogeneous expression of markers of PGCs/gonocytes, including c-KIT, BLIMP1, AP2gamma, OCT3/4, NANOG, LIN28 (and many more)
- OCT3/4 is the most specific and sensitive marker for CIS, strongly staining the nucleus of all CIS cells, but not normal spermatogonia
- The CIS counterpart in dysgenetic gonads with a low level of virilization (i.e., no or limited testicular differentiation) is known as gonadoblastoma
  - Gonadoblastoma is composed on CIS-like cells intermixed with stromal cells expressing FOXL2 (Granulosa differentiation), whereas Sertoli cells (SOX9 positive) are associated with CIS. Gonadoblastoma can mimic CIS, to be differentiated by SOX9 and FOXL2. Presence of gonadoblastoma is proof for the presence of DSD
- Overdiagnosis of CIS is possible due to germ cell maturation delay. This can be avoided using immunohistochemical detection of SCF (KITLG)
  - In a testicular biopsy taken during the first year of life in an individual with possible germ cell maturation delay (e.g., cryptorchidism), overdiagnosis is possible. Distinguishing morphological criteria are not strictly informative; immunohistochemistry with OCT3/4 is also discriminatory. It can be solved using immunohistochemistry for SCF, specifically present in the premalignant cells
- No informative animal model has been identified yet

- Molecular features
  - Chromosomal constitution
    - Seminomas and CIS are hypertriploid and the nonseminomas hypotriploid type II GCT show various losses and gains of (parts of) chromosomes: loss of chromosomes 4, 5, 11, 13, 18, and Y and gain of chromosomes 7, 8, X, and 12
    - Yolk sac tumors show recurrent chromosomal imbalances, including loss of 1p, 4, and 6q, and gain of 1q and 20q
    - All invasive tumors show gain of 12p, mostly due to formation of isochromosomes (i12p). Regional high level amplification can also be observed. No obvious candidate gene(s) has been identified so far, although various have been suggested (CCND2, NANOG KRAS2, etc.)
      - Studies indicate that gain of 12p is progression related (occurs when CIS cells become independent of interaction with Sertoli cells). Cyclin D2 (CNND2) is expressed in all type II GCTs, as well as in CIS, while NANOG is expressed in CIS as well as seminoma and embryonal carcinoma. It is most likely that multiple genes located on 12p are relevant in the pathogenesis. It matches with the observation that gain of 12p can be found in extended in vitro cultures of human embryonic stem cells
    - X chromosome is gained in the majority of TGCT
      - Familial predisposition of type II GCT has been linked to the X chro-

mosome. Additional X chromosome is also relevant in the context of Klinefelter syndrome patients, although these patients only develop mediastinal type II GCTs (not testicular). A role of the X chromosome might also be suggested from data of patients with specific forms of DSD. Supernumerical X chromosomes are inactivated in nonseminomas by methylation. This, in parallel to normal embryogenesis, result from function of the non-(protein)-coding *XIST* gene. This phenomenon is correlated with hypomethylation of the promoter region, reported to be useful as molecular target for this type of cancer

- Epigenetic modifications
  - CIS and seminomas show a hypomethylated DNA status, in contrast to the various histological types of nonseminomas, this parallels normal embryogenesis
  - Histone modification proteins BLIMP1 and PRMT5
    - The complex of the transcription factor BLIMP1 and protein arginine methyltransferase-5 PRMT5 protein is expressed in CIS and seminoma. Proposed function of BLIMP1/PRMT5 complex is suppression of premature differentiation and maintenance of pluripotency in PGCs/gonocytes by dimethylation of H2A/H4 at arginine 3 and repression of gene expression (see above). It suggests that histone H2A and H4 arginine 3 dimethylation suppress differentiation of CIS and seminoma, while loss of these histone modifications might induce reprogramming and differentiation to embryonal carcinomas and the various subtypes of differentiated nonseminomas
- Embryonal stem cell genes
  - Expression pattern of mRNA in seminoma and embryonal carcinoma shows high levels of mRNA of genes related to the pluripotency (OCT3/4, NANOG, LIN28), in close similarity to the PGCs/gonocytes and embryonal stem cells
    - In embryonal stem cells, pluripotency is regulated by interaction of POU5F1 (OCT3/4) with a member of the SOX family. Studies in cell lines derived from seminoma and embryonal carcinoma provide evidence that the functional partner of POU5F1 in seminomas is SOX17, and in embryonal carcinomas SOX2. The role of POU5F1/SOX17 complex is likely not in regulation of pluripotency but in prevention from apoptosis
  - Two specific variants of the protein encoding OCT3/4 are recognized, of which the A (or I) type is a nuclear protein and is related to pluripotency
    - The B (or II) variant is localized in the cytoplasm and is not related to regulation of pluripotency. Detection of OCT3/4 mRNA is hampered by existence of two variants as well as the presence of pseudogenes. This may result in false positive RT-PCR observations in variety of non-GCTs
  - Expression pattern of NANOG is similar to OCT3/4
- Involvement c-KIT (CD117)
  - Receptor tyrosine kinase
  - c-KIT is expressed mainly in CIS and also, but less, in seminoma, but not in embryonal carcinoma
  - c-KIT is downregulated during the progression of CIS to seminomas
  - Amplification of c-KIT is found in a selected number of seminomas
  - Activating gene mutations in exon 17 are detected mostly in (bilateral) seminomas
    - It is supposed that activation of c-KIT in early germ cells during fetal phase of germ cell differentiation can potentially lead to survival of immature germ cells in the niche of spermatogonia. It has also been proposed

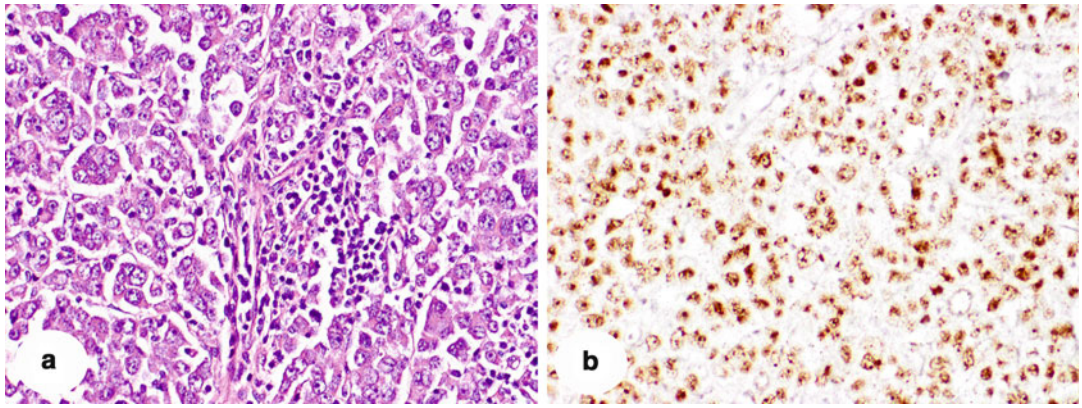

Fig. 12.6 Representative example of a spermatocytic seminoma, stained with (**a**) H&E and (**b**) DMRT1

that c-KIT plays a role in the initiation of the germ cell malignancy but might not be relevant for the further steps of progression

- Mutational status
  - GCTs show overall an exceptional low mutation rate of genomic DNA
    - This is supported by mutation analysis of individual genes as well as by high-throughput investigation on the mutation status of the kinome. Various deep sequencing projects are currently undertaken. The uniqueness of this low mutation rate is likely to be (again) related to the embryonic cell of origin. Embryonic stem cells keep one of the two DNA strands protected against any form of mutations, the so-called immortal DNA strand. This reduces the probability of transmitting of the DNA anomalies to the next generation
  - BRAF and microsatellite instability
    - Overall, type II GCTs, with the exception of teratomas, show an exceptional sensitivity to DNA damaging agents. However, not all nonteratomatous elements are sensitive to cisplatin-based chemotherapy. Various putative mechanisms for resistance are proposed based on limited studies and number of cases. Intriguing findings are the role of microsatellite instability (MSI), BRAF mutations, disturbed apoptotic, etc. MSI is found in about 30% of the refractory cancers, in a significant number related to hypermethylation of the promoter region of hMLH1. Interestingly, this seems to be partially overlapping with activating mutations within the BRAF oncogene (V600E) in all patient groups

Type III Germ Cell Tumors (Spermatocytic Seminoma)

- Clinical features
  - Rare tumor, up to 4% of all GCT of the testis
  - Incidence 0.4 per 1,000,000
  - Occur in older male compared to type II GCT, average age 52 years
  - Most tumors are unilateral
  - Bilateral tumors can occur
  - Serum markers (AFP, HCG, LDH) are negative
  - Metastases from a pure spermatocytic seminoma are very rare
  - Sarcomatoid dedifferentiation rarely occurs within a spermatocytic seminoma and is associated with a progressive disease
  - No association with cryptorchidism
  - Excellent prognosis with surgery alone
- Gross, microscopic, and immunohistochemical features (see Fig. 12.6)
  - Soft tumor with mucoid grayish-white cut surface, and friable texture

  - Background of edematous stroma
  - Tumor cells are of various size: large eosinophilic cells, small dark cells, and mono- or multinucleated giant cells
  - High mitotic activity
  - CIS/IGCNU/TIN is not present
  - Specific precursor lesions might be present (so-called intratubular spermatocytic seminoma in situ)
  - Immunohistochemical markers of classic seminoma (OCT3/4, PLAP, c-KIT) are negative
  - Spermatogonial markers DMRT1, OCT2, SSX2–4, and SAGE1 are positive in spermatocytic seminoma (the last suggesting a heterogeneous origin)
    - In the histogenetic model, spermatocytic seminoma arise from mature spermatogonia or spermatocytes
- Molecular features
  - Gain of chromosome 9 is consistent
  - DMRT1 is an interesting 9p gene
  - Paternal pattern of genomic imprinting
  - Many testis cancer antigens and genes related to spermatogenesis are expressed
  - HRAS and FGF3 genes are frequently mutated, especially in the cases in elderly men
  - The canine seminomas are the animal model for type III GCTs

Sex Cord/Gonadal Stromal Tumors

- Sex cord tumors are rare; these tumors constitute about 4% of all testicular neoplasms. Metastases can occur, mainly in adult patients, but no histological or molecular prognostic markers had been found so far to predict the clinical behavior

Leydig Cell Tumors

- Clinical features
  - Two age peaks, in children mostly between 5 and 10, and in adults
  - Hormone producing tumors: testosterone, androstenedione
  - Serum estrogen level might be elevated
  - In children, clinical presentation might be pubertas praecox or/and gynecomastia
  - Adults frequently present with testicular mass, 30% develop also gynecomastia
  - Bilaterality is rare
  - 10% of Leydig cell tumors are malignant
  - Malignant tumors do not respond to chemotherapy or radiation
- Gross, microscopic, and immunohistochemical features
  - Solid, yellowish tumor
  - Well circumscribed, sometimes nodular
  - Average tumor size 2–5 cm
  - Necrosis and extratesticular extension might occur
  - Typical histologic features include sheets of eosinophilic cells with distinct cell borders
  - Reinke crystals are pathognomonic and seen in up to 40% of the cases
  - Pseudoglandular and microcystic pattern can be seen
  - Lipomatous changes might occur
  - Malignant behavior correlates with the size (>5 cm), higher mitotic rate (>3 mitotic figures per 10 high power fields), necrosis, vascular invasion, invasion in the neighboring structures, and high proliferative activity
  - Tumors are positive for vimentin, inhibin, and LH-R

Sertoli Cell Tumors

- Clinical features
  - Very rare tumors (<1% of all testicular neoplasms)
  - Mean age at the time of the diagnosis 45 years
  - Some tumors are associated with genetic syndromes
  - Large cell calcifying Sertoli cell tumor (LCCST) can be sporadic or associated with Carney and Peutz–Jeghers syndromes

  - Estrogen production might lead to gynecomastia
  - Most Sertoli cell tumors are benign
- Gross, microscopic, and immunohistochemical features
  - Solid tumor, gray-whitish
  - Nests, tubules, sheets, mostly bland cytology
  - Frequently, stromal hyalinization is present
  - LCCST present with nests of large eosinophilic cells embedded with hyaline stroma (with calcifications in some cases)
  - In Peutz–Jeghers syndrome, multifocal bilateral Sertoli cell proliferation occur mostly in young patients
  - Immunohistochemically, Sertoli cell tumors are positive for inhibin, cytokeratin, vimentin, and SOX9

Granulosa Cell Tumors

- Clinical Features
  - In similarity to the ovary, juvenile and adult types are distinguished
  - Adult type is rare, metastases occur in up to 20% of the patients
  - Juvenile tumors occur mostly in maldescended testis, in young children
- Gross, microscopic, and immunohistochemical features
  - Adult type granulosa cell tumors
    - Solid and well circumscribed, with varying size
    - Several histological pattern can occur, including solid, trabecular, insular, microfollicular
    - Typically, microfollicles surround eosinophilic material (Call–Exner bodies)
    - Tumor cells show grooved nuclei
    - Tumor cells are reactive for vimentin, inhibin, smooth muscle actin
  - Juvenile granulosa cell tumors
    - Often cystic
    - Solid and follicle-like zones are admixed
    - Follicles lined by stratified epithelium
    - Tumor cells are reactive for vimentin, inhibin, smooth muscle actin, and focally to anti-Müllerian hormone
- Molecular features of sex cord/gonadal stromal tumors
  - Activating mutations in LH-receptor had been detected in pediatric Leydig cell tumors
  - Most ovarian adult type granulosa cell tumors harbor a somatic missense mutation in the FOXL2 gene, but no information is available yet for the testicular granulosa cell tumors

Summary of Molecular Pathology of Testicular Cancer

- Neoplasms of the testis comprise a heterogeneous group of tumors, of which the majority is of the germ cell origin. GCTs of the testis can be classified by the cell of origin, as well as the molecular findings into three main groups
- Type I GCT is a common neoplasm in the neonates and children and arises from very early germ cells, which are blocked in their differentiation. The most common type I GCT is teratoma, which is a benign tumor. Yolk sac tumor is a malignant type I GCT, developing from transformed early germ cells or, rarely, arises in a type I teratoma
- Type II GCT are malignant GCTs with complex morphology and different histological subtypes, all arising from a common precursor lesions, the carcinoma in situ
- Seminoma and embryonal carcinoma as well as carcinoma in situ share (partly) the same gene expression pattern and pattern of genomic imprinting as early fetal germ cells
- Transition from the precursor lesion to an invasive cancer is associated with gain of the short arm of chromosome 12, in which multiple genes might be involved
- Knowledge of cell of origin of type II GCT has led to the successful employment of

OCT3/4 as a highly specific marker of CIS, seminoma, and embryonal carcinoma

- Type III GCTs represent a benign lesion composed of germ cells in the maturation stage of spermatogonia/spermatocyte. They consistently show gain of chromosome 9, and are positive for a number of immunohistochemical markers, including DMRT1

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Molecular Pathology of Cutaneous Melanoma and Nonmelanoma Skin Cancer

13

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Contents

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Ultraviolet Light and Skin Cancer

- UV light exerts strong genotoxic effects; overwhelming epidemiological and experimental evidence point to solar UV radiation as a strong etiologic factor for both melanoma and nonmelanoma skin cancer (NMSC)
- Based on radiation wavelength (λ), the UV spectrum is conventionally divided into:
  - UVA [λ 320–400 nm, including UVA_1 (λ 340–400 nm) and UVA_2 (λ 320–340 nm)]
  - UVB (λ 290–320 nm)
  - UVC (λ 100–290 nm)
- UVB radiation constitutes only 5% of the total terrestrial sunlight UV; UVC radiation is completely absorbed by stratospheric O_2 resulting in O_3 production
- Depth of penetration of UV radiation into the skin is proportional to its wavelength: UVB does not penetrate past the basal layer of the epidermis, while UVA reaches into the papillary dermis and the superficial layers of the reticular dermis
- UV-induced DNA-damage
  - Although UVA is at least 100 times less mutagenic than UVB, its higher abundance in the terrestrial radiation spectrum makes it a relevant mutagen for the skin. Additionally, the active spectra for specific types of DNA-damage are still debated
  - UVB radiation causes DNA-damage mainly through direct photochemical reactions, leading to dimerization of adjacent pyrimidines and characteristic UV

L. Cheng and J.N. Eble (eds.), *Molecular Surgical Pathology*,
DOI 10.1007/978-1-4614-4900-3_13,

photoproducts (i.e., *cis-syn* cyclobutane pyrimidine dimers (CPDs), pyrimidine (6–4) pyrimidone photoproducts [(6–4) PPs], and Dewar valence isomers)
    - >80% of sunlight UVB-induced photoproducts in normal mammal cells are CPDs
- UVB also damages DNA through indirect mechanisms. Excitation of endogenous chromophores such as melanin results in production of ROS and subsequent formation of oxidized DNA bases such as 8-oxo-7, 8-dihydro-2′-deoxyguanosine (8-oxo-dG); 8-oxo-dG may lead to G–T transversions and, to a lesser degree, G–A transitions
- Mutagenic effects of UVA have long been believed to occur solely by indirect photosensitization reactions involving free radical-mediated oxidation of DNA purines (mainly 8-oxo-dG), possibly augmented by the Fenton reaction. Recent evidence suggests that also UVA_1 and UVA_2, albeit less efficiently than UVB, can induce CPDs through a direct photochemical process, with predilection for TT dypiridimine sites
    - A significant part of the mutagenic effect of UVA in vivo appears to rely on CPDs as UVA-induced oxidative DNA-damage is repaired with high efficiency in normal mammalian cells
- UV mutagenesis is typically distinguished by high frequency of C–T transitions and, less commonly, CC–TT tandem base substitutions at dypirimidine sites; this distinctive spectrum of mutations, which is collectively called the "UV signature," points to a role for cytosine-containing CPDs
- Bulky pyrimidine dimers photoproducts are repaired by the nucleotide excision repair (NER) system
    - Inherited genetic defects in the NER pathway such as in xeroderma pigmentosum (XP) and Cockayne syndrome (CS) lead to UV light hypersensitivity and dramatically increased incidence of skin cancers
    - Unrepaired CPDs prevent normal DNA replication stalling DNA polymerases, leading to stalled replication forks; a backup system of "bypass" DNA polymerases, such as polymerase-η (Pol-η), is specialized in translesional DNA synthesis (TLS) at sites with CPDs
    - Repair of CPDs by TLS is prone to result in C–T and CC–TT transitions
- UV-induced CPDs preferentially involve 5-methylcytosines (5mC), which are frequently found in CpG islands
- Oxidative base lesions are recognized by specific glycosylases and repaired through the base excision repair (BER) pathway; to date, no significant link has been found between defects in the BER system and skin cancer

- Effects of UV radiation on the immune system
    - UV radiation further effects skin carcinogenesis through proinflammatory and immunosuppressive effects
    - Longstanding UV exposure can result in a chronic inflammatory response mediated by proinflammatory cytokines (i.e., IL-1 and TNFα), ROS and RNS intermediates, PAF, and eicosanoids (such as PGE_2)
    - Cutaneous UV irradiation induces immunosuppressive effects through local and systemic reactions
        - UV exposure of human skin dampens both delayed-type and contact hypersensitivity reactions
    - The immunosuppressive effect of UV light on human skin, which peaks at 300 nm (UVB range) and 370 nm (UVA range), is obtained through several mechanisms, including:
        - Reduction of the number of Langerhans cells and their antigen-presenting ability
        - Th1 to Th2 shift in the adaptive immunity mediated by release of IL-4 and IL-10 as well as downregulation of IL-12
        - Production of *cis*-uronic acid, PAF, and PGE_2
- Melanin, pigmentation, and photoprotection
    - Production of melanin by skin melanocytes represents the predominant mechanism of photoprotection in the skin; levels of skin pigmentation inversely correlate with melanoma and NMSC risk

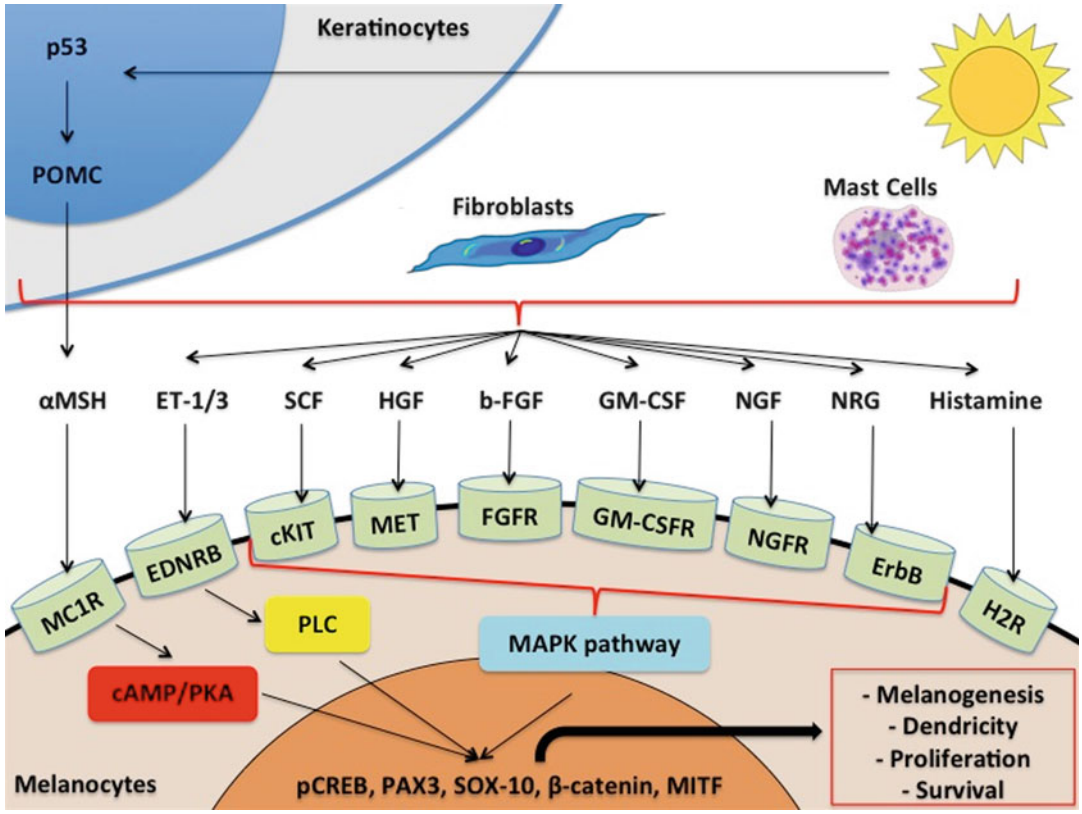

Fig. 13.1 Molecular interactions between melanocytes, keratinocytes, and dermal microenvironment in physiologic regulation of melanocytes functions, and influence of ultraviolet radiation

  - After the tyrosinase-mediated conversion of tyrosine to DOPA-quinone, two types of melanin can be produced: the brown/black eumelanin and the yellow/red pheomelanin
  - Skin pigmentation is highly correlated with eumelanin content and eumelanin/pheomelanin ratio, as well as with activity of melanogenic enzymes such as tyrosinase, Dct/Trp2, and Trp1
  - Eumelanin exerts strong photoprotective effects by transforming UV radiation into heat through internal conversion, quenching ROS, and showing a higher stability compared to pheomelanin; conversely, pheomelanin may even promote formation of ROS
- UV-induced stress response (tanning response)
  - UV irradiation induces a stress response aimed at decreasing the impact through activating pigment synthesis, DNA repair, antioxidative mechanisms, cell cycle arrest, and proapoptotic pathways
    - The effects are mediated through a complex network of autocrine and paracrine interactions between keratinocytes and melanocytes that are initiated through a DNA-damage mediated induction of p53 in keratinocytes (Fig. 13.1)
    - The principal determinant of melanin synthesis and release is the α-MSH–melanocortin receptor 1 (MC1R)–cAMP–PKA–CREB–microphthalmia-associated transcription factor (MITF) pathway
    - Further factors controlling pigmentation include SCF-, endothelin-, HGF-, FGF-, PGE_2- GM-CSF-, and NGF-mediated signaling
  - Additional players are the DNA-damage response (mediated through the ATM/

ATR–p53 and NER pathways), p38–MAPK, JNK, AKT, and the antioxidative system
- UV radiation and NMSC
  - Epidemiological and experimental data have established a causative link between UV light and NMSC
  - Incidence of basal cell carcinoma (BCC), squamous cell carcinoma (SCC) precursors, and SCC strongly correlates with exposure to natural (solar) and artificial (indoor tanning) UV radiation
    - NMSCs show frequent mutations of *TP53*, which harbor UV signatures
  - UV radiation may induce NMSC acting both as initiator and promoter:
    - Keratinocytes can be initiated by UV-induced *TP53* mutations, which interfere with the induction of DNA repair, cell cycle arrest, and elimination of keratinocytes with irreparable DNA-damage by apoptosis. This promotes cancer progression by providing a survival advantage and by increasing the acquisition of subsequent mutations in response to UV exposure
  - Critical oncogenic mutations induced by UV radiation involve NOTCH family members and *HRAS* in SCC as well as *PTCH1* in BCC
- UV radiation and melanoma
  - Epidemiologic and molecular data link UV radiation to some forms of melanoma:
    - Genomic analyses of cutaneous melanomas revealed an unusually high load of somatic mutations compared to other solid tumors, with a clear UV signature that implicated the majority of somatic mutations be caused by UV radiation
    - Sun-exposure early in life in susceptible individuals is a risk factor for melanocytic nevi and a distinct type of melanoma (nonchronically sun-damaged skin melanoma) favoring intermittently exposed sites and occurring predominantly at younger ages (<55 years)
    - High degrees of cumulative exposure to UV radiation promote development of a different type of melanoma, primarily affecting the chronically sun-damaged skin of individuals older than 55 years (chronically sun-damaged skin melanoma)
    - UV radiation is unlikely to play a role in the pathogenesis of acral and mucosal melanoma
  - The role of different UV wavelengths in the pathogenesis of melanoma is not fully resolved, with data supporting independent roles of UVA or UVB
    - Animal studies using the *Xiphophorus* fish and transgenic mice implicate UVB as the main inducer of melanoma
    - Epidemiological evidence of a dose-dependent association between the use of tanning beds primarily emitting UVA and cutaneous melanoma also supports a role of UVA, and has been attributed to the rising incidence of cutaneous melanoma in young females
  - The role of oxidative damage in cutaneous melanoma mutagenesis is questionable; UVA oxidative damage has been postulated as a main factor for inducing *BRAF* V600E mutations by T–A transversions and/or G–A and transitions

Melanoma

- Definition
  - A malignant neoplasm originating from melanocytes of the skin, mucosa, uvea, or, very rarely, other tissues
    - The denomination "melanoma" refers to a spectrum of different biological subtypes, characterized by distinctive genetic, epidemiological, clinical, and histological features
    - All melanomas are, by definition, considered malignant so that the term "malignant melanoma" represents a tautology
- Epidemiology of cutaneous melanoma
  - Cutaneous melanoma (CM) incidence is continuously increasing since several decades, whereas overall mortality has remained constant. Changes in behavior

resulting in increased sun-exposure levels have been attributed to the increase
    - The discrepancy between rapidly rising incidences with stable mortality raises the possibility that other factors may inflate the incidence, as there have been no significant improvements in CM therapy during the observation period. As in other cancers such as those of the breast and the prostate, part of the increase is likely to be due to stepped-up surveillance and increasing biopsy frequency with a resulting increase of biologically indolent disease forms that current diagnostic methods cannot reliably separate from CM with lethal potential
    - A shift in diagnostic criteria has been implicated as an additional contributing factor
- As a consequence of the increase in incidence, CM currently ranks as the fifth most common cancer in males and the sixth most common in females in the US population
- Light complexion, freckling, the number of melanocytic nevi, and a poor ability to tan are major phenotypic risk factors to develop CM
    - When adjusted for the size of the area at risk, CM most frequently arise on face and neck area, followed by trunk and proximal limbs
- CM in individuals younger than 65 years old primarily arise on the nonchronically sun-exposed skin, such as the trunk and the extremities. By contrast, in older individuals, CM preferentially arise on chronically sun-exposed sites. The paradoxical finding that CM on highly exposed sites take longer to develop is one of the main observations indicating the existence of at least two biologically distinct forms of CM, supported by histopathological and genetic findings (see below)
- The total number of nevi and an increased number of acquired nevi with increased size (so-called dysplastic or atypical nevi) are additional risk factors for CM originating from intermittently exposed skin

Table 13.1 Target genes under microphthalmia-associated transcription factor transcriptional regulation

Melanocytic differentiation
TYR, TYRP1, TYRP2/DCT, SILV/Pmel17, MLANA, OA1, RAB27A, SLC45A2, LYST, HPS4
Proliferation and survival
TBX2, CDK2, DIAPH1, CDKN1A/p21, CDKN2A/p16, BCL2, BIRC7/LIVIN, MET, APEX/Ref1, cKit, Ngfr
Additional targets
SLUG/SNAI2, GPNMB/osteoactivin, melastatin/TRPM1, DICER

    - CM arising on glabrous skin; i.e., palmoplantar surfaces and nail apparatus (so-called acral melanomas [AM]) and melanoma originating from the mucosa (mucosal melanoma [MM]) are reported at comparable absolute incidences throughout world populations. However, their relative frequencies are significantly higher in African and Asian populations, primarily due to the rarity of CM on the sun-exposed skin
        - The pathogenesis of AM and MM appears to be independent of UV exposure or sensitivity
- Melanocyte development and CM
    - Melanocytes are derived from the neural crest and colonize the epidermis and hair follicles of the skin during embryonic development. They are also found in notable numbers in mucosal membranes, uvea, stria vascularis of the inner ear, and leptomeninges
    - Melanocytes migrate from the neural crest through two main routes. A dorsolateral pathway takes them through the superficial mesenchyme from which they colonize epidermis and hair follicles of the skin. A ventromedial pathway also exists in which melanocytes emanate from a shared progenitor with Schwann cells within developing nerves
    - MITF plays a key role in melanocytic differentiation, function (i.e., pigmentation), proliferation, and survival (Table 13.1)
    - Additional players in regulating migration and differentiation of melanocyte precursors include:
        - Cell–cell contact interactions (i.e., N-CAM, E-CAM)

Table 13.2 Low penetrance cutaneous melanoma genes and loci

Confirmed, strong association after comprehensive meta-analyses of available GWAS studies:
MC1R, TYR, TYRP1, SLC45A2, MTAP, *VDR*, 20q11.13 (*ASIP MYH7B*, *PIGU*)
Additional genes/loci with weaker/inconsistent association:
TPCN2, KITLG, NCKX5, IRF4, OCA2, *MITF*, *TERT*, *TRF1*, 1p22, 10q25

    - Soluble factors and specific receptors (i.e., SCL–cKIT, ET1/3–EDNRB, glutamate–GRM1, HGF–Met, Neuregulin–ERBB3, soluble mediators within the WNT canonical and noncanonical pathways)
    - Transcription factors (PAX3, SOX10, CREB, β-catenin)
  - Dysregulation of related pathways is thought to play key role also in CM development, with different sets of molecular pathways being critical in the pathogenesis of distinct biologic types of CM
- Genetic predisposition to CM
  - Approximately 10% of CM occur in a familial setting of which about one-third are due to mutations of *CDKN2A*, primarily by affecting the *p16* gene product expressed from this locus; a small minority of familial CM is caused by germline mutations affecting *p14/ARF* or *CDK4*
  - Germline mutations in *BAP1* have also been linked to CM and uveal melanoma and germline mutations of MITF to CM.
  - In addition, several low penetrance genes have been identified the majority of which affect skin pigmentation and tanning ability (Table 13.2). The most important one to date is the *MC1R*, which is highly polymorphic in Caucasians resulting in decreased tanning ability, freckling, and red hair
- Somatic genetic aberrations of CM
  - Recent studies provided genetic and molecular data supporting the existence of different biological subtypes of CM; distinct CM subtypes would be characterized by different predisposing genotypes, environmental risk factors, molecular drivers, clinicopathological features, and responsiveness to specific therapies
- Oncogenes in CM
  - A number of oncogenes have been recently identified, whose constitutive activation through genetic mutations is believed to play a key role in development of different biological subtypes of CM. Several oncogenes show reproducible associations with clinical features suggesting that they delineate distinct biological subtypes of the disease
  - A common consequence of the major oncogenic mutations so far identified is the, direct or indirect, constitutive activation of the MAPK pathway (receptor tyrosine kinase (RTK)–RAS–RAF–MEK–ERK) (Fig. 13.2)
- *BRAF* and CM
  - Approximately 50% of all CM carry an activating *BRAF* mutation, most commonly affecting codon 600 in exon 15 (V600E or, less frequently, V600K)
    - *BRAF* V600 mutations are typically mutually exclusive with *NRAS* mutations
  - *BRAF* mutant melanomas seem to be more frequent in individuals with higher numbers of acquired, benign melanocytic nevi
    - Up to 80% of acquired common melanocytic nevi also bear *BRAF* activating mutations establishing them as clonal neoplasms
    - *BRAF* mutations are prevalent among CM arising from a preexisting nevus, but not all nevus-associated CM have BRAF mutations
  - *BRAF* mutant CM have characteristic morphologic and clinical features such upward scatter of intraepidermal melanocytes, increased cell size and pigmentation of neoplastic cells and low to moderate degrees of solar elastosis in the sub- and adjacent dermis
- RAS and CM
  - Approximately 15% of all CM have RAS mutations, with NRAS by far representing the most commonly mutated RAS family member

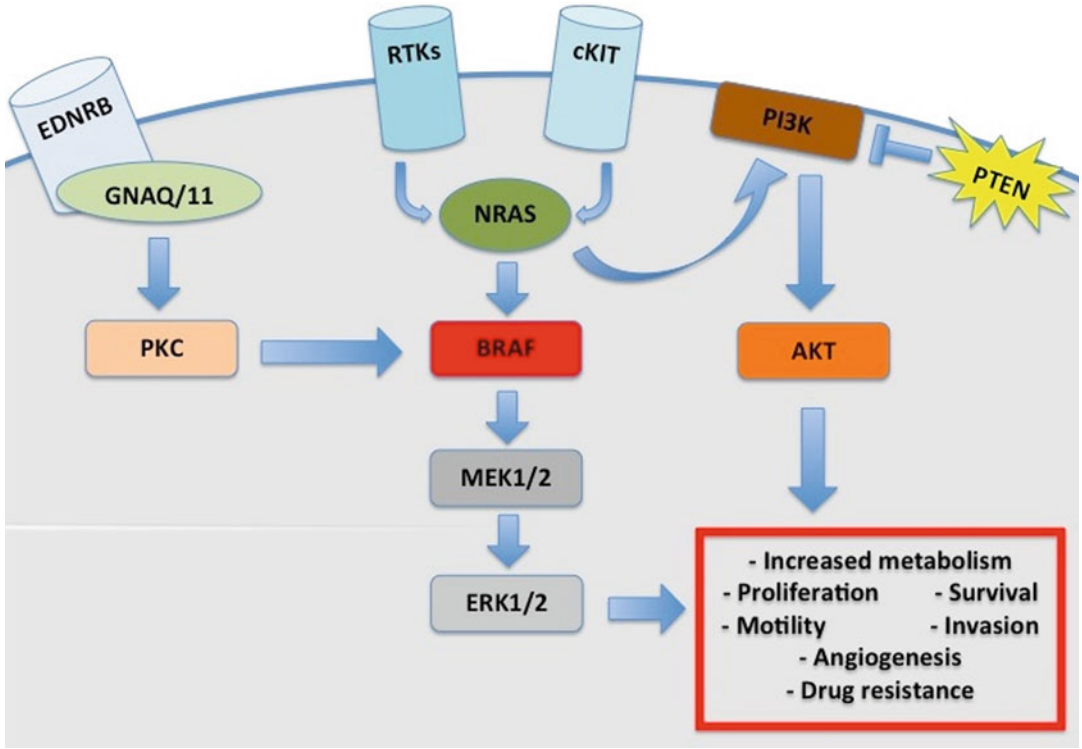

Fig. 13.2 Molecular pathways commonly activated by recurrent somatic events driving melanomagenesis. Different mutations converge on combined activation of the MAPK pathway and the PI3K–AKT pathway

  - ° *HRAS* mutations are found in approximately 1% of CM, and in up to 20% of benign Spitz nevi, with a predilection for the intradermal desmoplastic variant
  - ° *KRAS* appear to be rarely, if ever, mutated in CM
  - ° *NRAS* mutations are also a frequent finding in medium and large-sized congenital nevi (64–81% of cases)
- – Conflicting data are available about whether *NRAS* mutations are associated with specific CM subtypes
  - ° Some studies found NRAS in association with CM arising on chronically sun-damaged sites, nodular melanoma and lentigo maligna melanoma subtypes, increased Breslow thickness, higher mitotic index, and shorter survival. Other studies did not find specific associations with clinical or histopathological features

- KIT and CM
  - – KIT is a RTK which upon binding with SCF activates several pathways, including MAPK, PI3K/AKT/mTOR, and STAT3 pathways
  - – During embryonic development, *KIT* activation is essential for migration of melanocytic precursors from the neural crest to the skin along the dorsolateral pathway
  - – Activating mutations of *KIT* are found in 10–20% of CM occurring on acral sites (palmoplantar surfaces and nail apparatus), mucosal membranes, and chronically sun-damaged skin, and tend to occur in a mutually exclusive pattern with mutations in *BRAF* and *NRAS*. *KIT* mutations are typically absent in CM arising on intermittently sun-exposed sites
- G-alpha-proteins and melanoma
  - – Activating mutations in the Gα-subunits *GNAQ* and *GNA11* are found in the majority

of blue nevi, dermal melanocytoses (i.e., nevus of Ota and of Ito), uveal melanomas, and melanocytomas of the central nervous systems. The mutations affect primarily codons 209 or 183 of GNAQ or GNA11, crippling the G-proteins' GTPase activity, leading to constitutive activation. The mutations are found in a mutually exclusive pattern and do not co-occur with mutations in other known CM oncogene such as *BRAF*, *NRAS*, or *KIT*
    - Mutations are not sufficient to cause as they are also found in benign lesions such as blue nevi. They are therefore considered an early event in progression and have no prognostic role in established melanoma. Patients with nevus of Ota are at increased risk of uveal melanoma
- Other mutations in CM
  - Among all human cancers, CMs on sun-exposed skin have a high number of somatic mutations (10^4–10^5). As a consequence, distinguishing between mutations that are pathogenetic ("drivers") and irrelevant alterations ("passengers") is not always straightforward
  - Nevertheless, several genetic events have been described to occur more frequently than expected by chance in CM (Tables 13.3 and 13.4)
    - Only a minority of CM harbor *TP53* mutations; however, p53 function is typically impaired through genetic alterations upstream of p53 including amplification of *HDM2* or loss of *p14/ARF*
- Chromosomal aberrations and CM
  - Typically melanocytic nevi do not show clonal chromosomal aberrations
  - By contrast, the majority (>95%) of CMs have gains or losses of one or more chromosomes. Chromosomal aberrations in CM occur in a reproducible pattern that shows differences between subtypes. Frequent copy number increases involve chromosome 1q (25%), 6p (28%), 7 (50%), 8q (34%), 11q, 17q, and 20q, while common losses involve 6q (28%), 8p (22%), 9p, 9q (82%), and chromosome 10 (63%)

Table 13.3 Additional recurrent somatic events with pathogenetic significance in melanoma

Inactivating mutations/chromosomal losses within the *PTEN* locus (10q23)
Amplifications and/or activating mutations of *AKT1/3*
Genetic alterations involving the CDK4/6–cyclin D–p16–pRB pathway (i.e., chromosomal losses and/or inactivating mutations of *CDKN2A,* amplifications of *CCND1* or *CDK4*, deletions of RB)
Amplifications of *MITF*
Activating mutations of *CTNNB1*
Recurrent Ser722Phe mutations of *TRRAP*

Table 13.4 Other somatic events with possible relevance in cutaneous melanoma

Amplifications of *SETDB1*
Activating mutations of *ERBB4, FLT1, PTK2B*
Activating mutations of different metalloproteinases (*MMP8, ADAM7, ADAM29, ADAMTS18*)
GRIN2A inactivating mutations

  - Both AM and MM exhibit a distinctive form of genomic instability, with multiple amplifications and deletions; amplification recur at specific sites that differ between AM and MM. The most commonly amplified sites in AM are 11q13 harboring *CCND1* and 5p15 harboring *hTERT*. In MM, amplifications most commonly affect chromosome 12q14 harboring *CDK4* and *HDM2*. Amplifications arise early in progression as they can already be detected in the in situ portion of these CMs.
  - Detecting chromosomal aberrations in melanocytic neoplasms by e.g. comparative genomic hybridization or fluorescence in situ hybridization (FISH) can assist in the classification of melanocytic neoplasms with ambiguous microscopic features. A 4-probe FISH panel has been devised for diagnostic discrimination of melanocytic tumors, consisting of probes targeting chromosomes 6p, 6q, 6 centromere, and 11q13. Aberrations at these loci support the diagnosis of CM, and has been associated with increased risk of metastasis
- Evolving melanoma taxonomy
  - The first classification of CM was developed by Wallace Clark and colleagues in

Table 13.5 Melanoma subtypes according to the 2006 World Health Organization classification

Cutaneous melanoma
Superficial spreading melanoma (SSM): large pleomorphic epithelioid melanocytes in the epidermis with nested and single cell upward migration ("pagetoid upward scatter"), good circumscription, and significant intracytoplasmic melanization
Lentigo maligna melanoma (LMM): lentiginous proliferation of atypical melanocytes on the background of histopathologic signs of marked sun-damage, with frequent involvement of the adnexal epithelium
Acral lentiginous melanoma (ALM): arising from acral sites (palms, soles, and subungual region), lentiginous proliferation of atypical melanocytes often exhibiting marked dendricity and lateral extension as single units
Nodular Melanoma (NM): absence of any epidermal component extending more than three rete ridges beyond the margins of the dermal component
Desmoplastic melanoma (DM): intradermal proliferation of spindled melanocytes in the context of marked stromal desmoplasia, with frequent evidence of lentigo maligna in the overlying epidermis; pure and mixed subtypes can be identified according to the absence or presence of an additional population of more conventional malignant melanocytes, respectively
Nevoid melanoma: histopathologically resembling a nevus, of the common (small cell melanoma) or Spitz (spitzoid melanoma) variants
Melanoma arising from a blue nevus
Melanoma arising in a congenital nevus
Melanoma of childhood
Persistent melanoma: recurring locally after incomplete removal of the primary lesion
Mucosal melanoma (MM)
Uveal melanoma (UM)

1973, distinguishing different "histogenetic" subtypes. This classification was expanded in 2006 to what is the current WHO classification of melanoma (Table 13.5). This WHO classification has not been widely adopted into clinical management, as subtypes partially overlap and do not reliably guide therapy stratification

– Recent advances in the molecular pathogenesis have generally supported the existence of distinct subtypes and provided additional features for classification. While a taxonomy that integrates clinical and mechanistic aspects is still evolving, one can currently distinguish the following seven melanoma subsets: melanoma arising on skin without chronic sun-induced damage (NCSD), on skin with chronic sun-induced damage (CSD), acral skin (glabrous skin of the palms and soles and nail apparatus; AM), mucosal sites (MM), and melanoma arising from nonepithelial-associated melanocytes such as the uveal tract, dermis, and CNS

- Melanomas arising on sun-exposed skin without signs of cumulative sun-induced damage (non-CSD melanomas)
  – The most frequent type of CM in populations of European descent, peaking in the fifth–sixth decades and decreasing thereafter, primarily occurring on trunk and proximal limbs (intermittently sun-exposed skin)
  – Occurs in patients with increased number of acquired melanocytic nevi
  – Histopathological features consisting of sharp demarcation from the surrounding skin, melanocytes of increased size and round shape with increased intracellular pigment ("pagetoid") preferentially arranged in nests rather than single units and exhibiting frequent upward scatter in the epidermis; NCSD melanomas show overlap with the SSM category of the WHO classification but also include nodular melanomas arising on nonchronically sun-damaged skin
  – Approximately up to 70% of NCSD melanomas carry *BRAF* activating mutations, with the remaining fraction having *NRAS*

(20%) and/or other, yet undiscovered, oncogenic alterations
  - Recurrent chromosomal aberrations include gains on 1q, 6p, 7, 8q, 17q, and 20q, and losses on 9p, 6q, and 10
    - The lower age of patients with *BRAF*-mutated CM along with the association with *BRAF* mutant nevi, which tend to occur in the first two decades of lives, suggests that *BRAF*-driven melanocytic neoplasms develop during a window of increased vulnerability early in life
- Melanomas arising on sun-exposed skin with signs of cumulative sun-induced damage (CSD melanomas)
  - The second most frequent subtype of CM in populations of European ancestry, most common in individuals older that 55 years and further increasing with age, and primarily affecting the head and neck area and the sun-exposed areas of distal limbs
  - Arising on chronically sun-damage skin as determined by the presence of marked solar elastosis. No association with the number of melanocytic nevi
  - Histopathological features include poor circumscription, smaller melanocytes primarily arranged as single units in the basal epidermis (lentiginous growth)
  - 20% of cases bear activating mutations in either *NRAS* and 10–20% have mutations or copy number increases of *cKIT*, whereas *BRAF* mutations are less prevalent (approximately 10%)
  - Frequent chromosomal aberrations include gains on 1q, 6p, 11q13, 17q, and 20q, and losses on 6q, 8p, 9p, and 13
- Acral melanomas
  - Definition: CM arising on the glabrous (nonhair-bearing) skin of the palmoplantar surfaces and the nail apparatus
  - Incidence increases with age, peaking in the seventh–eighth decades, and is comparable among world populations; UV light is not considered to be an etiologic factor
  - No association with number of acquired melanocytic nevi
  - Histopathological features include poor circumscription, lentiginous growth
  - *cKit* mutations and/or amplifications are seen in approximately 20–30% of cases, 20% have *BRAF* mutations, and approximately 15% have *NRAS* mutations in a nonoverlapping pattern.
  - Other genetic features include marked genomic instability characterized by multiple focal amplifications, most often involving the *CCND1* and *hTERT* loci on 11q13 and 5p15, respectively. The amplifications arise early during progression and are detectable at the in situ stage. Additional recurrent DNA copy number changes include gains on 6p, 7, 8q, 17q, 20q, amplifications on 5p13 and 12q14, and losses on 6q, 9p, 10, 11q
- Mucosal melanomas
  - Definition: melanomas occurring on mucosal membranes, including the oropharynx, paranasal sinuses, conjunctiva, and anogenital region
  - Similar incidences across all world populations; no association with acquired melanocytic nevi; UV light is a pathogenetic factor
  - Histopathological features include poor circumscription, lentiginous growth
  - *cKit* mutations and/or amplifications are seen in approximately 20–30% of cases, 15% have *NRAS* mutations in a nonoverlapping pattern. *BRAF* mutations are rare.
  - Marked genomic instability similar as in AM with differences in the genomic region affected by focal amplifications. Most frequently amplified sites are the *CDK4*/*HDM2* locus on 12q; additional frequent DNA copy number changes include gains on 1q, 6p, 7, 8q, 11q13, 17q, 20q, amplifications on 1q31 and 4q12, and losses on 3q, 4q, 6q, 8p, 9p, 10, 11p, 11q
- Desmoplastic melanomas
  - Definition: a rare variant of invasive CM characterized by a deeply invasive proliferation of spindled cells within a desmoplastic stroma with tendency for extensive local invasion and high incidence of local recurrence
  - Most often occurring on CSD skin of elderly adults with fair skin; less frequently, younger patients and/or sun-protected sites are affected

  - Typical presentation is an amelanotic plaque/nodule, often in the context of a preceding visible pigmented lesion
  - The histopathological hallmark is a deeply infiltrative, asymmetric growth of spindled cells dispersed in a fibrotic stroma. Two histologic subtypes are recognized:
    - Pure DM: spindled neoplastic cells represent >90% of overall neoplastic population
    - Mixed DM: presence of a distinct population of malignant melanocytes resembling conventional melanoma, constituting >10% of the total neoplastic cells
    - Pure DMs are characterized by low frequency of nodal and visceral metastases; conversely, risk of nodal and systemic metastatic disease in mixed DMs is not significantly different from conventional CMs, suggesting that mixed DMs are derived from conventional CMs with secondary desmoplastic change
    - An intraepidermal (in situ) proliferation of neoplastic melanocytes is observed in a majority of cases; if the latter is absent, valuable diagnostic clues include detection of hyperchromatic nuclei and increased mitotic rate in spindled cells, perineural invasion, and patchy lymphocytic aggregated throughout the lesion
  - IHC features: strong positivity for S100 in most (but not all) cases, but common lack of expression of MITF, tyrosinase, HMB45, and MART1; staining with Ki67, SOX10, and p75/NTR may be useful
  - The genetic features of DM are yet to be characterized; common genetic events observed in CM (including *BRAF, NRAS,* and *cKit* mutations) are not observed in DM
- Melanoma of childhood
  - Definition: Varying age cutoffs are used, but the entity is probably best defined as melanomas occurring in prepubertal children, because melanomas in older children express a similar clinical behavior as adult CMs and have similar genetic alterations (high frequency of *BRAF* mutations) and histologic features (often consistent with SSM), and prognosis
  - The majority of cases (up to 75%) occur after age 14 years; if postpubertal cases are included, melanoma accounts for 1–3% of all pediatric malignancies
  - The category of pediatric melanoma encompasses a heterogeneous spectrum of conditions, including:
    - Transplacental melanomas, transmitted from an affected mother to the fetus in utero
    - Melanomas arising in large congenital melanocytic nevi, usually having a non-epidermal origin
    - Melanomas occurring in association with predisposing conditions such as xeroderma pigmentosum or oculocutaneous albinism
    - Malignant spitzoid tumors
  - The definition of malignant spitzoid tumors is based on demonstrated progression to systemic metastatic disease. A significant portion of borderline Spitz tumors or atypical Spitz tumors can show regional micrometastasis or even macrometastasis but does not progress to systemic metastatic disease. Malignant spitzoid tumors tend to have clonal chromosomal aberrations that can assist in their diagnosis
    - No histologic features of primary tumors have been found to reliably predict the malignant behavior of atypical spitzoid tumors
    - Sentinel lymph node positivity alone does not appear to be a reliable predictor of outcome as in adult CM
    - Despite the lack of a comprehensive genetic characterization, malignant spitzoid tumors appear to constitute a separate category, with distinctive biologic behavior and prognostic implications; further studies are needed to address this issue
- Uveal melanomas
  - Definition: melanomas originating from melanocytes of the choroid, ciliary body, and iris
  - Most common primary intraocular malignancy in adults, with approximately 1,500 new cases annually in the USA

Table 13.6 Tumor, node, and metastasis (TMN) staging categories for cutaneous melanoma

T		
Classification	Thickness (mm)	Ulceration status/mitoses
Tis	NA	NA
T1	≤1.00	a: Without ulceration and mitosis <1/mm^2
		b: With ulceration or mitoses ≥1/mm^2
T2	1.01–2.00	a: Without ulceration
		b: With ulceration
T3	2.01–4.00	a: Without ulceration
		b: With ulceration
T4	>4.00	a: Without ulceration
		b: With ulceration
N		
	Number of metastatic nodes	Nodal metastatic burden
N0	0	NA
N1	1	a: Micrometastasis[a]
		b: Macrometastasis[b]
N2	2	a: Micrometastasis[a]
		b: Macrometastasis[b]
		c: In transit metastases/satellites without metastatic nodes
N3	4+	Metastatic nodes, or matted nodes, or in transit metastases/satellites with metastatic nodes
M		
	Site	Serum LDH
M0	No distant metastases	NA
M1a	Distant skin, subcutaneous, or nodal metastases	Normal
M1b	Lung metastases	Normal
M1c	All other visceral metastases	Normal
	Any distant metastasis	Elevated

NA not applicable; *LDH* lactate dehydrogenase
[a]Micrometastases are diagnosed after sentinel lymph node biopsy
[b]Macrometastases are defined as clinically detectable nodal metastases confirmed pathologically

- Increased frequency in populations with fair skin and lightly colored eyes
- 95% of patients develop liver metastasis.
- Histopathologically, highly pigmented melanocytes show a spindle, epithelioid, or mixed morphology
- Prognostic features include: old age, large size of primary tumor, ciliary body involvement, epithelioid morphology, and monosomy of or loss of heterozygosity of chromosome 3, trisomy of chromosome 8, class 2 expression signature
- Gain of function mutations of the *GNAQ* or *GNA11* oncogenes in approximately 90% of tumors in a mutually exclusive pattern. No mutations in *BRAF, NRAS*, or *cKIT*. Biallelic loss of the histone deubiquitinase *BAP1* on chromosome 3 correlates with metastatic progression and poor prognosis

• Staging of cutaneous melanoma
  - The 2009 AJCC Melanoma Staging Guidelines include tumor thickness, ulceration, and mitotic rate (at least 1 mitosis/mm^2) as defining criteria (Table 13.6)
  - Other features of possible prognostic relevance include lymphovascular or perineural invasion, microscopic satellitosis, and host response ("brisk," "nonbrisk," or "minimal/absent")

- Immunohistochemical markers of melanoma
  - Antibodies against S100, gp100 (HMB45), MART1/Melan A, and tyrosinase, MITF, and SOX10 are commonly used diagnostically to detect melanocytic differentiation
    - Desmoplastic melanomas often lose expression of HMB45, MART1, MITF, and tyrosinase

Nonmelanoma Skin Cancer

- Definition
  - NMSCs are a group of keratinocyte-derived neoplasms consisting of:
    - BCC: a neoplasm composed of epithelial cells morphologically resembling the keratinocytes of the epidermal basal layer
    - SCC: a neoplasm of keratinocytic origin exhibiting variable degrees of differentiation
    - Precancerous conditions such as
      - Actinic keratosis (AK) and actinic cheilitis
      - Arsenic-, radiation-, tar-, and PUVA-induced keratoses
      - HPV-associated epidermal dysplasias (i.e., bowenoid papulosis and dysplastic lesions found in epidermodysplasia verruciformis (EV))
    - Bowen disease (BD) and erythroplasia of Queyrat, variants of SCC in situ occurring on the skin and genital mucosa, respectively
    - Keratoacanthoma (KA), a variant of SCC that is characterized by rapid growth, crateriform architecture with highly differentiated squamous cells, and a tendency to spontaneously regress
- NMSC epidemiology and associations
  - BCC is the most frequent cancer in humans (≅25% of all malignancies in the USA), accounting for approximately 75% of NMSC
  - Incidences of both BCC and SCC are consistently increasing in Caucasians, mainly due to population aging and increase of recreational UV exposure
  - UV radiation exposure is a key etiologic factor for both BCC and SCC
    - Intermittent sun-exposure and a history of blistering sunburns in the first two decades of life are risk factors for BCC
    - Cumulative sun-exposure is the primary risk factor for SCC and AK
  - After the first diagnosis of NMSC, the risk of developing subsequent NMSCs in the following 5 years is estimated to be about 50%
  - Additional risk factors and associations for development of NMSC are described in Table 13.7
- Genetic predisposition to NMSC
  - Syndromes caused by defects in DNA repair such as xeroderma pigmentosum and Cockayne syndrome or defects in pigmentation as in albinism results in increased risk for NMSC
  - Other heritable genetic alterations of lower penetrance have been associations with NMSC risk and typically are associated with light complexion (*MC1R*, *POMC*, *ASIP, TYR, IRF4, SLC45A2*). Variation in genes involved in the immune response have also been identified as risk factors (*IL10*, *IL12*, *IL4R*, *TNFR2*, *HTR2A*)
  - In addition, no further specified risk factors have been linked to chromosome 6p25 (near *EXOC2*) and 13q32 (near *UBAC2*)
  - Genetic loci associated with increased frequencies of BCC, but not SCC, are *KRT5*, *KLF14*, *TERT–CLPTM1L*, and not further refined loci at 1p36, 1q42, and 9p21
- Clinical features of BCC
  - BCC can present as several clinicopathological variants
    - Nodular BCC is most common and clinically characterized as a papule, plaque, or nodule with telangiectases, pearly border, and frequent ulceration
      - 70% of nodular BCC present on the head and neck area (predominantly the face), and 25% on the trunk and limbs
      - Superficial BCC: erythematous flat papule or plaque, typically presenting on the trunk or proximal limbs

Table 13.7 Risk factors for the development of nonmelanoma skin cancer

Risk factor	Basal cell carcinoma (BCC)	Squamous cell carcinoma (SCC)
Age	More frequent with increasing age	
Gender	Men 1.5–2× as frequently affected as women	
Skin complexion	More frequent in individuals with fair complexion, red hair, poor tanning ability, propensity to freckling	
Sun-exposure	Intermittent sun-exposure, history of blistering sunburns in childhood/adolescence, tanning beds Cumulative sun-exposure may increase risk on the facial area	Cumulative sun-exposure
Viruses	No known association	HPVs, in particular in immunocompromised patients and in epidermodysplasia verruciformis
Other	PUVA therapy Tanning beds Ionizing radiation Arsenic (contaminated water; Fowler solution)	PUVA therapy Tanning beds Ionizing radiation Arsenic (contaminated water; Fowler solution) Polycyclic aromatic hydrocarbons Mineral oils Cigarette smoking
Clinical conditions	Chronic immunosuppression (to a lesser degree than SCC) Chronic wounds (to a lesser degree than SCC)	Chronic immunosuppression (organ transplantation, hematologic malignancies, AIDS) Chronic wounds Chronic inflammation (discoid lupus erythematosus, erosive/hypertrophic lichen planus, lichen sclerosus et atrophicus) Linear porokeratosis
Genetic syndromes	Xeroderma pigmentosum Albinism Nevoid basal cell carcinoma (Gorlin–Goltz) syndrome Bazex–Dupré–Christol syndrome Rombo syndrome Oley syndrome (?)	Xeroderma pigmentosum Albinism Epidermodysplasia verruciformis Epidermolysis bullosa (dystrophic > junctional) Muir–Torre syndrome (keratoacantomas) Ferguson–Smith syndrome (keratoacantomas) Witten and Zak syndrome (keratoacantomas)
Previous history of NMSC	Increased risk of BCC and SCC	

- Sclerotic (morpheaform) BCC: nondescript plaque with scar-like features and ill-defined border
- "*Ulcus rodens*": exophytic nodular BCC with extensive central ulceration surrounded by rolled edges
- Fibroepithelioma of Pinkus: a soft, exophytic papule or nodule resembling a common dermal nevus of skin tag, often on the lower back
- Pigmented BCC: any above variant with brown to black, speckled or diffuse pigmentation, simulating melanoma

– BCC rarely metastasize (to the lung or bone) or cause death, but the propensity for local invasion can result in significant morbidity, even death if the neoplasms is not eradicated
– Multiple BCCs, in particular when occurring at young age, can be indicative of nevoid basal cell carcinoma syndrome (NBCCS), an autosomal-dominant syndrome caused by mutations in the hedgehog signaling pathway (Tables 13.8 and 13.9)
– A number of clinicopathological features are associated with increased risk of recurrence after surgical excision (Fig. 13.3)

Table 13.8 Nevoid basal cell carcinoma syndrome (NBCCS)

Synonyms: basal cell nevus syndrome, Gorlin–Goltz syndrome
Autosomal dominant transmission with complete penetrance and variable expressivity
Causative germline mutations in *PTCH1* (9q22.3), including insertions, deletions, missense, nonsense, and splice site mutations
Somatic inactivation of the wild type *PTCH1* allele by deletion, mutation, or loss of heterozygosity
In a minority of tumors no somatic event (either genetic or epigenetic) is detected, implying that *PATCH1* haploinsufficiency can be sufficient in some circumstances
Clinical signs
Neoplasms: multiple basal cell carcinoma; medulloblastoma; fetal rhabdomyoma/rhabdomyosarcoma; keratocystic odontogenic tumors of the jaw; ovarian and/or cardiac fibromas
Skin: epidermal cysts; palmar and plantar pits; facial milia
CNS: calcification of the falx and/or tentorium cerebella; cerebral cysts
Eyes: microphthalmia; congenital cataract; coloboma of the iris, choroid, and/or optic nerve; orbital and conjunctival cysts; strabismus and/or nystagmus
Head: macrocephaly; frontal bossing; enlarged mandibular coronoid; cleft lip and/or palate
Skeleton: increased length; kyphosis; spina bifida; bifid ribs; polydactyly/syndactyly of hands and/or feet
Additional features: mesenteric cysts; kidney abnormalities; hypogonadotropic hypogonadism
Multiple cutaneous basal cell carcinoma
Starting to occur from childhood onwards
Predilection for sun-exposed regions
High sensitivity to radiation
The infundibulocystic histotype is increased in frequency
Rarely metastases develop, primarily affecting lungs and bones
Systemic (i.e., GDC–0449) and local (i.e., LDE225) inhibitors of SMO have induced partial/complete resolution of cutaneous basal cell carcinoma in NBCCS patients
Objective responses to systemic treatment have been observed also for medulloblastomas and odontogenic keratocysts of the jaw

Table 13.9 Additional genetic syndromes associated with multiple basal cell carcinomas

Bazex–Dupré–Christol syndrome
Until now, approximately 20 families have been reported
X-linked dominant transmission with variable penetrance and expression; genetic defect unknown, but involved locus mapped to Xq25–27.1
Clinical features include hypotrichosis, follicular atrophoderma (dorsa of hands), milia, multiple trichoepitheliomas, hypohidrosis, facial hyperpigmentation
Hypotrichosis is often noticed early after birth, other symptoms manifest during childhood
Multiple BCC may develop from the first decade onwards
Rombo syndrome
So far, three families have been described
Autosomal dominant transmission, but genetic defect still unknown
Clinical features include hypotrichosis, vermiculate atrophoderma (cheeks and elbows), milia, multiple trichoepitheliomas, peripheral vasodilation with cyanosis
Follicular atrophy of sun-exposed skin and cyanosis are first noticed in childhood
Multiple BCC develop from the fourth decade onwards
Oley syndrome
Described by Oley et al. in 1992
Inheritance mode and genetic defect unknown
Probably a clinical variant of Bazex–Dupré–Christol syndrome with spontaneous regression of manifestations during adolescence

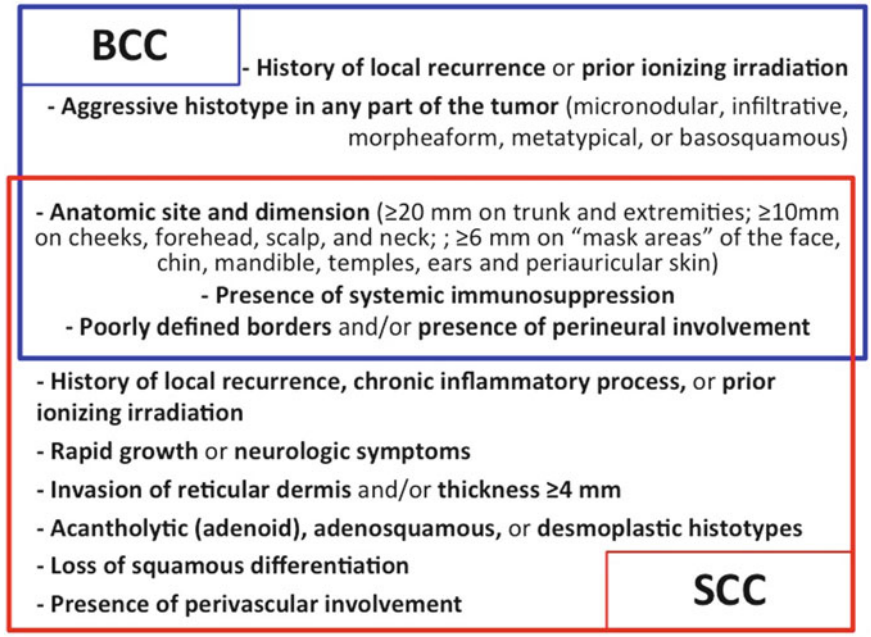

Fig. 13.3 Clinical and histologic risk factors for aggressive behavior in invasive nonmelanoma skin cancer. Many of them are shared by both basal cell carcinoma (BCC) and squamous cell carcinoma (SCC)

- Pathological features of BCC
  - Typical histopathological attributes include:
    - Basaloid cells arranged in round or angulated aggregates that emanate from the epidermis and show peripheral palisading and a characteristic retraction artifact from the surrounding stroma often rich in mucin
  - Several histological variants are distinguished and are associated with varying risk of recurrence after surgery:
    - Low risk: superficial BCC, nodular BCC, adenoid BCC, fibroepithelioma of Pinkus
    - High risk: micronodular BCC, infiltrative BCC, morpheaform BCC
  - The IHC profile of BCC resemble that observed in the outer root sheet of the hair follicle
    - BCC express CK5 and CK14, which are characteristic of the normal basal layer. BCCs also express CK15, CK19, SOX9, and Ber–EP4 (as observed in the embryonic hair germ)
  - In addition, BCC cells commonly express androgen receptors, diffuse BCL2 protein, and α6 and β1 integrins, while surrounding stroma stains for CD34 and nestin
- Molecular features of BCC
  - The vast majority of BCCs have somatic mutations in genes involved in the hedgehog signaling pathway primarily involving the inactivating mutations or deletions of the patched (*PTCH1*) gene (90%) or activating mutations of the smoothened (*SMO*) gene (10%)
  - Genomic loss of 9q occurs in 30–50% of BCCs, with the minimally deleted area spanning the *PTCH1* gene at 9q22.3
  - *TP53* is mutated in 50% of cases
  - *PTCH1* and *TP53* mutations have nucleotide substitutions characteristic for UV-radiation, underscoring the role played by sun-exposure in BCC pathogenesis
  - Additional recurrent DNA copy number changes in BCC include gains of 6p, 7, 9p, 18, and X

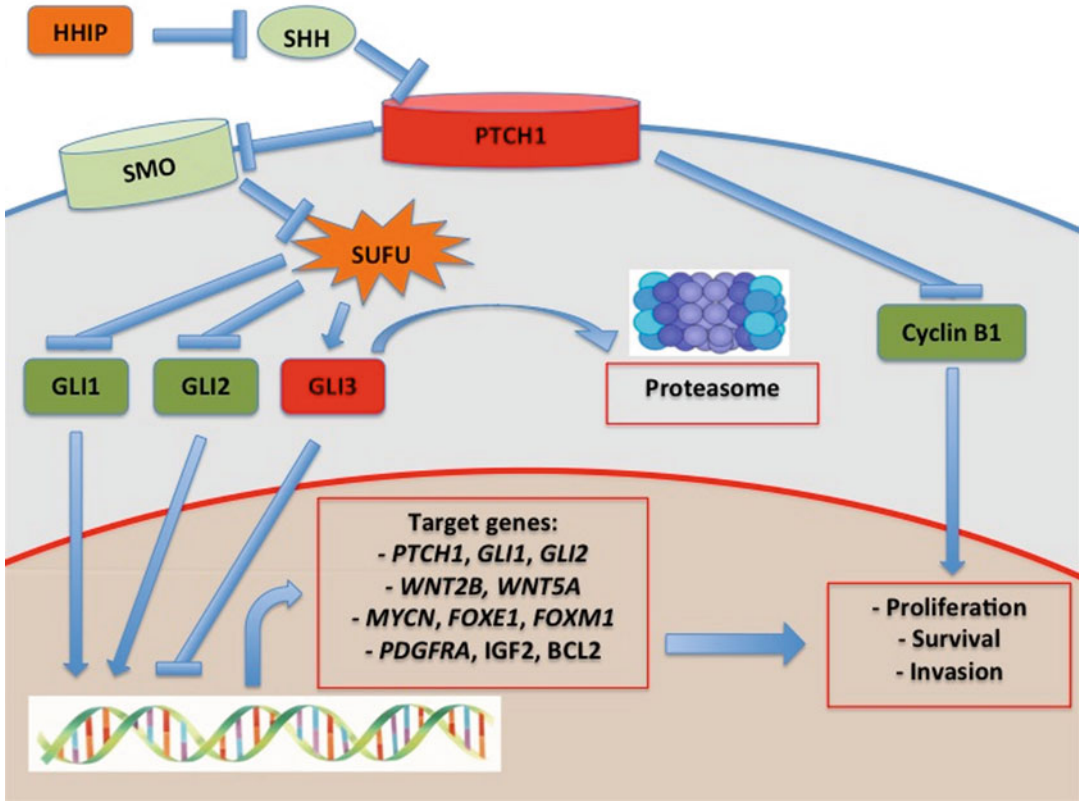

Fig. 13.4 Dissection of the molecular events involved in the hedgehog pathway. Most of the consequences deriving from PTCH1 loss of function are exerted through the SMO–Gli axis, but Gli-independent effects have been identified as well

  - Trisomy of chromosome 6 may correlate with local aggressiveness and metastatic behavior
  - Activating *RAS* mutations and inactivating mutations in *CDKN2A* have been detected in a small minority of BCC
- Hedgehog signaling and BCC
  - Hedgehog (HH) signaling was initially identified in *Drosophila melanogaster*, where it is critical for regulating the antero-posterior polarity of the developing larva
  - The mammalian hedgehog family includes the Indian hedgehog (*IHH*), desert hedgehog (*DHH*), and sonic hedgehog (*SHH*) genes (Fig. 13.4)
    - SHH signaling is crucial in early development, regulating the formation of skin, neural tube, musculoskeletal system, hematopoietic cells, and for hair follicle growth and cycling
    - IHH signaling is critical for cartilage differentiation; DHH signaling regulates the development of perineurium of peripheral nerves and spermatogenesis
  - Crucial effectors of SHH signaling are PTCH1, SMO, and GLI proteins
    - Active SHH is released in the extracellular space and acts as ligand for its receptors PTCH1 and PTCH2, which, in the absence of SHH, keep the G-protein-coupled receptor SMO in an inactive state. Binding of SHH to PTCH1 relieves SMO inactivation. PTCH and SMO are mainly located at the primary cilium of cells. Activation of SMO results in activated signaling from the GLI family of

transcription factors, including GLI1 (transcriptional activator), GLI2 (activator or repressor depending on post-translational modifications), and GLI3 (transcriptional repressor). GLI-mediated signaling increases the transcription of SHH target genes such as *PDGFRA*, *MYCN*, *FOXE1*, *FOXM1*, *RUNX3*, *BCL2*, *WNT2B, WNT5A, PTCH1*, *GLI1*, *GLI2*, *HHIP, MTSS1*, and *IGF2*
    - *PTCH1* (9q22.3) encodes a 12-pass membrane protein receptor which can bind and sequester SMO
    - *PTCH2* (1p33–34) shares 73% amino acid homology with *PTCH1*, but does not seem to play a major role in the SHH pathway in BCC
    - *SMO* (7q32) encodes a 7-pass GPCR protein
  - Additional components of the SHH pathway include:
    - The suppressor of fused homolog (SUFU), which binds to GLI proteins in the cytoplasm and exert an inhibitory function
    - STK36 (fused homolog, FU), which stabilizes GLI1 by inhibiting GLI1 ubiquitination
    - HHIP, a transmembrane glycoprotein that compete with PTCH1 for binding of SHH
  - In adult tissues, SHH signaling is mostly turned off; SHH signaling abnormal reactivation is involved in the pathogenesis of several neoplasms, including cutaneous BCC. Aberrant production of SHH, inactivating mutations and/or deletions of *PTCH1*, activating mutations of *SMO*, and genetic defects of *SUFU* are all mechanisms which can lead to abnormally active SHH signaling in BCC and other cancers such as medulloblastoma
    - There is evidence that little more than aberrant SHH signaling activation is needed for BCC initial development
  - SHH signaling and cell cycle regulation
    - Aberrant expression of SHH was proved to downregulate p21/CIP, promoting G1/S cell cycle progression
  - Depending on the role exerted by SHH signaling, malignant neoplasms can be classified in the following categories:
    - Group I: HH signaling is important for tumor initiation and maintenance (BCC, medulloblastoma, rhabdomyosarcoma)
    - Group II: HH signaling is important for tumor maintenance but not initiation (colorectal cancer, pancreatic AdC)
    - Group III: HH signaling is implicated in tumorigenesis, but its role is still undefined (prostate AdC, breast cancer, hematologic neoplasms, lung carcinoma, ovarian carcinoma, gastric cancer, esophageal AdC)
- Cell of origin of BCC
  - Epithelial cells and their precursors within the interfollicular epidermis and hair follicle epithelium are considered possible cells of origin for BCC
  - Different histologic patterns of BCC may derive from different cells of origin
- Targeted therapies for BCC
  - Targeting the SHH signaling pathway is the rational basis for the development of therapeutic approaches for BCC
  - Cyclopamine was the first SMO antagonist to be discovered and demonstrated to be effective against preexisting BCC in animal models of BCC
  - Refined inhibitors of SMO have been developed and show increased efficacy, fewer side effects, and better pharmacokinetic profiles
    - Systemic administration of the small molecular inhibitor GCD-0449 lead to tumor shrinkage in patients with locally advanced and metastatic BCC and reduced tumor burden in patients with NBCCS
    - Small molecule inhibitors are also under development for topical treatment of BCC; e.g., LDE225 and CUR61414
- Clinical features of SCC and precursor lesions
  - Actinic keratosis (AK) is a noninvasive precursor occurring on chronically sun-damaged skin
  - Synonyms: solar keratosis, senile keratosis, actinic cheilitis (i.e., AK occurring on the lip borders)

- Association with history of high cumulative sun-exposure and high number of solar lentigines
- Often multiple lesions ("field effect")
- Scaly erythematous macule, thin papule, or plaque, rough on palpation (more easily felt than seen), occasionally pigmented
- Overall risk of transformation to invasive SCC for individual lesions is low (<0.1%/year) but relevant due to high multiplicity and persistence of lesions
- Bowen disease (BD) is a full thickness dysplasia representing SCC in situ arising de novo (without adjacent AK)
- Commonly affecting on chronically sun-damaged skin but can involve sun-protected areas such as the genitalia
- Scaly, erythematous, circumscribed plaque
- BD occurring on the mucosal surfaces of the anogenital region is also known as erythroplasia of Queyrat
  - Invasive SCC
- Significantly more frequent in light-skinned individuals, but also the most frequent cutaneous malignancy in dark-skinned populations
- Typical presentation: an ulcerated, non-healing, keratotic papule, plaque, or nodule with an erythematous base on sun-exposed skin
- Adjacent AK or BD is often present in the background
- Development on sun-protected areas is favored by chronic inflammation/infection, systemic immunosuppression, and exposure to ionizing radiation or chemical carcinogens
- Overall risk of metastasis between 2% and 5% varying depending on clinical and pathological features (Fig. 13.3)
- Probability of extensive local invasion, recurrence after excision, and systemic metastasis is higher when high risk clinicopathological features are present (Fig. 13.3)
  - Verrucous carcinoma
- A well-differentiated variant of invasive SCC
- Almost no risk of systemic metastases but high propensity to local invasion (erosion of underlying bone structures is possible)
- Variants: giant condyloma of Buschke and Löwenstein: verrucous carcinoma on the genital skin; oral florid papillomatosis: verrucous carcinoma on the oral mucosa; carcinoma cuniculatum: verrucous carcinoma on the plantar surfaces
  - KA
- A rapidly growing, dome-shaped tumor with a central keratin plug presenting on sun-damaged skin. Marked tendency for spontaneous, complete involution within 3–6 months
- Variants: multiplicity of lesions in nonfamilial (Grzybowski syndrome) or in familial settings (Muir–Torre syndrome [MTS], Ferguson–Smith syndrome, Witten and Zak syndrome)
- Rare cases reported to cause visceral metastases, lending support to the notion that KA is a more benign form of cutaneous SCC

- Histological features of SCC and precursor lesions
  - AK
    - Pleomorphic keratinocytes, primarily in the basal layer with overlying parakeratosis, sparing the adnexal ostia
    - Epidermis may range from thinned (atrophic AK) to significantly acanthotic (hypertrophic AK)
    - In the dermis, superficial perivascular or lichenoid lymphohistiocytic infiltrate (rich in plasma cells in actinic cheilitis), and evidence of marked solar elastosis
    - Several histopathological variants are recognized, including acantholytic AK, digitated AK, pigmented AK, bowenoid AK
    - Attempts of histopathological grading of epidermal dysplasia (i.e., classification into keratinocyte intraepithelial neoplasia I, II, and III) have failed to show adequate reproducibility or significant correlation with risk of invasion
  - BD
    - Full-thickness intraepidermal dysplasia with loss of ordered maturation, nuclear pleomorphism, atypical mitoses, and scattered apoptotic cells

- Acanthotic epidermis with parakeratotic foci and bulbous rete ridges; underlying basal layer often preserved ("eyeliner sign")
- Variants: Pagetoid BD, clear cell BD change. Can be distinguished from Paget disease by negative IHC staining for CAM5.2 and CK7

– Invasive SCC
- Buds and strands of neoplastic keratinocytes invading into the underlying dermis
- Variable degree of differentiation, ranging from formation of horn pearls with preservation of intercellular bridges poorly differentiated tumors with only single cell or complete absence of keratinization
- Recognized histopathological variants include acantholytic (adenoid) SCC, spindle cell SCC, desmoplastic SCC, neurotropic SCC, and clear cell SCC

– Verrucous carcinoma
- Invading broad columns and lobules of well-differentiated neoplastic keratinocytes contiguous with an overlying epithelium that shows hyperkeratosis and papillomatosis
- Preserved keratinization and blunt, pushing borders at the edges of invading lobules
- Modest cytological pleomorphism, rare mitoses, formation of intraepithelial microabscesses

– KA
- Symmetrical, exoendophytic, cup-shaped proliferation of well-differentiated, ground glass-like keratinocytes with sharply demarcated peripheral border
- Central core with keratinous horn plug; frequent intraepithelial microabscesses with neutrophils and eosinophils. Low mitotic rate
- Regressing KA: increased number of apoptotic cells, loss of central keratin plug, flattened epithelium, underlying inflammatory foreign body reaction

• Molecular features of SCC and related lesions

– Syndromes with germline mutations resulting in a genetic predisposition specifically to SCC include epidermodysplasia verruciformis (Table 13.10). Multiple KA (at times with sebaceous differentiation) are a characteristic feature of MTS and the autosomal dominant Ferguson–Smith syndrome caused by inactivating germline mutation of *TGFBR1*

Table 13.10 Epidermodysplasia verruciformis (EV)

A rare, mainly autosomal recessive disease characterized by increased susceptibility to HPV cutaneous infection and HPV induced oncogenesis
Classical EV-associated genes include *EVER1* (*TMC6*) and *EVER2* (*TMC9*), encoding transmembrane channel-like proteins that localize to the endoplasmic reticulum. *EVER1* and *EVER2* defects result in deficient cell-mediated immunity and impaired immune response against EV-associated β-HPV strains (mainly HPV5 and HPV8)
Rare cases with X-linked recessive transmission have been reported
Cutaneous lesions start to occur in the late first decade and are polymorphous, featuring plane warts, red, brown, or achromic plaques, and pityriasis versicolor-like macules
Malignant transformation involve approximately 30% of cutaneous lesions starting to occur from the third decade onwards, with predilection for sun-exposed areas
Histologic features of EV-associated skin lesions are those of plane warts, with mild hyperkeratosis, hypergranulosis, and acanthosis along with blue–grayish pale keratinocytes with perinuclear halos
Progression toward squamous cell carcinoma is paralleled by increased cytological atypia, architectural disorder, and increased mitotic index

– *TP53* inactivating mutations are found at high frequencies in SCC (41–69%) and precursor lesions such as AK (50–60%)
- *TP53* mutations typically bear the UV exposure and appear to represent early events in UV-induced development of SCC
- *TP53* mutant keratinocytes have a survival advantage as they are no longer capable of undergoing apoptosis after obtaining irreparable UV-induced DNA-damage
- Inactivation of a single *TP53* allele appears to be already frequent in precursor lesions, while loss of the second allele is associated with a vast expansion in

simple mutations, significant chromosomal instability, and progression to invasive SCC

- *NOTCH1* and *NOTCH2* mutations are found in 75% of invasive SCC of the skin; these mutations probably represent loss of function events. Loss of a competent NOTCH pathway impairs terminal differentiation of keratinocytes and increases their proliferative potential and motility
  - Approximately 75% of Notch receptors mutations in invasive SCC resulted from G>A transitions after homozygous loss of *TP53*
  - The distribution of missense mutations in *NOTCH1* and *NOTCH2* reflects abrogated function, spanning Notch ectodomains as well as the N terminal portion of the intracellular domains; conversely, Notch gain of function mutations characteristic of leukemias are clustered in the negative regulatory region and the C terminal PEST domain
  - Truncation mutations, occurring at high frequency in cutaneous SCC, are likely to result in nonsense-mediated decay or disable Notch signaling activity
  - The heterozygous status of Notch mutations in cutaneous SCC suggest a role for haploinsufficiency and, possibly, dominant-negative effects in selected cases
  - Notch receptor mutations appear to be absent in BCC and more frequent in SCC from the skin than from visceral sites
- *RAS* activating mutations, predominantly involving *HRAS*, are found in approximately 15% of AK and 20% of invasive SCC
  - Average frequencies of *HRAS, NRAS*, and *KRAS* in skin invasive SCC are approximately 9, 7, and 5%, respectively
  - When *RAS* mutations are found, however, they are regarded as a crucial early event in skin SCC pathogenesis
  - In experimental models, *RAS* constitutive activation alone was not sufficient to drive SCC development, resulting in growth arrest due to oncogene-induced senescence
  - SCC tumorigenesis required the combination of *RAS* mutations with additional oncogenic events, including forced coexpression of CDK4 or IκBα, inactivation of *TP53*, or loss of p14/ARF function
- The classical two-stage chemical carcinogenesis protocol for skin papillomas and SCC induction in mice exploits HRAS constitutive activation
  - 7,12-dimethylbenz(α)anthracene (DMBA) acts as initiator by causing *HRAS* activating mutations in codon 12 or 61, with 12-O-tetradecanoylphorbol-13-acetate (TPA) promoting proliferation of mutant keratinocytes through PKC activation
  - Studies using conditional expression of constitutively active *KRAS* mutant combined with loss of *TP53* in different epidermal and hair follicle compartments showed that the either interfollicular epidermis, the hair follicle bulge stem cells, or their immediate progeny, but not the transient amplifying matrix cells, could generate benign papillomas and invasive SCC
- The PI3K/AKT/mTOR signaling is typically increased in invasive cutaneous SCC, as suggested by IHC pAKT staining, and promotes proliferation and survival of neoplastic keratinocytes; however, classical cancer-associated mutations in this pathway are rarely found in cutaneous SCC
  - Activating mutations in *FGFR2*, *PIK3CA*, or *AKT1* are rarely found, if ever, in skin SCC
  - Similarly, loss of function mutations in *PTEN* (7%) are a relatively infrequent event
- Similarly to the PI3K/AKT/mTOR pathway, the EGFR signaling pathway plays a significant role in cutaneous SCC pathogenesis, but *EGFR* mutations are only rarely found in skin samples

- SCC exhibits a higher number of chromosomal aberrations compared to BCC, which increase during the progression from AK to invasive cancers
- Individual AK already show a substantial number chromosomal aberrations, with recurrent LOH at 3p, 9p, 9q, 13q, 17p, and17q
- Recurrent regions of LOH in SCC include 2q, 3p, 4p, 5q, 8p, 9p, 10p, 13q, 17p, and 17q
  - The presence of recurrent regions of LOH shared by AK and invasive SCC support the potential for AK to evolve into invasive SCC
- Recurrent chromosomal gains in invasive SCC involve 3q, 5p, 7p, 11q, 18q, 19p, and 20q
- Chromosomal losses involving the *CDKN2A* locus on 9p21 are recurrent events in AK (20%) and SCC (30–50%), with a high rate of *CDKN2A* somatic mutations observed in SCC (9–42%); loss of function of p16 and/or ARF may be involved in invasive transformation of AK
- Recurrent gains and amplifications of the *cMYC* locus at 8q24 have been described in SCC, with MYC overexpression correlating with progression from AK to invasive SCC and loss of differentiation
- KA show a lower degree of genomic instability than SCC, with some tumors exhibiting no detectable genetic aberrations
  - Recurrent chromosomal aberrations include gains of 3q, 6p, 11q, 17p, and 17q, and losses at 3p and 9p
  - *HRAS* mutations seem to be more frequent in KA than in SCC, being associated with gains of 11p
  - Differently from SCC, KA exhibit preservation of p16 function as well as reduced BCL2 and high BAK expression in regressing lesions
- Multiple KA (at times with sebaceous differentiation) are a characteristic feature of MTS (see below)
- The genetic cause of the autosomal dominant Ferguson–Smith syndrome, featuring multiple self-regressing KAs, was recently unraveled with the finding of loss of function *TGFBR1* mutations
  - Missense mutations in the same gene may cause Marfan syndrome-related disorders characterized by developmental defects with vascular involvement but no reported predisposition to cancer

- SCC, immunosuppression, and viral oncogenesis
  - Differently from the carcinogenetic involvement of high risk mucosal HPVs in cervical cancer, the role of HPVs in cutaneous SCC is less established
  - In patients with epidermodysplasia verruciformis (EV) (Table 13.10), precursor pityriasis versicolor-like lesions and subsequent development of overt SCC on sun-exposed skin have been unambiguously linked with infection by β-HPVs
  - In addition, organ transplant recipients (OTRs) are at markedly increased risk of developing SCC (significantly more than BCC) and numerous, extensive HPV-induced warts which may undergo malignant transformation
  - Because β-HPVs are very common and can persist for long time on normal skin, a causal relation between cutaneous SCC in immunocompetent patients and β-HPVs has been hard to establish or refute
    - Recent data from whole exomic sequencing suggest that HPVs are simple passengers in sporadic SCC within the general population, without playing any causative role
    - Only one of 31 SCC evaluated showed evidence of HPV transcripts (HPV92, a β-HPV) and only at very low levels (0.0001% of total reads)
    - The study leaves open the hypothesis that a HPVs may be involved in the initiation, but not the maintenance, of cutaneous SCCs: β-HPVs antiapoptotic oncoproteins might prevent UV-induced apoptosis, UV-induced mutant keratinocytes to survive, and progress to carcinomas
    - Such type of hit and run mechanism has been demonstrated in experimental systems (including progressions of BPV4-induced alimentary tract papillomas to malignant carcinomas)

- OTRs exhibit a more than 50-fold increase in incidence of cutaneous SCC
  - In the pathogenesis of OTR-associated SCC, a prominent role is played by the interaction of immunosuppression, chronic sun-exposure, and HPV infection
  - HPV DNA is found in approximately 70–90% of OTR-associated cutaneous SCC, including strains occurring in common benign cutaneous warts (HPV1 and HPV2), EV (HPV5 and HPV8), low risk oncogenic genital warts (HPV6 and HPV11), and high risk oncogenic warts (HPV16 and HPV18)
- However, recent data point to a significant increased risk in OTRs undergoing cyclosporine (CyA)-based immunosuppressive protocols
  - By inhibiting calcineurin, CyA inhibits NFATc1 translocation into the nucleus
  - As a consequence, ATF3 transcriptional activity would become unrestrained, preventing p53 from activating an effective oncogenic senescence response against aberrant growth signals, such as those induced by constitutively activated RAS
  - In advanced SCC, CyA may also favor tumor progression by increasing TGFβ signaling and promoting epithelial–mesenchymal transition
- CyA-sparing regimens, such as those including rapamycin and related agents, could result in a significant reduction of SCC incidence among OTRs
  - Rapamycin, temsirolimus, and eversirolimus may also antagonize cutaneous SCC development and progression by inhibiting mTOR complex 1 downstream of EGFR, RAS, and PI3K/AKT signaling

Cutaneous Adnexal Neoplasms

- The group of cutaneous adnexal tumors includes a broad spectrum of entities, with complex nosology and different molecular bases. Hence, a comprehensive review of such category is beyond the aims and scope of this chapter

Trichoblastoma and Trichoepithelioma

- Definition
  - A benign adnexal tumor with basaloid cells differentiating toward the hair germ
    - Trichoepithelioma (TE), also known as cribriform trichoblastoma (TB), is currently regarded as a subset of TB with more conspicuous follicular differentiation (Table 13.11)
  - Variants: trichogerminoma, lymphadenoma cutis, trichoblastic fibroma
- Clinical features
  - Solitary or multiple slow growing, asymptomatic papules or nodules
  - Predilection for the head and neck area, particularly the central face
  - Most frequent neoplasm arising in nevus sebaceous (followed by syringocystadenoma papilliferum, trichilemmoma, and sebaceous adenoma)
    - The overwhelming majority of alleged "BCC developing in nevus sebaceous" in the past was TB
- Pathologic features
  - Well-circumscribed dermal proliferation of basaloid cells with peripheral palisading but no cytological atypia

Table 13.11 Main histological differences between trichoblastoma (TB) *sensu stricto* and trichoepithelioma (TE) (cribriform TB)

TB	TE
Large nodules deep in the dermis	Smaller and more superficial
Tightly packed nodules of basaloid cells	Small clusters, strands, and cords of basaloid cells
No cornifying cysts	Superficial keratinous cysts, with infundibular or isthmic differentiation
Absent to rare papillary mesenchymal bodies	Frequent papillary mesenchymal bodies
Frequent mitoses	Rare mitoses
Predominance of epithelial component	Prominent peritumoral stroma

  - Dense fibrocytic stroma surrounding the tumor
  - Clefting between peritumoral and normal stroma, not between tumor islands and surrounding stroma as observed in BCC
  - Papillary mesenchymal bodies: rudimentary follicular papillae with basaloid cells engirdling primitive mesenchyme, immunoreactive for CD10 and CD34
  - Occasional signs of sebaceous and/or apocrine differentiations, reflecting the common origin of structures belonging to the folliculosebaceous apocrine unit
  - Often, increased number of CK20-positive Merkel cells and/or S100-positive melanocytes within the tumor
  - Basaloid cells positive for CK15 and CK19; faint expression or negativity for Ber–EP4, BCL2, and androgen receptors may help in the distinction with BCC
- Molecular features
  - The pathogenesis of several cases of multiple familial trichoepithelioma (MFT)-associated TE has been linked to *CYLD* inactivating mutations (see below)
  - No *PTCH1* mutations have been found in either TB or TE, although LOH 9q22.3 was reported in almost 50% of sporadic TE
- Desmoplastic trichoepithelioma
  - Benign adnexal tumor classified as a variant of TE, but exhibiting distinct clinicopathological features
  - A solitary, firm, pink–reddish papule or plaque with central dell, resembling morpheaform BCC
  - Predilection for the face of adult females
  - Histopathologically, superficial proliferation of pale basaloid cells arranged in thin strands; keratinous microcysts with isthmic or infundibular differentiation; sclerotic stroma with occasional foci of granulomatous reaction; absence of ductal differentiation
  - Several IHC markers, including androgen receptors, CD20, NGFR/p75, and familial adenomatous polyposis (FAP), have been proposed to aid in the differential diagnosis with sclerosing BCC but are not universally accepted

Cylindroma and Spiradenoma

- Definition
  - Two types of cutaneous adnexal neoplasms with follicular apocrine differentiation often showing overlapping features and probably representing two ends of a common clinicopathological spectrum
  - Multiple cylindromas and spiradenomas, together with TE, may be observed in Brooke–Spiegler syndrome (BSS) (Table 13.12); familial cylindromatosis (FC) and MFT are allelic to BSS
- Clinical features
  - Cylindromas and spiradenomas present as slow growing, skin-colored to erythematous dermal papules or nodules, with predilection for the head and neck region and the upper trunk
    - Spiradenomas may be distinguished by bluish color and paroxysms of localized pain
    - Glabrous surfaces of the skin are always spared
  - Multiple cylindromas and spiradenomas, together with TE, may be observed in BSS (Table 13.12); FC and MFT are allelic to BSS
- Pathological features
  - Cylindromas are characterized by a dermal proliferation of closely set epithelial lobules arranged in a "jigsaw pattern"
    - In each lobule, peripheral basaloid cells surround larger pale cells; ductal differentiation is possible
    - Lobules are surrounded by prominent eosinophilic PAS-positive basement membrane material and exhibit eosinophilic PAS-positive globules within them
  - Spiradenomas present as well-circumscribed epithelial nodules in the dermis
    - The two cell populations typical of cylindroma are intermixed in nodules of spiradenoma, together with numerous scattered lymphocytes
    - Occasional ductal and/or cystic structures
    - The association between spiradenomas and BSS further supports an apocrine rather than eccrine nature

Table 13.12 Brooke–Spiegler syndrome (BSS) and the CYLD protein

BSS is an autosomal dominantly inherited syndrome predisposing to multiple cylindromas, spiradenomas, and trichoepithelioma (TE)
Clinical features include:
Progressive development of multiple cylindromas (favoring the scalp), spiradenomas, and/or TE (preferentially arising on the central face)
Initial presentation typically in the second decade
Penetrance increases with age, but some carriers remain free of clinically detectable lesions
Marked phenotypical variability regarding both clinical and histological findings
Involvement of trunk and genital regions and development of neoplasms around puberty, point to a significant role for hormonal factors rather than UV irradiation
Histopathologically, BSS-associated neoplasms do not differ from their sporadic counterparts
Up to 88% of BSS, familial cylindromatosis, and multiple familial trichoepithelioma individuals carry germline inactivating mutations of the *CYLD* gene on 16q12.1
To date, 59 unique *CYLD* germline mutations have been reported, including frameshift, nonsense, missense, splice-site mutations
BSS-associated *CYLD* mutations are restricted to exons 9–20, with over 75% concentrated in exons 16–20; exons 4–8 are retained, resulting in a truncated protein lacking the C terminal catalytic region ($CYLD^m$)
The expression of $CYLD^m$ may have a dominant negative effect
The remaining wild type CYLD allele is inactivated in neoplasms by somatic deletion, mutation, or LOH
CYLD encodes the ubiquitin (Ub) carboxyl-terminal hydrolase CYLD
CYLD is a widely expressed, constitutive active deubiquitinase (DUB) belonging to the class of Ub-specific proteases (USP)
CYLD preferentially removes Lys-63-linked rather than Lys-48-linked Ub chains
Polyubiquitination through Lys-63 controls nonproteolytic events in DNA repair, endocytosis, and kinase-mediated signaling responses
CYLD DUB activity results in specific effects on multiple pathways, including:
Inhibition of the "classical" NFκB pathway through interaction with TRAF2, TRAF6, TRIP, and NEMO/IKKγ
Inhibition of the "alternative" NFκB pathway by negative regulation of the BCL3–p50/p52–cyclin D1 axis
Inhibition of the JNK/AP1 pathway through inhibition of MKK7/JNKK2, c-Jun, and c-Fos
Inhibition of the p38MAPK pathway by interaction with TAK1
Inhibition of the canonical Wnt pathway by deubiquitinating Dvl on its DIX polymers
CYLD has emerged as a tumor suppressor gene in several types of human cancer
Depending on the setting, alteration of CYLD expression is achieved through different mechanisms, including genetic aberrations, transcriptional repression, phosphorylation, and ubiquitination
CYLD transcriptional repression in melanoma correlates with increased BCL3 nuclear activity and worse prognosis

  - Hybrid tumors with intermediate features between cylindroma and spiradenoma may often be seen ("cylindrospiradenomas")
  - Malignant transformation of preexisting lesions into cylindrocarcinoma, spiradenocarcinoma, and BCC is an uncommon but possible occurrence
- Molecular features
  - Biallelic inactivation of the *CYLD* tumor suppressor gene is typical of BSS- and FC-associated neoplasms and frequent also in sporadic lesions (Table 13.12)
    - CYLD is a deubiquitinating enzyme that removes lys63-linked ubiquitin chains and acts as a negative regulator of NFκB. Inactivating mutations of CYLD enhances activation of the transcription factor NFκB
  - Presence of *MYB–NFIB* fusion transcripts and/or MYB nuclear expression was found in 67% of sporadic cylindromas
    - The *MYB–NFIB* fusion oncogene is found in a large proportion of adenoid cystic carcinomas of the breast and head and neck, neoplasms whose histological features resemble cylindromas
    - Additional fusion oncogenes which may be shared by salivary gland neoplasms

and adnexal tumors include the *CRTC1–MAML2* and *EWSR1–POU5F1* transcripts, reported in both salivary mucoepidermoid carcinomas and cutaneous hidradenomas

Pilomatricoma

- Definition
  - A benign adnexal neoplasm characterized by follicular differentiation towards the hair matrix
  - Synonyms: pilomatrixoma, calcifying epithelioma of Malherbe
- Clinical features
  - Solitary pink–bluish dermal nodule or cyst on nonglabrous skin, especially head and neck region
  - Typically firm consistency, related to fibrosis and calcification
  - Usually asymptomatic, but inflammation and erythema may occur
  - Predilection for children and young adults, but may occur at any age
  - Multiple lesions may be idiopathic (sporadic or familial), or occur in association with Gardner syndrome (*APC* mutations) or Sotos syndrome (nuclear receptor-binding SET domain containing protein (*NSD1*) gene mutations)
- Pathologic features
  - Well-circumscribed, nodulocystic epithelial tumor in the dermis
  - Two cell populations with matrical differentiation
    - Basaloid cells often with mitoses at the periphery
    - Eosinophilic cells with residual outlines of nuclei (shadow cells) in the center
    - Transition between the two cell populations is often abrupt; at times, transitional cells with intermediate features and trichohyalin granules may be observed
    - Varying degree of calcification, and even ossification, granulomatous inflammatory reaction and fibrosis
  - Basaloid cells show prominent nuclear expression of β-catenin, but stain negatively for CK15 and SOX9
- Molecular features
  - Pilomatricomas are characterized by increased β-catenin transcriptional activity and canonical Wnt signaling activation
  - Activating mutations in exon 3 of the *CTNNB1* gene, encoding β-catenin, have been identified in 25–100% of sporadic pilomatricomas
  - Reported exon 3 *CTNNB1* mutations disrupt β-catenin phosphorylation by the axin/APC/GSK3β/CKIα complex required for its destruction and promote its nuclear localization, leading to constitutive Wnt signaling
    - Gardner syndrome, which frequently features pilomatricomas and/or epidermal cysts with matrical changes, is a FAP variant; FAP-associated *APC* mutations lead to β-catenin stabilization
  - *CTNNB1* mutations may be relatively specific for matrical differentiation
    - Sporadic BCCs with foci of matrical differentiation harbored *CTNNB1* mutations
    - Activating *CTNNB1* mutations have been found in craniopharyngiomas, ameloblastomas, and calcifying odontogenic cysts, neoplasms which all may feature the abrupt transition between peripheral basaloid cells and central eosinophilic cells with shadow cells differentiation
- Pilomatrical carcinoma
  - Pilomatrical carcinoma is a rare, low grade carcinoma distinguished by matrical differentiation and high propensity for recurrence
  - The mutation spectrum of *CTNNB1* is similar to pilomatricoma
    - Recurrence of a pilomatricoma has been related to increased risk of malignant transformation
  - Pilomatrical carcinomas show a significant predilection for adults and chronically sun-damaged skin and are rare in children
  - Pathological features favoring a diagnosis of pilomatrical carcinoma include presence of bona fide necrosis, marked nuclear pleomorphism, or infiltrative growth

Sebaceous Adenoma and Sebaceoma

- Definition
  - Sebaceous adenoma (SA): a benign neoplasm of the sebaceous gland
  - Sebaceoma (synonym: sebaceous epithelioma; SE): a proposed variant of SA characterized by a predominance of undifferentiated seboblastic cells over mature sebocytes
- Clinical features
  - Asymptomatic, frequently lobulated papule or nodule, skin-colored to yellowish
  - Predilection for adults and for the head and neck region
  - Multiple lesions, at times with a cystic appearance, may arise in the setting of MTS
    - Presence of any sebaceous neoplasm (either benign or malignant), but not sebaceous hyperplasia, should raise suspicion of MTS (see below for additional diagnostic approaches)
- Pathological features
  - Well-circumscribed dermal tumor, with lobulated configuration typically emanating from the undersurface of the epidermis and comprised of two cell populations in variable proportion, mature sebocytes (usually prevailing toward the center) and undifferentiated basaloid seboblasts (more abundant at the periphery)
    - Cystic lesions are more commonly associated with MTS
  - Mitotic figures may be observed in seboblasts, but no cytologic atypia or necrosis are found
    - SA *sensu stricto* are characterized by more superficial location, smaller size, significant predominance of sebocytes, and multiple peripheral layers of seboblasts
    - SE feature deeper, larger proliferation of seboblastic cells (>50%), with foci of sebocytic differentiation
    - Lesions with intermediate features may be observed indicating that SA and SE represent a spectrum
  - Helpful IHC markers in the differential diagnosis include:
    - Adipophillin (membranous labeling of intracytoplasmic globules) is the most sensitive and specific marker of sebocytic differentiation
    - CK7 preferentially stains the seboblastic population
    - The combination of cytoplasmic EMA positivity and lack of Ber–EP4 expression may aid in the distinction between SE and BCC
  - Complete loss of nuclear immunoreactivity for MLH1, MSH2, or MSH6 in lesional tissue may be employed as a surrogate for PCR-based assessment of mismatch repair (MMR) defects and microsatellite instability (MSI) (see below)
- Molecular features
  - Sebaceous neoplasms can be associated with MTS or somatically acquired MSI (see below)
    - Depending on the study, MSI was detected in 25–100% of sebaceous neoplasms, while was rare in other skin neoplasms. It can be due to MTS or somatic alterations of the mismatch repair system and has been reported to be more frequent in tumors arising outside the head and neck area and in benign than malignant sebaceous tumors
  - Approximately one-third of SA/SE have somatic mutations in LEF1 at 133 G>A, in exon 1
    - The causative double-nucleotide substitution results in a dominant negative LEF1 protein with impaired interaction with β-catenin, hampering β-catenin transcriptional activity
    - Expression of a functionally analogous LEF1 mutant protein in *K14ΔNLef1* transgenic mice led to development of cutaneous tumors with sebaceous differentiation
    - Expression of the mutant LEF1 protein in follicular stem cells/seboblasts results in decreased canonical Wnt signaling and increased PPARγ and IHH signaling, promoting sebaceous differentiation and proliferation
- Sebaceous carcinoma (SC)
  - Malignant counterpart of SA/SE
  - Two clinicopathological subsets are conventionally recognized:

Table 13.13 Suggested clinical criteria for a diagnosis of Muir–Torre syndrome (MTS)

[1] Visceral malignancy + [2] Any sebaceous neoplasm of the skin (SA, SE, SC, or KA with sebaceous differentiation)
–OR—
[1] Multiple visceral malignancies+[2] Multiple KA+[3] Family history of MTS

SA sebaceous adenoma; *SE* sebaceoma; *SC* sebaceous carcinoma; *KA* keratoacantoma

    - Periorbital SC, representing almost three quarters of SC
    - Extraocular SC
  - Nondescript appearance, frequently with rapid growth and possible ulceration
    - Initial misdiagnosis of periorbital SC as chalazion may hinder timely treatment
  - High propensity for local recurrence and, if treatment is delayed, significant risk of nodal and visceral metastases
    - The conventional notion that extraocular SC behaves more aggressively than cutaneous forms has been questioned and may be artifactual
  - Histopathologically, distinctive features are infiltrative growth, seboblasts with cytologic atypia and frequent necrosis, irregular areas of sebocytic differentiation
    - Intraepidermal, pagetoid spread of pale malignant cells is a frequent occurrence in periorbital SC
  - High nuclear Ki67 and p53 staining may help in establishing the malignant nature of any sebaceous neoplasm

Muir–Torre Syndrome

- Definition
  - An autosomal-dominant hereditary cancer syndrome clinically defined by the association of skin tumors (sebaceous neoplasms [SA/SE/SC] and/or multiple KA) and various internal cancers (Table 13.13)
    - MTS is currently regarded as a clinicopathological subset (1–3%) of the hereditary nonpolyposis colorectal carcinoma syndrome (HNPCC, Lynch syndrome)
- Clinical features
  - High penetrance, if also familial cases of HNPCC are included
  - KA may occur in up to 20% of patients with MTS, regardless of development of sebaceous tumors
    - Hybrid tumors with histologic features of both KA and sebaceous neoplasms ("seboacanthomas") are rare but appear to be highly specific for MTS
  - The spectrum of MTS-associated visceral malignancies is broad, including colorectal, genitourinary, breast, hematological, and upper gastrointestinal neoplasms
    - Almost half of MTS patients are thought to develop at least two internal malignancies
    - Incidence of specific cancers does not overlap with what observed in Lynch syndrome
    - Similarly to HNPCC-associated malignancies, both internal and cutaneous neoplasms occurring in MTS patients tend to show a less aggressive behavior than sporadic counterparts
  - In a significant proportion of MTS patients, the occurrence of skin tumors precedes (22–32%) or accompanies (9–12%) the diagnosis of visceral internal malignancy
    - Prompt recognition of a newly developed sebaceous neoplasm may lead to early diagnosis of MTS-associated visceral neoplasms
  - Cases have been reported of latent MTS phenotype being unmasked by immunosuppressive therapy with calcineurin inhibitors (CNI), leading to development of sebaceous neoplasms
    - Sebaceous hyperplasia is a known side-effect of CNI-based systemic immunosuppression
  - Apposite clinical guidelines have been suggested for establishing a diagnosis of HNPCC (Amsterdam II criteria) and for identifying tumors with MSI (revised Bethesda criteria)
    - Compared to the Amsterdam II criteria, the revised Bethesda criteria are characterized by increased sensitivity but lower

positive predictive value for MMR germline mutations

- Pathological features
  - MTS-related sebaceous lesions more frequently show KA-like changes and/or increased tumor-infiltrating lymphocytes, whereas the alleged association with a cystic architecture has been questioned
- Molecular features
  - Approximately two-thirds of both MTS and HNPCC cases are associated with hereditary MMR defects and MSI in both visceral and cutaneous neoplasms
    - As a general rule, neoplasms in individuals with heterozygous mutations in MMR genes show inactivation of the healthy allele by somatic inactivation via genetic or epigenetic
  - The mutation spectrum of MTS differs from that in HNPCC. Approximately 90% of MTS cases exhibiting MSI have been linked to *MSH2* mutations, with only a minority (≅10%) related to *MLH1* mutations; *MSH6* mutations are rare but *MSH3*, *PMS1*, or *PMS2* mutations have not been reported
    - MSI-associated sebaceous neoplasms occur in a *MSH2*-deficient mouse model
  - 30–40% of MTS and Lynch syndrome cases show no relation with inherited MMR genes defects; *MYH* hereditary biallelic mutations may account for a proportion of such cases
    - MYH protein is a adenine-specific glycosylase involved in the BER system
    - The two most common (>80%) inactivating *MYH* mutations in the Caucasian kindreds are Y165C and G382D
    - Homozygous or compound heterozygous germline mutations to *MYH* have been linked to both (1) an attenuated or classic FAP phenotype lacking *APC* germline defects (*MYH*-associated polyposis), and (2) a MMR-independent HNPCC phenotype
    - Biallelic *MYH* germline mutation carriers are at increased risk of gastrointestinal, ovarian, and bladder neoplasms
    - Recently, MSS–MTS cases, characterized by retained expression of MMR proteins in associated sebaceous neoplasms, were associated with biallelic *MYH* germline inactivation
  - Loss of FHIT expression may be relevant for the genesis of sebaceous neoplasms in the setting of MSS–MTS
    - *Fhit*$^{+/-}$ transgenic mice developed a MTS-like phenotype after intragastric administration of NMBA, including gastroenteric and sebaceous neoplasms
    - Periocular SC in patients with MSS–MTS showed loss of immunoreactivity of FHIT, secondary to somatic inactivation by genetic or epigenetic mechanisms
    - FHIT plays a critical role in the response to cellular stress and may exert a repressive effect on β-catenin transcriptional activity
- MTS screening methods
  - Any newly diagnosed sebaceous neoplasm (SA/SE/SC) could be the presenting sign of MTS and should be followed by appropriate investigations, although no definite agreement has been reached regarding MTS screening algorithm
  - A detailed family history of internal malignancy should be always obtained
  - IHC staining for MLH1, MSH2, and MSH6 is a first-line cost-effective option for evaluating the functional competence of the MMR system in lesional tissue from any sebaceous neoplasm
    - MLH1 and MSH2 form functional, stabilizing complexes with PMS2 and MSH6, respectively; MLH1 and MSH2 are required constituents of the complexes, whereas a isolated lack of PMS2 or MSH6 can be partially compensated by PMS1 and MSH3, respectively
    - Results of IHC staining for MMR proteins can guide subsequent genetic analyses (i.e., absence of MSH6 nuclear expression without loss of IHC staining for MSH2 points to a selective defect of MSH6)
    - Combined evaluation of MLH1/MSH2/MSH6 nuclear expression by IHC has been shown to be a highly sensitive (92%), specific (95%), and reproducible test with rapid turnaround time and very good correlation with MSI status, as measured by conventional PCR-based methods

Table 13.14 The Merkel cell lineage

Merkel cells are postmitotic, slowly adapting mechanoreceptor cells belonging to the APUD system
Merkel cells preferentially reside in specialized epithelial structures in the interfollicular nonglabrous epidermis, the so-called touch domes (TDs)
Bona fide TDs markers are Tbc1d10c and CD200
Rare Merkel cells are found in human epidermis outside of TDs; their number increases in response to sun-exposure
Several murine lineage-tracing models demonstrated that Merkel cells are derived from asymmetric cell division of specialized K14-positive basal keratinocytes rather than from neural crest cells
Merkel cells epithelial progenitors are found in the TDs of adult skin and can give rise to both the neuroendocrine and squamous epidermal lineages

  - Lesional MSI can be assessed at a genetic level evaluating the Bethesda markers of five specific microsatellites (BAT25, BAT26, D2S123, D5S346, and D17S250)
    - MSI-H is defined as detection of MSI in at least two Bethesda markers
    - Genetic analysis of MSI is considered less cost-effective and does not provide any information about specific MMR protein defects
  - Any positive result of IHC and/or MSI genetic testing, especially if supported by additional anamnestic and/or clinicopathological clues for MTS, should warrant genetic investigation for MMR germline defects

Merkel Cell Carcinoma

- Definition
  - A rare, highly aggressive skin neoplasm of epithelial and neuroendocrine differentiation, presumed to derived from cutaneous Merkel cells (Table 13.14)
  - Synonyms: primary neuroendocrine carcinoma of the skin, primary cutaneous small cell carcinoma
- Clinical features
  - Most common in white males in their seventh to eighth decades
  - Increased incidence, early onset, and more aggressive behavior are seen in immunosuppressed patients (HIV-infection, HSC or solid organ transplantation, chronic lymphocytic leukemia, and lymphomas)
  - Predilection for chronically sun-damaged skin (head, neck, extremities), suggesting that UV exposure plays a role in Merkel cell carcinoma (MCC) development
  - Typical presenting as a painless, solitary, pink–purple nodule, which rapidly grows over a period of weeks
  - MCC clinical features are summarized by the acronym AEIOU (Table 13.15)
    - Up to 89% met three or more of the AEIOU criteria in one study
  - MCC age-adjusted incidence in the USA tripled between 1986 and 2001 up to 4 cases/10^6/year
    - Factors accounting for the rise in MCC incidence include increased UV light exposure, rising elderly and immunosuppressed populations, progresses in immunodiagnostic techniques
  - MCC 2-year mortality rate is 28% higher than that recorded in melanoma
- Pathologic features
  - Typically a highly cellular, strongly basophilic, asymmetric dermal-based tumor, with frequent subcutaneous extension but sparing of epidermis, papillary dermis, and adnexa; occasionally, an intraepidermal component may be observed
  - The intermediate variant is the most common: monotonously uniform cells with dense round nuclei and typical nuclear

Table 13.15 The AEIOU acronym for clinical diagnosis of Merkel cell carcinoma

A—Asymptomatic/lack of tenderness
E—Expanding rapidly (doubling in <3 months)
I—Immunosuppression
O—Older than 50 years
U—UV-exposed sites

chromatin ("salt and pepper") pattern; sparse cytoplasm with paranuclear plaques consisting of intermediate filaments; diffuse and/or nested pattern of growth; high mitotic activity along with frequent single apoptotic cells and/or necrotic areas
  - Additional histologic subtypes, without clinical significance, include the small cell variant (small round cells with very scant cytoplasm and hyperchromatic nuclei) and the trabecular variant (polygonal cells with abundant cytoplasm in a trabecular or ribbon-like arrangement)
  - Rarely, expression of squamoid, adnexal, or melanocytic differentiation and/or association with SCC, BCC, or melanoma
  - IHC plays a key role in MCC diagnosis
    - Conventional IHC profile is CK7-negative/CK20-positive (with CK20 expressed in a characteristic paranuclear dot-like pattern), but double-positive, double-negative, and CK7-positive/CK20-negative cases have been documented
    - Additional IHC markers may include CAM5.2, AE1/AE3, neural (CD56, neurofilament, NSE) and neuroendocrine (synaptophysin, chromogranin) markers, CD99, CD117, and TdT
    - Differential diagnosis with clinicopathological mimickers may be aided by negativity for TTF1, MASH1, CDX2, S100, and CD45/LCA
    - Nuclear expression of Merkel cell polyomavirus (MCPyV) T-Ag in MCPyV-positive MCC cases (see below)
    - IHC markers which may be associated with more aggressive behavior and poor clinical outcome include expression of at least one of the p63 isoforms, expression of p53, high proliferative index (Ki67/MIB1)
  - Beginning in late 2009, a new consensus AJCC/IUAC staging system was adopted
- Molecular features
  - Before the discovery of MCPyV, the pathogenesis of MCC was largely unclear
  - Merkel cells share similar IHC features with MCC cells and are the only cutaneous cells forming electron-dense neurosecretory granules; it has been postulated (but never proved) that MCC arise from Merkel cells
  - Neither *PDGFRA* nor *cKit* activating mutations have ever been detected in MCC; cKit expression does not correlate with aggressive behavior; cKit and PDGFR inhibitors (imatinib mesylate) failed to show effectiveness in patients with advanced cKit-positive MCC
  - The MAPK pathway appears to be largely inactive in MCC; no *BRAF* mutations have ever been detected in MCC
  - PTEN expression is significantly reduced in MCC; heterozygous losses including chromosome 10q23 frequently occur in MCC
  - Inhibition of apoptosis may play a critical role in MCC pathogenesis, as suggested by overexpression of BCL2 and survivin
  - Losses at 13q including the *RB* locus appear infrequent, while dysregulation of the p14/ARF–MDM2–p53 pathway seems to be relevant in MCC; mutations in *TP53* occur in approximately 20% of MCC cases, often carrying a "UV signature"; silencing by methylation at the *p14/ARF* promoter (but not at the *p16/INK4a* promoter) was observed in 40% of cases
- MCPyV and MCC
  - Given the increased risk for MCC observed with immunosuppression, MCC was a prime cancer candidate for a viral cause
  - The 5,387 bp DNA genome of a novel human polyomavirus, called MCPyV (Table 13.16), was identified by digital transcriptome subtraction technique from MCC
  - MCPyV DNA was originally found to be clonally integrated in the tumor genome of approximately 80% of MCC
  - MCPyV DNA prevalence in MCC specimens by PCR has been variably reported between 43% and 100% in different studies
  - Obstacles to proving a link between MCPyV and MCC are the relative infrequency of MCC, the near-ubiquitous diffusion of asymptomatic MCPyV infection in the general

Table 13.16 The *Polyomaviridae* family

Small, nonenveloped double-stranded DNA viruses with icosahedral capsids, infecting multiple species
In addition to MCPyV, four polyomaviruses are known to naturally infect humans:
JCV (linked to AIDS-associated progressive multifocal leukoencephalopathy)
BKV (associated with nephropathy after renal transplantation)
KIV
WUV
Phylogenetically, JCV, BKV, KIV, and WUV appear to group with the SV40 polyomavirus; MCPyV is more closely related to the murine polyomavirus
Natural history of HPV infections is largely unknown; common features include routes of transmission (respiratory and fecal–oral), high prevalence in the general population, seroconversion occurring in the first decades of life, and latency establishment
Polyomaviruses share conserved early, late, and noncoding regulatory (NCRR) regions
Early tumor (T) antigens (LT-, 57kT-, and sT-Ag) are generated through alternative splicing and play key roles in viral replication and tumorigenesis
Large T (LT)-Ag possesses helicase activity unwinding the viral replication origin, and promotes the early to late switch in gene expression
Small T (sT)-Ag is expressed after LT-Ag and cooperates with the latter for optimal viral replication
Viral capsid proteins (VP1 and VP2) encoded by the late region self-assemble in virus-like particles inside infected cells
Although the SV40 virus, which infects the rhesus macaques, can induce tumor formation in experimentally infected hamsters, MCPyV was the first polyomavirus for which strong evidence supports a causal role in human cancer

population, and the existence of MCPyV-negative subset of MCC, whose pathogenesis is unrelated to MCPyV infection

- Incidental low level infection is frequently found in non-MCC skin (either healthy or not) and other human tissues, characterized by episomal structure and low load of MCPyV genome
- Multiple evidence support a role of MCPyV in MCC pathogenesis, with MCPyV infection and genomic integration occurring prior to MCC clonal expansion and progression
- MCPyV DNA is clonally integrated in most of MCPyV-positive MCC, with primary MCPyV-positive MCC and corresponding metastases showing identical integration sites in individual patients
- In MCPyV-positive MCC, the T-Ag is expressed in tumor cells but not in healthy surrounding tissues; LT-Ag nuclear expression by IHC is strongly associated with MCPyV DNA detection by qPCR and DNA sequencing, potentially representing an effective and practical method to identify MCPyV-positive MCC
- A minority of MCPyV-positive MCC harbors <0.1 viral DNA copies per one copy of reference gene and lacks of LT-Ag expression, exhibiting additional clinicopathological features typical of MCPyV-negative MCC; possible explanations are an incidental, passenger infection by MCPyV or progression to a MCPyV-independent status (hit and run model, such as described in animal models of polyomavirus-induced oncogenesis)
- Deletion mutations, found only in tumor-derived MCPyV, impair viral permissivity, revealing a strong selective pressure to silence independent and unlicensed DNA replication from the integrated viral genomes
  - In most cases, the C terminal origin-binding, the helicase, and the p53-binding domains are lost due to deletion mutations, while the nuclear localization signal (NLS) and the N terminal RB-binding (LXCXE motif) domain are retained
  - The sT-Ag and its putative PP2A interaction domain remain unaffected by MCC-specific mutations, potentially playing further oncogenic activity
- Disruption of RB function by LT-Ag appears critical for sustained MCC growth,

without requirement for additional RB phosphorylation
  - Only MCPyV-positive cell lines, but not MCPyV-negative cell lines, are "addicted" to MCPyV T-Ag expression
  - UV radiation may promote MCPyV-mediated MCC oncogenesis through upregulation of MCPyV sT-Ag expression in infected keratinocytes, induction of immunosuppression, and mutagenesis leading to MCC-specific T-Ag mutations
  - The general population is often seropositive for circulating anti-VP1 antibodies, which have limited potential as markers for MCC; conversely, anti-T-Ag antibodies, which are only rarely detected among MCPyV-negative MCC patients and healthy controls, strongly correlate with MCPyV-positivity as well as tumor burden in MCC patients; further studies are required to test their potential as MCC markers
  - Studies aiming to determine potential clinicopathological correlations between MCC and MCPyV status have provided conflicting results
    - Patients with MCPyV-positive MCC seem to exhibit better MCC-specific and overall survival than patients with MCPyV-negative MCC; LT-Ag expression in MCC by IHC may predict improved prognosis
    - MCPyV-positivity appears to be associated with the CK7-negative/CK20-positive phenotype by IHC
    - *TP53* mutations and p53 overexpression by IHC are associated with absence of MCPyV DNA, pointing to a role of p53 in MCPyV-negative MCC tumorigenesis

Dermatofibrosarcoma Protuberans

- Definition
  - A locally aggressive sarcoma of intermediate malignant behavior and controversial histogenesis, characterized by frequent local recurrence but low metastatic potential
- Clinical features
  - Most common in young to middle-aged adults, but can occur at any age; congenital cases have been described
  - Predilection for the shoulder and pelvic girdles, trunk, proximal extremities
  - Onset typically as a skin-colored or red–brownish firm plaque or nodule, with a scar-like appearance; at time resembling a vascular malformation
  - Slow, progressive, poorly defined growth, often reaching several centimeters in size; multinodular morphology in advanced stages
  - 20–50% rate of local recurrences after surgical excision, but only rare risk for nodal or visceral metastases (less than 5%); wide local excision or micrographic surgery are mandatory to reduce risk of local recurrence
- Pathologic features
  - Poorly circumscribed proliferation of small wavy to spindled cells in the deep dermis, displaying a monotonous storiform pattern
  - Neoplastic cells exhibit uniform cytological features, with scant cytoplasm, mild degree of pleomorphism, and only few mitoses
  - Striking tendency to invade the subcutaneous adipose tissue, initially along the adipose septa, later in a diffuse multilayered (honeycomb) pattern
  - IHC positivity for CD34 (80–90% of cases) and apoD; negative staining for fXIIIa, S100, CD31, and SMA
  - Several histopathologic variants exist: pigmented dermatofibrosarcoma protuberans (DFSP)/Bednar tumor (characterized by scattered S100-positive, CD34-negative melanocytes colonizing the neoplasm), myxoid DFSP, atrophic DFSP, granular cell DFSP, and palisading DFSP
  - Occasionally, areas of dedifferentiation resembling fibrosarcoma (DFSP–FS) or, more rarely, pleomorphic high grade sarcoma (DFSP–PleoSarc); these changes carry an increased risk of metastases

  - DFSP may show giant cell fibroblastoma (GCF)-like areas; DFSP may recur as GCF, and vice versa; evidence of common molecular aberrations (see below) support that DFSP and GCF are related tumors
- Molecular features
  - More than 95% of DFSP cases (including histopathological variants) feature one of the following cytogenetic aberrations:
    - Presence of supernumerary ring chromosomes containing low level amplified sequences from chromosomes 17q22–qter and 22q10–13.1
    - A recurrent (balanced or unbalanced) linear chromosomal translocation t(17;22) (q22;13.1) (less common, but frequent in pediatric cases)
  - The common outcome is a chimeric gene resulting from the fusion of *COL1A1* on chromosome 17q21–22 (coding for the α1 chain of type 1 collagen) with *PDGFB* on chromosome 22q13 (coding for the PDGFβ chain)
  - The chimeric protein is posttranslationally processed to generate a functional PDGFβ, leading to PDGFβ-mediated autocrine and/or paracrine activation of PDGFRβ on neoplastic cells
  - *COL1A1–PDGFB* chimeric transcripts have been found in DFSP–FS, DFSP–PleoSarc, DFSP with areas of GCF, GCF, and their hybrid lesions
    - Fibrosarcomatous evolution into DFSP–FS has been attributed to *TP53* mutations, microsatellite instability, or genomic gains of the *COL1A1–PDGFB* fusion gene
  - Alternative chromosomal rearrangements have been sporadically described, including t(2;17), t(9;22), t (5;8), t(X;7), and a complex unbalanced translocation involving chromosomes 3, 5, 7, and 22
  - Variable level of *PDGFB* copy gains or amplifications can be an associated finding, having unresolved biological or prognostic significance
  - Location of *COL1A1–PDGFB* chimeric gene breakpoints:
    - The *PDGFB* breakpoint is always located in the first intron, removing genomic elements which negatively regulate *PDGFB* transcription and translation
    - The *COL1A1* breakpoints span between exons 5 and 49, without any significant clustering. No correlation exists between *COL1A1* breakpoint location and clinical, pathological, or prognostic features of DFSP. The *COL1A1* side of the chimeric gene is thought to act merely as a *cis* element to upregulate *PDGFB* expression
  - Detection methods for DFSP cytogenetic alterations include: G-banded cytogenetic analysis (50% sensitivity), multiplex RT-PCR on FF or FFPE samples (74–96% sensitivity, depending on assay and design of primers), and FISH analysis on FF or FFPE samples (95% sensitivity)
  - Failure to detect the chimeric gene alone does not exclude a diagnosis of DFSP, despite pointing to an alternative diagnosis
  - FISH analysis appears as the most appropriate method for routine detection of fusion gene (location of *COL1A1* breakpoints does not have any clinicopathological significance); multiplex RT-PCR and sequencing analysis should be reserved for validation of negative FISH results and for research purposes
  - Documentation of rearrangements and/or chimeric transcript is mandatory for diagnosing atypical/pediatric cases and assessing potential sensitivity to targeted therapy with tyrosine kinase inhibitors (TKIs)
  - TK inhibitors targeting PDGFRβ (including imatinib mesylate, sunitinib, and sorafenib) are indicated in locally advanced and metastatic DFSP with confirmed *COLA1–PDGFB* fusion gene; presence of chimeric gene is not predictive of degree of response to targeted therapy, but its absence is predictive of treatment failure
- GCF
  - A rare, low grade mesenchymal neoplasm with a typical predilection for young boys; usually arising on the trunk. Histological

features are sparse cellularity and a myxoid stroma; spindled bland fibroblasts and stellate cells are observed admixed with CD34-positive giant cells, which often line "angiectoid" or pseudovascular spaces. Parts of the tumor may resemble DFSP
- Molecular aberrations are identical to the ones described in DSFP; GCF and DFSP are currently seen as related neoplasms

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Molecular Pathology of Head and Neck Cancer

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Squamous Cell Carcinoma

Overview of Head and Neck Squamous Cell Carcinoma

- Head and neck cancer includes diverse tumor types arising from various structures: craniofacial bones, soft tissues, salivary glands, skin, and mucosal membranes
  - 90% are head and neck squamous cell carcinomas (HNSCC)
- HNSCC is the sixth most common cancer in the world with more than 560,000 new cases diagnosed annually and over 300,000 deaths per year
  - HNSCC arises from the mucosal lining of the upper aerodigestive tract and demonstrates squamous differentiation
  - Historically, the most commonly affected patients are older males with a long history of cigarette smoking and alcohol consumption
- Surgery, chemotherapy, and radiation are the currently available treatments
- 5-year survival rate in head and neck cancer has remained unchanged at 50% over the past 20 years; novel methods of cancer detection and therapeutic options need to be developed
- Genetic, epigenetic, and viral agents have been identified as etiologic factors in the

L. Cheng and J.N. Eble (eds.), *Molecular Surgical Pathology*,
DOI 10.1007/978-1-4614-4900-3_14,

development of head and neck cancer, which is now considered a heterogeneous group of cancers based on molecular studies of tumor biology
  - HNSCC tumors now have distinct genetic profiles that differ in their risk factors, pathogenesis, and clinical behavior
  - Specific molecular alterations and molecular therapy have been associated with improved treatment response and prognosis
  - Biomarker refers to an objectively measured characteristic which may provide an indication of an outcome in question, such as response to treatment, metastasis, or survival
  - Various biomarkers and their application for diagnosing, staging, monitoring, and prognosticating HNSCC are being developed
  - Human papilloma virus (HPV) and epidermal growth factor receptor (EGFR) are the two most well studied and frequently used biomarkers in HNSCC
- Improved molecular characterization of primary tumors and surgical margins will soon allow clinicians to detect and treat earlier lesions, predict response to treatment, and tailor specific therapy

Risk Factors and Molecular Pathology in HNSCC

- Tobacco smoking is a well-known risk factor for HNSCC; the risk of developing HNSCC correlates to the duration and amount of smoking
  - Increased risk of developing HNSCC is attributed to the nitrosamines and polycyclic hydrocarbons in tobacco smoke
  - The molecular targets of cigarette smoke and tobacco-induced mutations are currently being studied
  - One example is TP53 mutations in HNSCC, which occur more frequently in patients who smoke than in those who do not smoke, but are not present in all patients who smoke
  - Individual differences in carcinogen metabolizing enzymes may modify individuals' risk of HNSCC development
- Alcohol consumption is an independent risk factor for developing HNSCC, especially for hypopharyngeal squamous cell carcinoma
  - Alcohol consumption and tobacco smoke synergistically magnify the risk of developing HNSCC
  - Acetaldehyde, the metabolite in alcohol, interferes with DNA synthesis and repair
  - Alcohol likely potentiates the effects of smoking because it is a chemical solvent and therefore prolongs mucosal exposure to the carcinogens in tobacco smoke
- HPV type 16 is the causative agent in up to 70% of oropharyngeal cancers (20–25% of all HNSCC), which is shifting the demographics of HNSCC toward younger patients with no alcohol or tobacco risk factors
  - HPV-18 and other subtypes can also be found in up to 5–10% of HNSCC
  - HPV-associated HNSCC is associated with oral HPV infection and sexual practices which increase oral viral exposure (these include early age of initial sexual activity, a high number of oral and vaginal sexual partners, frequent oral-genital and oral-anal contact, and decreased use of barriers during intercourse)
  - HIV positivity increases the frequency of oral HPV detection
  - Marijuana is an independent risk factor for HPV-positive HNSCC; the risk increases with intensity, duration, and cumulative years of marijuana use
  - Cannabinoids from marijuana bind the CB2 receptor on B cells, T cells, NK cells, macrophages, and dendritic cells in human tonsillar tissue, which suppresses immune responses to HPV infection inducing tumorigenesis
  - Tonsillar anatomy consists of crypts lined by reticulated squamous epithelium, which serves to transport foreign antigens from the external environment to the tonsillar lymphoid tissue allowing for the

direct passage of lymphocytes and antigen-presenting cells, which also allows for HPV deposition
  - The microanatomy of the crypt epithelium likely contributes to the clinical observation that small oropharyngeal carcinomas often present with advanced regional metastases

Histopathology of HNSCC

- Squamous dysplasia refers to alterations in the surface epithelium prior to invasion beyond the epithelial basement membrane
  - Changes include abnormal cellular organization, increased mitotic activity, and nuclear enlargement with pleomorphism
  - Dysplasia is graded 1–3 based on the severity of atypia (grade 3 dysplasia is also known as carcinoma in situ)
  - Progression beyond the basement membrane is invasive squamous cell carcinoma (SCCA)
- HNSCC has different pathological variants based on tumor differentiation; the most common type of HNSCC is moderately differentiated SCCA
  - Spindle cell variant consists of a proliferation of noncohesive spindle cells resembling a sarcoma more than a carcinoma
  - Verrucous carcinoma is an exophytic mass with markedly thickened squamous epithelium with "church spires" of parakeratotic squamous cells and broad pushing borders without atypia and no potential for metastasis
  - Papillary variant of HNSCC has a prominent exophytic component of papillary growth; papillary fronds are lined by malignant squamous cells
  - Basaloid squamous variant is highly aggressive and consists of solid lobules of cells with peripheral palisading, scant cytoplasm, and dark nuclei
  - A subtype of basaloid SCCA consists of HPV-16 positive tumors which have a significantly increased overall survival despite being more likely to present with lymph node metastases

Genetic Model of HNSCC

- A multistep process of genetic and epigenetic alterations results in the transformation of normal mucosa or epithelium into HNSCC
  - A tumor progression model hypothesis was developed for other tumors and has been applied to HNSCC
  - Neoplasms are the result of tumor suppressor gene inactivation and/or protooncogene activation
  - There is a defined order of genetic events leading to a specific tumor phenotype
  - The net accumulation of genetic alterations determines the tumor phenotype
- Allelic loss appears to be more common than allelic gain
- A tumor progression model has been proposed for head and neck cancer based on microsatellite analysis for allelic loss at ten major chromosomal loci in benign hyperplasia, dysplasia, carcinoma in situ, and invasive cancer
  - The accumulation and not necessarily the order of genetic events determines tumor progression into invasive carcinoma
  - The accumulation of genetic alterations tends to follow a sequential order
  - There is an increased rate of allelic loss in areas of apparently benign mucosa adjacent to premalignant lesions lending support to the concept of "field cancerization" derived from a common clone
  - Importantly for future screening possibilities, genetic damage often precedes microscopic changes
- Field cancerization was proposed by Slaughter 40 years ago to explain multifocal tumor origin as 10–40% of patients with HNSCC will develop a second tumor of the aerodigestive tract
  - Field cancerization refers to a large area of mucosa or epithelium surrounding the

primary tumor which possesses an increased potential to develop malignancy
  - Contemporary molecular biology using microsatellite analysis and X chromosome inactivation has shown synchronous and metachronous lesions in head and neck cancer that originate from a common clone
  - Lesions separated by time or distance may have a common clonal origin which evolve into cancer through an accumulation of successive genetic alterations
  - Therefore, genetically damaged cells may be present in surrounding mucosa without any histopathologic evidence of dysplasia, accounting for local recurrence despite surgical resection with negative margins determined histopathologically
- Identifying early events within a tumor progression model will allow for the clinician to observe or treat lesions which may appear histopathologically normal, but have a high risk for progression to malignancy

Common Genetic Alterations in Head and Neck Cancer

- The p53 and retinoblastoma (Rb) tumor suppressor pathways are the most common pathways altered in HNSCC
- Loss of p53 in chromosome region 17p13 is almost universal in HNSCC, as in other malignancies
  - p53 mutation frequency is directly proportional to worsened HNSCC histologic appearance
  - Loss of function of p53 is seen in the transformation from a preinvasive to invasive phenotype; further genetic alterations subsequently continue through the cell cycle without repair
  - A mutation rate of 50–79% has been observed in HNSCC tumors
  - p53 mutations may be an obligatory genetic alteration in HNSCC
  - p53 expression has been associated with poor prognosis and progression of disease
  - HPV 16 viral products (E6, E7) inhibit the p53 pathway by degrading wild type p53 protein
  - Therefore, HPV-positive HNSCCs are less likely to contain a p53 mutation than HPV-negative HNSCCs
- The disrupted Rb pathway occurs most commonly from a loss of the p16/p14^{ARF} genes in chromosome region 9p21–22
  - This is the most common genetic change in HNSCC and occurs early in the progression of head and neck tumors
  - p16 prohibits cells from entering the cell cycle by inhibiting cyclin-dependent kinases 4 and 6 (CDK4 and CDK6), disrupting Rb phosphorylation and leading to G1 cell cycle arrest
  - p14ARF inhibits MDM2 from inhibiting p53 and therefore causes an antiproliferative effect in wild type cells
  - Disruption of these tumor suppressor genes in HNSCC is from genetic mutations, loss of heterozygosity, or inactivation by promoter hypermethylation
  - The frequency of loss of heterozygosity at 9p21–22 in HNSCC is 70%
  - In HPV-positive cancer, wild type p16 protein is consistently expressed and immunohistochemistry of p16 protein can be used as a marker of HPV status in HNSCCs
- Epidermal growth factor receptor (EGFR) overexpression is a predictor of increased locoregional recurrence and decreased overall and disease-free survival in HNSCC
  - EGF induces cell division, migration, adhesion, differentiation, and apoptosis via a tyrosine kinase-dependent pathway
  - More than 90% of HNSCC tumors overexpress EGFR
  - Overexpression of EGFR increases with increasing severity of dysplasia in premalignant lesions
  - EGFR downstream effectors also regulate cellular growth properties
  - Signal transducer and activator of transcription (STAT3) is a downstream effector of EGFR, which has antiapoptotic

properties in a head and neck cancer xenograft model
  - EGFR has been identified as a potential target for cancer therapeutics; adenoviral E1A gene products downregulate EGFR expression in HNSCC cell lines and induce apoptosis, which is reversible with EGFR expression
- Loss of chromosome 3p heterozygosity is another early genetic alteration observed with a 60% frequency in HNSCC
  - There is no consensus on the specific locus responsible for the tumor suppressor phenotype of chromosome 3p
  - Three distinct regions of loss have been mapped
- HER2 is an oncogene with homology to EGF and is overexpressed in 40% of oral squamous cell carcinomas
  - HER2 overexpression in breast cancers is related to a poor prognosis
  - Trastuzumab (Herceptin) is a monoclonal antibody which binds and blocks the growth factor receptor of HER2, resulting in greater survival rates
- Amplification of Cyclin D1 in the chromosome region 11q13 is reported in 33% of HNSCC tumors
  - Cyclin D1 is a protooncogene, also known as PRAD1 and CCD1
  - Cyclin D1 activates Rb via phosphorylation, causing the cell to progress from the G_1 phase to the S phase
  - Constitutive activation causes increased proliferation and therefore tumorigenesis
  - Both amplification of cyclin D1 and inactivation of p16 result in increased phosphorylation of Rb and movement of cells from G_1 to the S phase in the cell cycle
- p12 is a tumor suppressor gene implicated in S phase-associated growth suppression, through binding with DNA polymerase α-primase and/or CDK2
  - Delivery of a p12 murine gene suppresses tumor cell growth in vivo in an orthotopic mouse model of HNSCC
  - Upon delivery of p12, an antitumor effect of p12 is noted
  - Delivery of a p12 murine gene suppresses tumor cell growth in vivo in an orthotopic mouse model of HNSCC
  - Upon delivery of p12: increases in TUNEL labeling and apoptotic indices and decreases in Ki67 cell proliferation labeling indices as compared with controls are noted, resulting in an antitumor effect consistent with the role of p12 as a tumor growth suppressor in vivo
  - p12 inhibits cell turnover and tumor growth and as such, may be a potent therapeutic agent suitable for further development in cancer gene therapy
- Matrix metalloproteinase (MMP) molecules are zinc-dependent endopeptidases, which degrade the extracellular matrix (ECM) and are involved in tumor invasion and metastasis in HNSCC
  - Tumor invasiveness has a direct correlation to MMP-13 expression in HNSCC tumors
  - MMP-2 is expressed more in metastatic cancer cells than in nonmetastatic cancer cells and correlates with more aggressive tumor growth and poor prognosis
  - MMP-9 is associated with poor prognosis
  - Antibodies against MMP-9 showed a decrease in invasive properties of HNSCC cell lines
  - The expression of MMP-2, MMP-9, and MMP-13 may serve as valuable markers of tumor progression because of their role in tumor invasiveness
  - Specific cancer treatments targeting MMPs include chelators that bind zinc ions, MMP signaling pathway inhibitors, and MMP antibodies

Practical Molecular Pathology Applications in HNSCC

Current Practical Applications

- Biomarkers can be objectively measured to evaluate the presence and progression of disease
  - As molecular pathways in HNSCC become more understood, there is an increasing number of potential biomarkers

  - Biomarkers have thus far been used mostly as prognostic indicators in HNSCC, though none has consistently proved reliable across multiple studies and none are currently used in routine surgical pathology practice
  - Their role is expanding to include early cancer detection, more accurate tumor staging, selection for targeted therapies, and post treatment cancer surveillance
- HPV types 16 and 18 are associated with an increased risk of development of oropharyngeal and to a lesser degree oral cavity squamous cell carcinoma
- The E6 and E7 viral proteins of HPV degrades p53 and Rb, which inactivates tumor suppressive mechanisms
- HPV-positive tumors are almost always associated with wild type p53 and overexpression of the p16 protein
- p16 immunohistochemical staining can be used alone as a surrogate marker for the presence of HPV
- HPV-positive HNSCC tumors are more common in younger patients without a significant alcohol or smoking history
- HPV-positive tumors are more likely to be poorly differentiated with higher cervical lymph node metastases, but a better overall prognosis and response to therapy, regardless of therapeutic regimen
- HPV-positive tumors also have a lower risk of second primary tumors
  - Mechanisms underlying the improved clinical outcome in HPV-positive tumors may include the combined effects of immune surveillance to viral-specific tumor antigens, an intact apoptotic response to radiation, and the absence of widespread genetic alterations (field cancerization)
  - Mechanisms to detect HPV include polymerase chain reaction (PCR) based assays, real-time or quantitative PCR, and in situ hybridization
- EGFR expression is a consistent marker of poor prognosis, but its utility in predicting response to EGFR-targeted therapies is not yet completely elucidated
  - Cetuximab, an EGFR monoclonal antibody, is the only currently approved EGFR targeting drug for use
  - Cetuximab with RT has been shown to be effective in locoregionally advanced HNSCC; Cetuximab with platinum-based chemotherapy has shown a survival benefit in recurrent and metastatic HNS
  - There is no correlation between the effectiveness of EGFR-inhibitor therapies and molecular or immunohistochemical testing for EGFR in tumor samples

Future Practical Applications

- Tumor localization in cases of regional metastases with an unknown primary can be aided by the detection of certain oncogenic viruses that target specific regions of the aerodigestive tract
  - Epstein-Barr virus (EBV) in a neck metastasis reliably suggests the nasopharynx as the tumor origin
- The presence of HPV in a cervical lymph node metastasis reliably points to an oropharyngeal primary tumor
- The application of this approach is currently limited because the majority of HNSCC tumors are not linked to an oncogenic virus
- Detecting genetic alterations early in the tumor progression cycle may result in earlier diagnosis when cure is more attainable
  - Identifying premalignant lesions (dysplasias) that are more at risk for transformation into invasive carcinoma is difficult by histopathology alone
  - Among the studied biomarkers, loss of heterozygosity at defined chromosomal loci may be the most promising technique to identify high risk premalignant lesions
- Several studies have shown that dual loss of heterozygosity at 3p and 9p can distinguish lesions which are likely to progress to invasive carcinoma from those that will not
- The detection of undiagnosed HNSCC is currently under investigation by using saliva as a substrate for biomarker assessment

  - Saliva has been used to assess HPV status, promoter hypermethylation profiles, p53 gene mutations, telomerase activity, and gene expression profiles
  - More recently, saliva has been shown to harbor clinical discriminatory transcriptomic (IL1B, IL8, SAT1, S100P, DUSP1, and OAZ1) and proteomic (IL1B, IL8, CD59, Profilin, MRP-14, Catalase, and M2BP) biomarkers
  - miRNAs have also been identified in both whole saliva and supernatant saliva. Two of these miRNAs, miR-125a and miR-200a, are differentially expressed in the saliva of HNSCC patients compared with that of healthy controls. miRNAs in saliva can be used as a noninvasive and rapid diagnostic tool for the diagnosis of oral cancer.
  - A nationwide oral cancer saliva biomarker validation study is currently ongoing in a prospective specimen collection, to meet the guidelines of the early detection network (EDRN) of the National Cancer Institute
- HPV 16/18 status may be useful in screening at risk patients for development of HNSCC
  - A large double-blind, randomized clinical trial showed that the HPV 16 vaccine in young women significantly reduced the incidence of HPV-16 related cervical intraepithelial neoplasia; a similar vaccine may prevent HPV-associated HNSCC
  - There is a potential to select a high risk population such as heavy drinkers and smokers, and screen them for potential molecular alterations, such as HPV DNA
  - Patients with concerning molecular alterations could undergo closer observation
- Microarray analysis distinguishes gene expression differences between normal and malignant tissue and has been used more recently in characterizing gene sets that identify subgroups of HNSCC
  - The unique "molecular signatures" are being studied in predicting disease course, response to therapy, and survival
  - Gene sets and microarray analysis have been used to predict response to chemoradiation (CRT) and radiation therapy (RT)
  - Probe sets have differed between responders and nonresponders to CRT and RT
  - Microarray analysis and genetic expression profiling have the potential to be used as markers of prognosis and response to CRT and RT in the future
  - Proteomic analysis of normal epithelia and well-differentiated, moderately, and poorly differentiated HNSCC has shown differences at each stage and evaluation of histologically normal mucosa in HNSCC may be able to predict the development of second primaries and local recurrence
  - Proteomics accounts for posttranscriptional and translational modifications and may be more accurate in understanding tumor biology
  - Methods in detecting protein expression include two-dimensional differential in gel electrophoresis, surface-enhanced laser desorption/ionization time of flight mass spectrometry, and matrix-assisted laser desorption ionization time of flight mass spectrometry
- Hypermethylation of CpG-rich promotor regions leads to tumor suppressor gene inactivation and can be detected on molecular detection assays
  - Physiologic promoter hypermethylation occurs in X chromosome inactivation and genetic imprinting, but has been implicated in various cancers by inactivating tumor suppressor genes
  - The promoter hypermethylation status in HNSCC patients has been assessed using a panel of four tumor suppressor genes: p16, O6-methylguanine-DNA methyltransferase (MGMT), GST-π, and death-associated protein kinase (DAPK)
  - At least one of the four genes exhibited promoter hypermethylation in 42–56% of head and neck squamous cell tumors
  - Creating an assay sensitive enough for clinic use has been difficult because not all

tumors have currently identifiable methylated genes in HNSCC

- Determination of telomerase activity in patients with HNSCC tumors could select patients likely to benefit more from chemotherapeutic agents which induce DNA double-strand breaks
  - Telomeres are tandem repeats of DNA capping the ends of chromosomes; telomere shortening during cell cycles leads to natural cellular senescence
  - Telomerase maintains telomere length in pluripotent germline cells and is absent in somatic cells
  - Telomerase activity has been reported in 90% of invasive HNSCC cell lines and in 100% of premalignant lesions
  - Nonsteroidal antiinflammatory drugs possibly inhibit telomerase in HNSCC treated with indomethacin and ibuprofen
- Molecular analysis of surgical margins (using p53 and eIF4E) may supersede conventional histopathologic criteria, which is currently associated with a high rate of local recurrence
  - Unique single-stranded DNA probes complementary to each type of p53 mutation have been used in analysis of surgical margins in HNSCC
  - In one study, patients whose margins were negative by molecular mutation analysis had a statistically significant decrease in local recurrence compared to margins with p53 mutations and histologically negative margins
  - eIF4E is a protooncogene which initiates translation and has also been investigated by molecular margin analysis
  - eIF4E overexpression was present in 98% of primary tumors and 52% of histologically negative margins; patients with clear histologic margins positive for eIF4E had significantly higher local recurrence rates than margins without eIF4E overexpression
- Predictive molecular pathology identifying patterns of genetic alteration could be used to predict the behavior and tumorigenic potential of premalignant head and neck lesions and determine which tumors would be more amenable to certain therapeutic interventions
  - HPV(+) and p16(+) are highly predictive for poorly differentiated tumors and basaloid SCCA. Additionally, HPV and p16 positivity demonstrate superior predictive value for lymph node metastasis above standard H&E histopathologic features
- COX2 activation increases the expression of SNAIL, a transcription factor. SNAIL in turn binds to the promoter region of cell adhesion molecule E-cadherin, blocking its expression. This pathway is thought to be critical in epithelial–mesenchymal transition and subsequent aggressive HNSCC behavior
- SNAIL positivity is significantly predictive of poorly differentiated, lymphovascular invasive, as well as regionally metastatic tumors. Because SNAIL positivity appears independent of HPV, p16, and EGFR expression, SNAIL can improve upon these markers' predictive limitations.
  - Analysis of p53 status has shown improved survival and response rates with wild type p53, but also with absent p53 expression
  - In the future, analysis of p53 function may be more important in predicting tumor response because wild type p53 can be inactivated by methods other than genetic alterations, such as HPV product inactivation
  - Increased pretreatment IL-6 levels were found to be an independent predictor of both recurrence and poor survival in a large longitudinal, prospective cohort study
- Several molecular targets for molecular therapy are under investigation
  - Targeted therapy toward EGFR has been studied most extensively and consists of monoclonal antibodies, tyrosine kinase inhibitors (inhibit the phosphorylation function of the cellular domain of EGFR), antisense oligonucleotides, and small interfering RNAs (attacks EGFR in extracellular and intracellular domains and at the translational stage)
  - EGFR expression status in primary laryngeal HNSCC was shown to positively correlate with the development of secondary tumors
  - Hyperphosphorylation of EGFR independently has been shown to be associated with increased lymph node metastases and higher nodal stage of disease

  - STAT proteins, specifically STAT3 is overexpressed in HNSCC tumors; in vitro and in vivo studies targeting STAT3 using oligonucleotide decoys show antitumor effects
  - Therapeutic HPV vaccines are being studied targeting the antigens E6 and E7 via viral vector vaccines, bacterial vector vaccines, peptide/protein vaccines, DNA vaccines, and cell-based vaccines
  - Gene therapy targeting replacement of the mutated tumor suppressor gene p53 has been difficult due to creating adequate delivery systems to all affected cells

Nonsquamous Cell Carcinomas

Nasopharyngeal Carcinoma

- Clinical
  - Nasopharyngeal cancer (NPC) is rare throughout the world, but more common in certain geographic areas such as southern Asia
  - NPC is a squamous cell carcinoma that develops around the ostium of the Eustachian tube in the lateral wall of the nasopharynx
  - Both environmental and genetic factors play roles in the development of NPC
  - Environmental factors include nitrosamine exposure in salted and pickled foods
  - The strong association of NPC with EBV makes NPC unique from other head and neck cancers
  - Treatment consists of radiotherapy with or without concurrent chemotherapy
- Histopathology
  - The World Health Organization classifies NPC into three subtypes
    - Type 1: Keratinizing squamous carcinoma
    - Type 2: Nonkeratinizing squamous carcinoma
    - Type 3: Poorly differentiated with highly variable cell types
  - Types 2 and 3 are EBV-associated and have better prognoses than type 1
- Molecular pathology
  - EBV has been implicated in the molecular abnormalities leading to NPC, which consist of a large variety of pathways and the alteration in expression of numerous proteins
  - EBV has tumorigenic potential due to a unique set of latent genes: latent membrane proteins (LMP1, LMP2A, LMP2B) and EBV-determined nuclear antigens (EBNA1 and EBNA2)
  - LMP1 is the principal oncogene of NPC and is present in 80–90% of NPC tumors and activates a number of signaling pathways
  - LMP1 is also involved in suppressing immunogenic responses against NPC
  - There is upregulation of cellular proliferation pathways such as the Akt pathway, mitogen-activated protein kinases, and the Wnt pathway
  - Dysfunctional cell adhesion results from abnormal E-cadherin and β (beta)-catenin function
  - There is dysregulation of p16, cyclin D1, and cyclin E resulting in aberrations in the cell cycle
  - NPC also contains antiapoptotic mechanisms with upregulated antiapoptotic factors bcl-2, survivin, and telomerase
  - High levels of p53 are also found in NPC
    - Most head and neck cancers contain low levels of p53 due to mutations
    - High LMP1 levels correlate with higher p53 expression, which fail to induce apoptosis because of p53 inactivation
    - It is unclear why p53 levels in NPC are high
  - p16 levels are decreased in NPC in two thirds of NPCs
  - There are ongoing studies to develop molecularly based treatments against NPC

Sinonasal Malignancies

- Malignancies of the nasal cavity and paranasal sinuses comprise only 0.2–0.8% of all malignant neoplasms
- Sinonasal malignancies most commonly arise in the maxillary sinus, followed by the nasal

cavity, the ethmoid sinus, and the sphenoid and frontal sinuses
- The most common malignancy of the sinonasal tract is SCCA, followed by adenocarcinoma, minor salivary gland tumors, and undifferentiated small cell tumors (olfactory neuroblastoma (ONB), melanoma, and sinonasal undifferentiated carcinoma)

Sinonasal Adenocarcinoma

- Sinonasal intestinal type adenocarcinoma (ITAC) is morphologically similar to colorectal adenocarcinoma and is associated with occupational exposures to wood or leather dusts
- ITAC has a range of microscopic features
  - Some tumors are indistinguishable from colonic adenocarcinoma
  - Other tumors resemble mucinous or signet ring cell carcinoma of the colon
- The immunophenotypical and genetic profile suggest that ITAC tumorigenesis may have some distinct molecular mechanisms from colonic adenocarcinoma
- The most commonly altered oncogenes are p53 (18–40% of ITACs) and p16 (60% of cases)
  - p53 mutations were detected in 86% of adenocarcinomas
  - Overexpression of p53 has been observed in 60% of cases
- There are frequent losses at 18q, which is the chromosome region which contains genes implicated in colorectal tumorigenesis, such as deleted in colon cancer gene (DDC)
- Mucinous ITACs follow a distinct molecular pathway from nonmucinous variants and pursue an aggressive clinical behavior based on microarray analysis
  - p53 expression greater than 20% was statistically significantly higher in nonmucinous ITAC
  - DDC was significantly lower in mucinous versus nonmucinous ITAC
  - The absence of E-cadherin was present significantly more in nonmucinous ITAC
- Research examining the molecular basis for sinonasal adenocarcinoma is ongoing to further develop diagnostic, therapeutic, and prognostic biomarkers

Small Round Blue Cell Tumors of the Sinonasal Area

- These tumors represent diverse malignancies of epithelial, hematolymphoid, neuroectodermal, and mesenchymal origin which are challenging to differentiate because of overlapping cytomorphologic features
- Small round blue cell tumors are a monotonous population of undifferentiated tumor cells with small-sized nuclei and scant neoplasm
- Immunohistochemistry is a technique which aids in diagnosis
- The discovery in recent years of chromosomal alterations in certain small round blue cell tumors is becoming an invaluable tool in pathologic diagnosis of these tumors

Poorly Differentiated, Nonkeratinizing Squamous Cell Carcinoma

- Poorly differentiated SCCA has histopathologic features which overlap with other small round blue cell tumors
- Immunohistochemistry is useful in diagnosis
  - Cytokeratin immunoreactivity helps distinguish poorly differentiated SCCA from ONB
  - The lack of immunoreactivity for synaptophysin, chromogranin, and CD56 distinguishes poorly differentiated SCCA from neuroendocrine type carcinoma
- Recently, an undifferentiated carcinoma with focal squamous differentiation arising in the midline of the sinonasal cavity has been described called NUT midline carcinoma (NMC), which is an aggressive lesion with a mean survival of 9 months
  - They are thought to arise from primitive neural crest-derived cells
  - NMC lack definitive clinical and histologic features, but are grouped together based on molecular rearrangements of the NUT gene on chromosome 15q14
  - Two-thirds of these tumors contain a t(15;19)(q14;p13.1) translocation resulting in a chimeric fusion oncoprotein encoding BRD4-NUT

  - Diagnosis is by FISH or RT-PCR to detect NUT rearrangement
  - There are ongoing studies to develop antibodies and inhibitors against MYB-NFIB
  - This tumor is unique because it is defined molecularly

Sinonasal Undifferentiated Carcinoma (SNUC)

- SNUC is a rare, highly aggressive carcinoma that presents with locally extensive disease
- This tumor tends to grow along the mucosal surface, extending into superficial mucosal glands, and into lymphovascular spaces
- Immunohistochemistry is not very useful in establishing a diagnosis as they are immunoreactive for pancytokeratin and simple keratins
- There are no known molecular diagnostic tests to establish the diagnosis of SNUC

Small Cell Carcinoma, Neuroendocrine Type (SCCNET)

- SCCNET is a high grade neoplasm that most frequently arises from the superior or posterior nasal cavity with frequent sinonasal extension
- Most are positive for cytokeratin and CD56; EBV-RNA is negative
- Cytogenetic studies for SCCNET have not yet been discovered

Olfactory Neuroblastoma (ONB)

- ONB originates from the olfactory bulb in the region of the cribiform plate
- The presence of fibrillary cell processes, Homer Wright rosettes, and S100-positive sustentacular cells when present are useful in diagnosis
- Recently, array comparative genomic hybridization (aCGH) studies have shown complex gene copy number profiles with a gain of 13q, 20q, and loss of Xp in high stage tumors

Sinonasal Mucosal Malignant Melanoma

- The amelanotic variant of mucosal melanoma can be especially difficult to accurately diagnose
- Diffuse immunostaining for S100, HMB45, and vimentin are useful in distinguishing this malignancy
- Diagnostic molecular studies are still being developed

Extraskeletal Ewing Sarcoma/Primitive Neuroectodermal Tumor

- CD99 immunoreactivity can be useful in distinguishing this tumor from other small round blue cell tumors, but is also positive in desmoplastic small round cell tumors, synovial sarcoma, and lymphoma
- Primitive neuroectodermal tumor has a characteristic EWSRI-FLII fusion transcript or t(11;22)(q24;q12)
  - Identifying this fusion transcript is diagnostic of this tumor

Desmoplastic Small Round Blue Cell Tumor

- Only a single case has been reported in the sinonasal tract
- Recognition of the t(11;22)(p13;q12) and associated EWSR1-WT1 fusion transcript is necessary for diagnosis

Rhabdomyosarcoma

- Embryonal and alveolar rhabdomyosarcoma occur in the sinonasal cavity
- The identification of the 2;13 and 1;13 translocations or respective PAX3-FOXO1 and PAX3 variant translocations is extremely useful in diagnosis as the immunohistochemical profile of rhabdomyosarcoma can often be misleading
  - These translocations create a chimeric oncogene

Extramedullary Plasmacytoma

- This tumor affects adults over the age of 65 and involves the sinonasal cavity in 75% of cases
- Diagnostic confirmation is based on immunohistochemistry or in situ hybridization for immunoglobulin mRNA with the identification of light chain restriction

Extranodal NK/T Cell Lymphoma

- NK/T cell lymphoma is an aggressive disease known for necrosis, vascular invasion, and destruction
- In situ hybridization demonstrating the presence of EBV virus by detecting EBV-encoded early RNAs in addition to an NK-cell immunophenotype is diagnostic

Salivary Gland Malignancies

Mucoepidermoid Carcinoma (MEC)

- Clinical
  - MEC is the most common salivary gland malignancy and appears in both minor and major salivary gland locations
  - MEC most commonly presents as a painless salivary gland mass (parotid gland is the most common location)
  - MEC occurs more commonly after radiation exposure
  - Clinical aggressiveness and rate of regional metastasis depend on the histologic grade
  - Treatment for low and intermediate grade MEC is wide local excision
  - Treatment for high grade MEC is wide local excision, elective neck dissection, and postoperative radiation therapy
- Histopathology
  - Three cell types are required for diagnosis: epidermoid, intermediate, and mucinous cells
  - Grading schemes rely on the percentage of the tumor composed of cystic spaces and more recently on point systems
- Molecular pathology
  - A translocation between the MECT1 gene and the MAML2 gene (t(11;19)(q12;p13)) has been identified, which initially appeared more prevalent in low and intermediate grade MEC than in high grade MEC; however, a recent study showed higher rates of this translocation in high grade MEC
  - The MECT1–MAML2 translocation can be identified using fluorescent in situ hybridization or assays based on reverse transcription PCR
  - Some studies have demonstrated this translocation in Warthin tumor
  - No other malignant salivary gland tumors have shown this translocation

Adenoid Cystic Carcinoma

- Clinical
  - Presents most commonly as a painless salivary gland mass which can occur in both major and minor salivary glands
  - Adenoid cystic carcinoma (ACC) is known for its propensity for perineural invasion and late recurrences both locoregionally and in the form of distant metastases
  - Treatment consists of wide local excision of the involved salivary gland and postoperative radiation for positive margins and/or perineural invasion
- Histopathology
  - ACC is a biphasic salivary gland tumor, containing both epithelial and myoepithelial cell components
  - Growth patterns fall into three groups: tubular, cribiform, and solid types
  - The presence of a significant solid component >30% is considered high grade and has a worse prognosis
- Molecular pathology
  - Interest in the molecular pathology of ACC began with the identification of c-kit (CD117) overexpression in ACC, but most studies have found no evidence of c-kit mutations
  - Imatinib, a monoclonal antibody against c-kit, has had low efficacy in ACC
  - c-kit is seen in other tumors (polymorphous low-grade adenocarcinoma and epithelial–myoepithelial carcinoma) and therefore cannot be used as a diagnostic marker in ACC
  - Recent studies have described a translocation fusing the MYB gene and the NFIB gene into a chimeric transcript (t(6;9)(q22–23;p23–24)) causing overexpression of MYB, which is specific to ACC and can be potentially used not only as a biomarker for ACC, but also to determine potential downstream therapeutic targets

  - Comparative genomic array technology and loss of heterozygosity studies have not shown widespread loss and gain mutations, which are more common in more aggressive tumors
  - Isolated genomic losses have been detected at individual genomic loci, including loss of heterozygosity of 12q, 1p, and 9p
  - p53 is likely involved in high grade transformation of ACC, as these tumors overexpress p53 and contain p53 gene mutations

Mammary Analog Secretary Carcinoma of the Salivary Gland

- Clinical
  - Newly described salivary gland tumor that histologically resembles secretory carcinoma of the breast and is an aggressive tumor
  - Most of these tumors have likely been misdiagnosed as acinic cell carcinomas because of histologic overlap
- Histopathology
  - The tumor grows in an infiltrative pattern with microcystic and tubular patterns
  - Secretary material within luminal spaces is PAS positive and diastase resistant
  - Low-grade, bland, monomorphic nuclei with vesicular type chromatin with prominent centrally placed nucleoli
  - Positive for CK7, vimentin, and S100 on immunohistochemistry
- Molecular pathology
  - Salivary gland mammary analog secretory carcinoma contains a translocation between ETV6 and NTRK3 genes, similar to the tumor version in the breast
  - The translocation can be detected using RT-PCR or fluorescent in situ hybridization

Thyroid Carcinoma

- The rapidly expanding knowledge of molecular genetics of thyroid cancer is beginning to translate into clinical practice to improve accuracy in preoperative diagnosis and prognostication

Papillary Thyroid Carcinoma (PTC)

- PTC is the most common type of thyroid carcinoma, accounting for 80% of all thyroid malignancies
- PTC carries point mutations of the BRAF and RAS genes and rearrangements of RET/PTC and TRK
  - All of these genetic alterations are able to activate the mitogen-activated protein kinase (MAPK) pathway
  - These mutually exclusive mutations are found in over 70% of PTCs
- BRAF is a serine threonine kinase belonging to the family of RAF proteins, which are intracellular effectors of the MAPK signaling cascade
  - Point mutations of the BRAF gene are the most common genetic alteration in PTC, occurring in 40–45% of tumors (the most common point mutation is Valine to Glutamate substitution at residue 600, V600E)
  - Point mutations in BRAF lead to constitutive activation of BRAF kinase and chronic stimulation of the MAPK pathway
  - BRAF V600E is found in PTC with classic pathology as well as the tall cell variant
  - BRAF V600E can also be seen in poorly differentiated and anaplastic carcinomas, especially those containing areas of well-differentiated PTC
  - BRAF V600E has not been found in follicular carcinomas and benign thyroid nodules, making it a specific marker of PTC and its related types
  - Studies have shown that BRAF V600E testing in fine needle aspiration (FNA) samples of thyroid nodules improves the accuracy of cytologic diagnosis (one large study showed a rate of malignancy of 99% in BRAF-positive nodules)
  - Molecular testing can be done using probe-specific real-time PCR, real-time allele-specific PCR, direct sequencing, and colorimetric assay
  - BRAF V600E may also be a good prognostic marker for PTC; It is associated with more aggressive tumor characteristics such as extrathyroidal extension, advanced

tumor stage at presentation, and lymph node, or distant metastases
  - BRAF V600E has also been shown to be an independent predictor of treatment failure, tumor recurrence, and tumor-related death
  - The prognostication of BRAF V600E has also been shown in T1 PTC tumors and papillary microcarcinomas (tumors less than 1 cm)
- The RET protooncogene encodes a cell membrane receptor tyrosine kinase and is highly expressed in thyroid parafollicular or C cells, but not in follicular cells; it is activated by the RET/PTC chromosomal rearrangement
  - 11 types of RET/PTC rearrangements have been reported with the two most common being RET/PTC1 and RET/PTC3
  - All fusions contain the intact tyrosine kinase domain of the RET receptor, enabling the RET/PTC protein to activate the MAPK signaling pathway
  - RET/PTC has been detected in some adenomas and benign lesions, but clonal RET/PTC (found in a significant portion of tumor cells) is specific for PTC
  - For frozen or freshly collected tumor tissue or FNA sample, RT-PCR is an adequate detection method
  - For formalin-fixed and paraffin-embedded tissue, FISH is the assay of choice; the assay should be set up to detect no fewer than 8–12% of cells with the rearrangement patterns
  - Clonal RET/PTC rearrangements are found in 10–20% of adult sporadic PTCs; RET/PTC occur in 50–80% of patients with a history of radiation exposure and in 40–70% of PTCs in children and young adults
  - Testing for RET/PTC rearrangements is of limited use in surgical specimens since PTC architecture is usually obvious, but they can be useful in preoperative diagnosis of thyroid nodules
  - Correlation between the RET/PTC rearrangement and prognosis remains unclear
- RAS genes encode G proteins located at the inner surface of the cell membrane, which send signals from tyrosine kinase receptors and G-protein coupled receptors along the MAPK, PI3K/AKT, and other pathways
  - In thyroid tumors, the most frequent mutations in RAS are NRAS codon 61 and HRAS codon 61
  - RAS mutations are found in all types of thyroid follicular cell-derived tumors
  - RAS mutations are in 10–20% of PTCs; almost all PTCs with a RAS mutation have a follicular variant histology
  - RAS mutations are found in 40–50% of follicular thyroid carcinomas (FTC) and in 20–40% of follicular adenomas
  - The RAS mutation cannot be used as a universal prognostic marker, though a predisposition for dedifferentiation and more aggressive behavior has been suggested in tumors with RAS mutations
  - Detection of a RAS mutation in a thyroid nodule is strong evidence for neoplasia, but does not establish that it is a malignancy
  - Its importance is as a marker for the follicular variant of PTC which is difficult to diagnose, especially on FNA

Follicular Thyroid Carcinoma

- FTC is the second most common type of thyroid malignancy (15%)
  - FTC is divided into conventional type and oncocytic (Hurthle cell) type
- FTCs contain RAS mutations or PAX8/PPARγ (gamma) rearrangements
  - These mutations are mutually exclusive and occur in 70–75% of FTCs
- The PAX8/PPARγ (gamma) rearrangement is a result of a t(2;3)(q13;p25) translocation that leads to the fusion between the PAX8 gene (encoding a paired domain transcription factor) and the peroxisome proliferator-activated receptor (PPARγ [gamma]) gene
  - PAX8/PPARg (gamma) is found in 30–40% of follicular carcinomas
  - The rearrangement results in overexpression of the PPARg (gamma) protein
  - Tumors with this rearrangement are usually in younger patients, smaller and more often have vascular invasion

  - The rearrangement is also found in as many as 38% of follicular variant PTCs
  - The PAX8/PPARg (gamma) rearrangement is also found in 2–13% of follicular adenomas
  - PAX8/PPARg (gamma) rearrangements and RAS point mutations rarely occur in the same tumor, suggesting two different molecular pathways in the development of FTC
  - Detection of PAX8/PPARγ (gamma) should prompt a further investigation into vascular or capsular invasion, though it is not fully diagnostic for a malignancy
  - PAX8/PPARγ (gamma) rearrangement appears to be useful in preoperative FNA diagnosis, but more studies confirming this are needed

Medullary Thyroid Carcinoma (MTC)

- MTC originates from thyroid parafollicular or C cells and accounts for 3% of all thyroid cancers
- Both familial and sporadic MTC frequently possess point mutations in the RET gene

Poorly Differentiated and Anaplastic Thyroid Carcinomas (ATC)

- Poorly differentiated and ATC arise de novo or from preexisting well-differentiated PTC or FTC
- Genetic alterations involving the PI3K/AKT pathway have a higher prevalence in poorly differentiated thyroid tumors
- Mutations in the TP53 and CTNNB1 genes are also common

Summary of Key Points in the Molecular Pathology of Head and Neck Cancer

HNSCC

- The most common cancer of the head and neck is by far SCCA
- HNSCCs arise from the epithelium lining the sinonasal tract, oral cavity, pharynx, and larynx with evidence of squamous differentiation
- Head and neck tumorigenesis is a multistep process resulting from the accumulation of multiple genetic and epigenetic alterations.
- Field cancerization refers to clones of phenotypically intact but genetically damaged cells which populate extended tracts of mucosa and give rise to secondary tumors
- There has been an increase in incidence of oropharyngeal SCCA in a population of younger patients without an alcohol and smoking history attributed to HPV infection
- HPV, especially type 16, is a causative agent in 70% of oropharyngeal cancers and result in different clinical and pathological tumor behavior
- HPV-positive tumors are more poorly differentiated and have a higher propensity for regional lymph node metastases, but have a better response to treatment with significantly improved overall survival
- The p53 and Rb tumor suppressor pathways are frequently disrupted in head and neck tumorigenesis with various mechanisms including genetic and epigenetic silencing (in the majority of HPV-negative HNSCC) and viral oncoprotein degradation by E6 and E7 in HPV-positive HNSCC
- Continuing research in the underlying molecular genetics of HNSCC will help further elucidate biomarkers to measure the presence, extent, and progress of disease
- Molecular targeted therapy against EGFR in HNSCC has been moderately successful; further research will assist in producing therapy inhibiting other signaling pathways involved in head and neck tumorigenesis
- The resolution of specific genetic profiles for each HNSCC is on the horizon with the goal of individualizing therapy targeting specific gene alterations and signaling pathways
- Early detection is the key to improve the survival of HNSCC patients
- Saliva, a local biofluid for oral cancer, has been shown to harbor clinical discriminatory proteomic and transcriptomic biomarkers, in addition to microRNAs

Nasopharyngeal Carcinoma

- The strong association between NPC and EBV distinguishes NPC from other squamous cell carcinomas of the head and neck
- The tumorigenic potential of EBV lies in its set of latent membrane proteins (LMP) and EBV-determined nuclear antigens, of which LMP1 is the principal oncogene
- The presence of EBV is necessary but not sufficient in the tumorigenesis of NPC in which multiple cell signaling pathways are implicated
- Studies are ongoing to develop molecularly based treatments against NPC

Sinonasal Malignancies

- Molecular cytogenetics is an ongoing field of study in malignancies of the sinonasal tract, which include SCCA, adenocarcinoma, minor salivary gland tumors, and the various small round blue cell tumors
- The molecular pathology of certain tumors in the sinonasal tract is currently most helpful in differentiating tumors which demonstrate the small round blue cell morphology
- Molecular markers for selected undifferentiated sinonasal tumors allows for identification of tumors in which other diagnostic criteria may not be sufficient, which include NMC, Ewing sarcoma, alveolar rhabdomyosarcoma, and desmoplastic small round cell tumor

Salivary Gland Malignancies

- The field of molecular biology in salivary gland malignancies remains in development for diagnostic, prognostic, and therapeutic biomarkers
- The MECT1–MAML2 translocation has recently been described in Mucoepidermoid Carcinoma and has not been found in other salivary gland tumors
- A translocation fusing the MYB and NFIB genes into a chimeric transcript has been discovered in ACC, which is a potential biomarker for diagnosis and developing downstream therapeutic targets

Thyroid Carcinoma

- Many genetic mutations and molecular alterations in PTC and FTC have been discovered
- The most clinical experience has been with the diagnostic use of the BRAF mutation, which is highly specific for malignancy
- Testing FNA samples for a panel of mutations which includes BRAF, RAS, RET/PTC, and PAX8/PPARγ (gamma) is suggestive of malignancy if any of these are present and helps to clarify clinical management in patients with indeterminate cytology

Acknowledgements Supported in part by the Felix and Mildred Yip Endowed Professorship and The Barnes Family Foundation Fund.

Suggested Reading

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Molecular Pathology of Soft Tissue and Bone Tumors

15

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Contents

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General Concepts

Definition

- Bone and soft tissue tumors are neoplasms arising from mesenchymal tissues of the body, which may be either benign or malignant, although most solid tumors for which molecular diagnosis is applied are malignant
- Malignant bone and soft tissue tumors are referred to as *sarcomas*, and are classified primarily by the type of tissue (e.g., liposarcoma [adipose], rhabdomyosarcoma [skeletal muscle], chondrosarcoma [cartilage]), rather than by anatomic site

Clinical Features

- Presentation, treatment, and outcome are heterogeneous, not only between different tumor types, but also within each tumor type. Some very coarse generalizations can be made, but exceptions are very common
- Presentation, treatment, and prognosis are dependent on disease *stage* (function of tumor

L. Cheng and J.N. Eble (eds.), *Molecular Surgical Pathology*,
DOI 10.1007/978-1-4614-4900-3_15,

size, local extension, and distant spread), and *grade* (function of the microscopic features of the tumor cells and the architecture/pattern of their growth)
- Sarcomas tend to spread to distant sites via blood vessels
- Bone and soft tissue sarcomas are much less common than carcinomas in the USA (according to American Cancer Society estimates for 2011)
  - ~14,000 new cancers/year out of a total of ~1,600,000 new cancers/year
  - ~5,500 deaths/year out of a total of ~570,000 deaths/year
- Primary osseous neoplasms are less common than primary soft tissue cancers (~3,000/year vs. ~11,000/year), with a mildly higher lethality rate (~1,500 deaths/year vs. ~4,000 deaths/year)
- Primary neoplasms of bone and soft tissue are more common in children (~7% of childhood cancers) than in adults (<1%)

Basic Principles

- Molecular pathology of sarcomas is also heterogeneous, but generally involves alteration(s) in genes encoding proteins critical for regulating cellular growth and proliferation, programmed cell death (apoptosis), differentiation, and/or motility
- Tumor suppressor genes (TSG) typically inhibit cellular functions such as growth, proliferation, and motility, but may also promote functions such as adhesion, apoptosis, differentiation, and DNA repair
  - TSGs are inactivated in cancer, by diverse mechanisms including deletion, point mutation, promoter methylation, or chromosomal rearrangement
  - Generally, both tumor suppressor alleles are *inactivated* in a cancer (homozygous), although cancer may arise in association with haploinsufficiency of some TSGs as a result of a single mutant allele
  - Many hereditary cancer syndromes involve an inherited dysfunctional TSG (e.g., *RB1* in rtinoblastoma, *TP53* in Li–Fraumeni syndrome), and neoplasia result when the second allele is affected by a sporadic mutation (second hit) in a given tissue. These syndromes have dominant inheritance patterns, even though both alleles are mutated in the tumors
- Oncogenes typically promote cellular functions such as growth, proliferation, and motility, but may also inhibit functions such as differentiation, adhesion, DNA repair, and apoptosis
  - Oncogenes are *activated* in cancer, by diverse mechanisms including polyploidy, polysomy, gene amplification, chromosomal rearrangement, and point mutation
  - Generally, one oncogene allele is altered in a cancer, unless the mechanism of alteration is amplification, polysomy, or polyploidy, in which case multiple copies are present
- Sarcomas usually have characteristic, recurrent chromosomal translocations that have, in some instances, become criteria for the diagnosis of these tumors (*see* Table 15.1)
  - Some translocation breakpoints interrupt genes directly, resulting in a novel fusion oncogenic protein, while other breakpoints may result in deregulated expression, generally overexpression, of the new fusion gene
    - For example, t(11;22)(q24;q12) in Ewing is sarcoma results in a hybrid protein containing the C terminal DNA-binding domain of FLI1 (11q24) and the N terminal transactivating domain of EWSR1 (22q12), under the regulation of the constitutively expressed *EWSR1* promoter. The result is increased transcriptional activation of genes with FLI1 binding sites, leading to increased cell growth and proliferation
  - Two common types of genetic alterations have been observed: translocations forming chimeric protein tyrosine kinases (ALK and ETV6–NTRK3) and translocations encoding a chimeric autocrine growth factor (COLA1A–PDGFB)
  - Many translocations have *chromosomal variants*, where one chromosome band is consistently rearranged, but may be translocated to different chromosome partner regions

Table 15.1 Specific chromosomal translocations in sarcomas

Tumors	Translocation	Molecular event
Alveolar rhabdomyosarcoma	t(2;13)(q35;q14)	*PAX3–FOXO1*[a]
	t(1;13)(p36;q14)	*PAX7–FOXO1*[a]
	t(2q35)	*PAX3*
Alveolar soft part sarcoma	t(X;17)(p11.2;q25)	*ASPSCR1–TFE3*
Angiomatoid fibrous histiocytoma	t(2;22)(q32;q12)	*EWSR1*[a]*–CREB1*
	t(12;16)(q13;p11)	*FUS*[a]*–ATF1*
	t(12;22)(q13;q12)	*EWSR1*[a]*–AFT1*
Clear cell sarcoma	t(12;22)(q13;q12)	*ATF1–EWSR1*[a]
	t(2;22)(q34;q12)	*CREB1–EWSR1*[a]
Dermatofibrosarcoma protuberans	t(17;22)(q22;q13)	*COL1A1–PDGFB*
Desmoplastic round cell tumor	t(11;22)(p13;q12)	*WT1–EWSR1*[a]
Endometrial stromal sarcoma	t(7;17)(p15;q21)	*JAZF1–SUZ12*
	t(6;7)(p21;p15)	*PHF1–JAZF1*
	t(10;17)(q22;p13)	*YWHAE–FAM22A/B*
Epithelioid hemangioendothelioma	t(1;3)(p36.3;q25)	*WWTR1–CAMTA1*
Ewing sarcoma/PNET[b]	t(11;22)(q24;q12)	*EWSR1*[a]*–FLI1*
	t(21;22)(q22;q12)	[b]*EWSR1*[a]*–ERG*
	t(20;22)(q13;q22)	*EWSR1*[a]*–NFATC2*
	t(16;21)(p11;q22)	*FUS*[a]*–ERG*
Infantile fibrosarcoma	t(12;15)(p13;q25)	*ETV6*[a]*–NTRK3*
Inflammatory myofibroblastic tumor[b]	t(2;19)(p23;p13.1)	[b]*ALK*[a]*–TPM4*
	t(1;2)(q22–23;p23)	*TPM3–ALK*[a]
Low grade fibromyxoid sarcoma	t(7;16)(q33;p11)	*FUS*[a]*–CREB3L2*
	t(11;16)(p11.2;p11)	*CREB3L1–FUS*[a]
Myoepithelioma, soft tissue	t(19;22)(q13;q12)	*EWSR1*[a]*–ZNF444*
	t(1;22)(q23;q12)	*EWSR1*[a]*–PBX1*
	t(6;22)(p21;q12)	*EWSR1*[a]*–POU5F1*
Myxoid chondrosarcoma, extraskeletal	t(9;22)(q22;q12)	*NR4A3–EWSR1*[a]
	t(9;17)(q22;q11)	*NR4A3–RBP56*
	t(9;15)(q22;q21)	*NR4A3–TCF12*
Myxoid liposarcoma	t(12;16)(q13;p11)	*FUS*[b]*–DDIT3*[a]
	t(12;22)(q13;q12)	*EWSR1*[a]*–DDIT3*[a]
Myxoinflammatory fibroblastic tumor	der(10)t(1;10)(p22;q24)	*TGFBR3–MGEA5*
Synovial sarcoma	t(X;18)(p11;q11)	*SS18*[a]*–SSX1*
		SS18[a]*–SSX2*

[a]Dual color, break apart probes available commercially
[b]Other chromosome partners have been described

- For example, 22q12 (*EWSR1*) may be translocated to 11q24 (*FLI1*), 21q12 (*ERG*), 7p22 (*ETV1*), 2q33 (*FEV*), 17q12 (*E1AF*), and 20q13 (NFATC2) in Ewing sarcoma
- Many translocations have *molecular variants*, where the breakpoints may vary within the involved gene(s), leading to fusion between different exons
  - *EWSR1–FLI1* fusions in Ewing is sarcoma commonly fuse exon 7 of *EWSR1* to either exon 5 or 6 of *FLI1*, although many other configurations have been described
- Some genes are involved in different translocations in different type of lesions
  - In addition to the Ewing sarcoma fusions, *EWSR1* is fused to *WT1* in

desmoplastic small round cell tumor, to *NR4A3* in extraskeletal myxoid chondrosarcoma to *ATF1* in clear cell sarcoma and angiomatoid fibrous histiocytoma, to DDIT3 in myxoid liposarcoma

  - Although most translocations are unique to a specific sarcoma, some translocations can be seen in different tumor types, including epithelial cancers
    - *ETV6–NTRK3* fusions are seen in congenital mesoblastic nephroma, secretory breast carcinoma, and isolated cases of acute myeloid leukemia
    - *EWSR1–ATF1* fusions are seen in angiomatoid fibrous histiocytoma, clear cell sarcoma, and hyalinizing clear cell carcinoma of the salivary glands
    - *EWSR1–POUF5* fusions are seen in soft tissue myoepithelioma, hidradenoma of the skin, and mucoepidermoid carcinoma of salivary glands
    - *ALK* rearrangements are seen in anaplastic large cell lymphoma, nonsmall lung carcinoma, and inflammatory myofibroblastic tumor
- Numerical abnormalities of whole chromosomes (e.g., trisomy, monosomy) or subchromosomal regions (e.g., amplification) tend to be less specific, secondary alterations in sarcomas
- Because different sarcomas may require different, specific assays, and sarcomas are sufficiently uncommon that control materials and expertise can be hard to accumulate, few labs offer a comprehensive menu for sarcoma testing
- *Nomenclature* for genes is inconsistent, and subject to revision. We have chosen to use the Human Genome Organization (HUGO *[sic]*)-standardized nomenclature, but when each gene is first discussed, we also include, in parentheses, the legacy name used in the original papers
  - For example, when the gene at the chromosome 22 breakpoint in t(11;22) is first presented, it is as *EWSR1* (*EWS*), because *EWSR1* is the standard nomenclature according to HUGO, while EWS was the name first given to this gene upon its discovery in Ewing sarcoma. Subsequently in the text, however, this gene will be referred to solely as *EWSR1*, for simplicity

Molecular Diagnostics of Soft Tissue and Bone Tumors

Test Indications

- Diagnosis, usually as an adjunct to morphology and immunohistochemistry (IHC)
- Prognosis
- Theranosis, selection of therapies targeted to specific molecular genetic abnormalities
- Risk assessment for hereditary cancer syndromes
- Minimal disease testing, either for monitoring success/failure of therapy or for screening (less well developed for sarcomas)

General Technical Considerations

- Sampling: cancers are somatic diseases, and the lesion itself must be analyzed, which is likely to require an invasive procedure
  - Less invasive techniques, including small biopsies, fine needle aspiration, brushing, and fluid collection, tend to yield small amounts of cancer cells admixed with benign cells that can interfere with some kinds of analysis
- Fixation: most archived tumor samples are embedded in paraffin after fixation in formalin (FFPE), which cross-links DNA–RNA–proteins, inactivating the proteins and protecting the nucleic acids from digestion
  - DNA isolated from FFPE tissues breaks into pieces roughly 500 bp or smaller during isolation, so techniques that require larger stretches of DNA are unreliable for these samples
  - Some other fixatives (e.g., Zenker, B5) contain heavy metals that inhibit enzymes (e.g., *Taq* polymerase) used for molecular diagnosis, and other tissue treatments (Bouin, bone decalcifying) contain acids

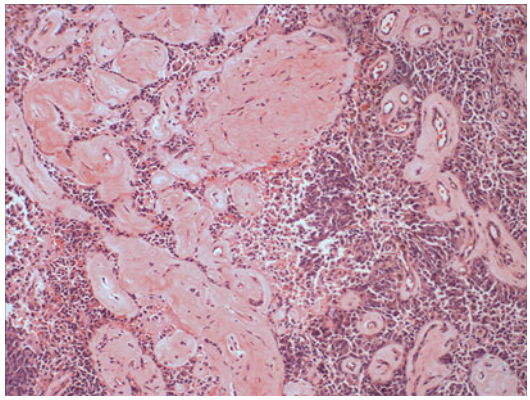

Fig. 15.1 A sarcoma showing intratumor heterogeneity. The malignant cells grow in sheets that are intimately intermingled with reactive, benign elements, including inflammatory cells, entrapped soft tissue, and blood vessels. A DNA sample from this section of a sarcoma would contain admixed malignant and benign DNA, which may make certain types of DNA analysis challenging

that damage nucleic acids and/or inhibit testing (e.g., fluorescence in situ hybridization or FISH)
  - Samples that are not fixed promptly undergo nucleic acid degradation from ubiquitous nucleases, which impairs analysis, especially for RNA
  - Tissue additives are available that report to preserve nucleic acids if applied at the time of fixation; however, they have yet to garner widespread use
- Heterogeneity: most tumors contain an admixture of the cancer cells with benign cells (*see* Fig. 15.1), including residual normal tissue infiltrated by the cancer, reactive elements (e.g., lymphocytes, macrophages, and fibroblasts) recruited and/or stimulated to try to contain the cancer, and foci of necrosis. In general, this is more of a problem for carcinomas than for sarcomas, which tend to overgrow tissue in continuous fashion
  - The cancer cells themselves may also be heterogeneous, such that genetic changes in one part of the tumor may not be seen in another part
  - Some techniques are particularly unreliable for heterogeneous samples, and partial purification of the cancer cells (e.g., microdissection or flow cytometric sorting) may be required before analysis. Direct sequence analysis, for example, requires that approximately 20% of the sample DNA contains the mutation in order for it to be reliably detected
  - Mutation screening (e.g., single-stranded conformational polymorphism [SSCP], heteroduplex-mismatch cleavage, denaturing gradient gel electrophoresis [DGGE], DNA analysis by denaturing high performance liquid chromatography [DHPLC], high resolution melting (HRM) analysis) methods are often less affected by heterogeneity, but may yield incomplete information requiring followup with another assay to define the exact molecular variation
  - Allele-specific amplification and hybridization techniques are considerably less affected by heterogeneity, but are restricted to the exact sequence variants tested
  - Heterogeneity is less of a limitation for in situ hybridization (ISH) techniques, where analysis is performed cell by cell with correlative histology to confirm that tumor cells are being analyzed and not the intermingled benign cells
  - IHC can be used to detect molecular abnormalities that alter the level of expression of a protein. IHC is less useful for detecting mutations that alter the function of a protein without changing its abundance, unless the mutation alters a specific epitope recognized by a monoclonal antibody

Basic Methodologies

- Karyotype analysis (Fig. 15.2) provides a general assessment of large chromosomal abnormalities, including numerical abnormalities and large rearrangements
  - Requires fresh tissue
  - Most sarcomas grow well in short-term culture, requiring 3–4 days
  - Cultured cells are arrested in metaphase, then stained, usually with Giemsa (GTG-banding)

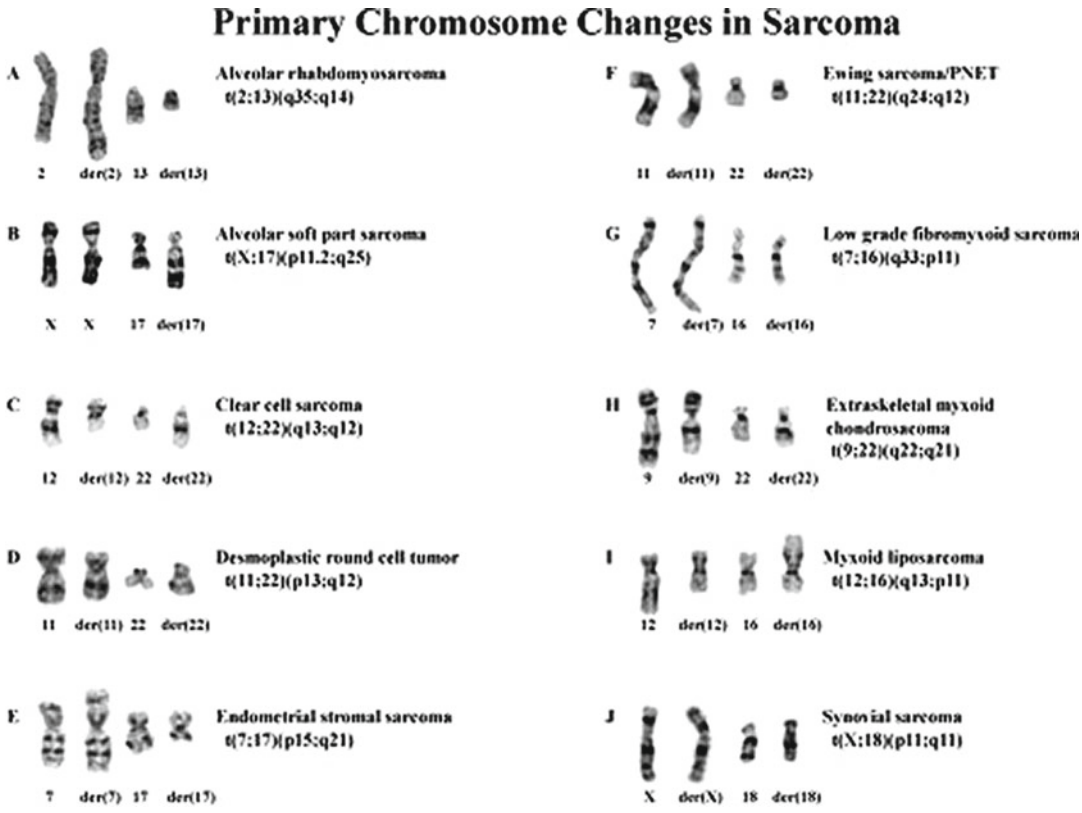

Fig. 15.2 Classic sarcoma partial GTG-banded karyotypes depicting recurrent chromosomal rearrangements observed in sarcomas

  - Admixture of benign stromal cells is a concern
  - Subtle and cryptic translocations can be very difficult to detect by standard GTG-banding, as can small deletions and amplifications
- ISH enables detection of specific chromosomal abnormalities, including subtle or cryptic rearrangements, and small deletions and amplifications
  - Requires a prior knowledge of a suspected aberration
  - Probes hybridize to unique locus-specific DNA sequences, which are usually genomic clones but may be cDNAs, and vary in size from about 1 to 100s of kb
  - May be done with radioactive (ISH), fluorescent (FISH), chromogenic (CISH), or silver (SISH) probes for detection
  - FISH, CISH, and SISH enable rapid detection
  - CISH and SISH enable immediate correlation of the hybridization signal with histopathology, which is particularly valuable for heterogeneous samples
    - The number of colors that can be distinguished with light microscopy limits CISH and SISH; most applications are for numerical alterations (e.g., aneuploidy, amplification)
  - FISH, by using different colored probes, enables simultaneous detection of more than one chromosomal region, which is particularly valuable for analyzing multiple abnormalities within a single cell, or for analysis of chromosomal translocations
    - Correlation of FISH signals with histopathology is more difficult

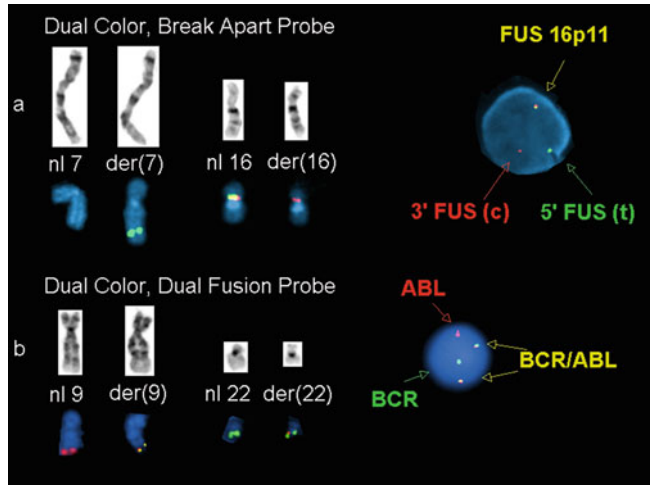

Fig. 15.3 Examples of two different types of hybridization strategies for locus-specific probes: (**a**) A break apart probe shows a *FUS* gene rearrangement at 16p11 from a t(7;16)(q34;p11), a characteristic abnormality in low grade fibromyxoid sarcoma. Two differently labeled probes (spectrum *orange* and spectrum *green*) hybridize to opposite sides of the breakpoint of the *FUS* gene located at 16p11. The 16p11 *FUS* region in its normal state would be seen as two immediately adjacent or fused *orange*+*green* (*yellow*) signals. However, if a rearrangement at 16p11 region has occurred, as in this example, separate *orange* and *green* signals would be seen. (**b**) A dual fusion probe shows a *BCR/ABL* fusion from a t(9;22)(q34;q11.2), a characteristic abnormality in chronic myelocytic leukemia. Two differently labeled probes (spectrum *orange* and spectrum *green*) each hybridize near one of the chromosomal breakpoints involved in a translocation. The 9q34 *ABL1* and 22q11.2 *BCR* regions in their normal state would be seen as two separate *orange* and *green* signals. However, if a t(9;22) has occurred, as in this example, two immediately adjacent or fused *orange*+*green* (*yellow*) signals would be seen

  - However, most laboratories do FISH on metaphase spreads from cultured samples or interphase nuclei, and/or CISH on FFPE tissue sections
    - Metaphase FISH has the same sample requirements as conventional karyotyping
    - Interphase FISH can be performed on intact nuclei (nondividing cells) from cytologic samples (touch or smear preparations, fluid cell suspensions), or from whole tissue samples (enzymatic disaggregation or histologic sections)
      - Standard histology (4 μm) sections can miss signals due to sectioning through the nucleus, and require analysis of many more cells
      - 50 μm thick sections enable evaluation of intact nuclei and definitive interpretation
      - Poor cell preservation/morphology can impair interpretation

- Two different types of hybridization strategies (break apart, dual fusion) can be used to detect translocations (*see* Fig. 15.3)
  - *Break apart* assay design uses differentially (usually red/green) labeled probes that hybridize to the 5′ and 3′ side, respectively, of one of the breakpoints in a translocation, with sufficient proximity that in normal (non-translocated) alleles, the two probes overlap optically to give a fused (yellow) signal; however, in a translocation, the red and green signals are separated and detected separately
    - Break apart probe designs offer the greatest sensitivity for detection of rearrangements that consistently involve one chromosomal region (e.g., *EWSR1*), but which may have many different chromosomal variants; however, this design cannot identify the translocation partner, and therefore cannot distinguish between chromosomal variants

    - Most commercial FISH probe kits are for break apart probe designs
  - *Dual fusion* assay design uses differentially labeled (usually red/green) probes that each hybridize near one of the chromosomal breakpoints involved in a translocation, such that a fused (yellow) signal is seen when the translocation is present, but separate red and green signals are seen in normal (untranslocated) alleles
    - Dual fusion probe designs can distinguish between chromosomal variants of translocations, but require a separate assay for each variant to be tested
- No ISH assays can distinguish between molecular variants
- ISH can also be used to detect gene amplifications and deletions, but scoring systems may vary, and take on greater significance when FISH is performed on 4- or 5-μm sections because of sectioning through the nucleus
- Polymerase chain reaction (PCR) enables analysis of very small genetic changes, including point mutations, microdeletions/insertions, and molecular variants of translocations
  - Fresh or frozen tissue is optimal, but FFPE tissues work consistently for amplicons less than approximately 500 bp; larger amplicons will amplify inconsistently
  - Simple detection involves slab gel or capillary electrophoresis
    - Sensitivity may be enhanced by a variety of techniques, including real time probe detection, and Southern transfer of the PCR product followed by oligonucleotide probe hybridization
  - PCR may be used to prepare DNA for other detection strategies (e.g., direct sequencing, oligonucleotide hybridization, restriction digestion) or mutation screening approaches
  - DNA PCR is generally not used often for translocations because breakpoints are usually in introns, requiring a very large PCR product; reverse transcription-PCR (RT-PCR) is preferable because exon primers can flank breakpoints and give a small enough amplicon for successful analysis
  - "Real time" PCR can be used to quantitate gene dosage
  - Contamination of PCR reactions with PCR products from previous assays is a problem for clinical testing, and countermeasures include
    - *Ultraviolet irradiation* of consumables to damage carryover PCR products
    - *Physical separation* of assay setup, amplification, and detection
    - *Meticulous cleansing* of work benches with bleach
    - *Dedicated supplies and reagents* for PCR use
    - *One-step PCR protocols*, as opposed to nested protocols
    - *Spinning PCR tubes* briefly before opening
    - *Closed tube detection systems*, such as "real time" PCR
    - *Uracil-N-glycosylase* endonuclease, used with a dNTP mix containing dUTP in place of dTTP, to destroy carryover PCR product
- Direct Sanger dideoxyterminator sequence analysis (*see* Fig. 15.4) is considered the diagnostic "gold standard" for detection of point mutations and small deletions and insertions
  - Amplification is usually required first
  - Intratumor heterogeneity is highly problematic, and most automated sequence analyzers cannot distinguish a mutation from background noise if the mutant allele accounts for less than approximately 20% of the total DNA
  - Sensitivity may be extended considerably by mutant enrichment strategies, including silencing of wild-type alleles with peptide nucleic acid (PMA) or locked nucleic acid (LNA) probes, selective amplification of mutant sequences with lower denaturation temperatures in the PCR (COLD-PCR), or restriction digestion of wild-type sequence between stages of a nested reaction
  - Method of choice for genes with a very wide spectrum of mutations, or for which the mutation spectrum is not fully characterized

Fig. 15.4 *KIT* mutation detection in a gastrointestinal stromal tumor (GIST) by direct sequence analysis. An 18 bp deletion in exon 11 is shown, which results in apparent sequence noise due to superposition of mutant sequence bearing the deletion and wild-type sequence lacking the deletion. By sequencing in both directions, the precise boundaries of the deletion can be determined

  - "Next generation" sequencing technologies that involve massive-scale parallel sequencing of individual template strands hold great promise for multiplexed sequence analysis of specific targets or whole exomes/genomes from samples
    - Because of low throughput and high cost, these technologies are currently best suited for research applications, but are likely to become standard clinical instruments as speed increases and cost decreases
- Allele-specific amplification or hybridization enables fast and sensitive diagnosis of specific point mutations
  - The exact mutation must be known; novel mutations will be missed
  - Allele-specific hybridization assays range from simple paper dot blots to massively multiplexed silicon chip microarrays
    - Large-scale analysis of thousands of genes at once can be performed with microarrays to study patterns of gene expression or amplification/deletion, which may be used to classify a solid tumor

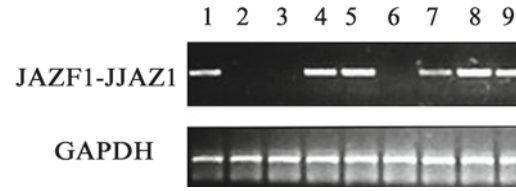

Fig. 15.5 RT-PCR for *JAZF1-SUZ12* (*JJAZ1*) fusion transcript in endometrial stromal sarcoma. *GAPDH* is used as a reference gene, to ensure adequate analysis of RNA from cases with no detectable *JAZF1-SUZ12* fusion transcript. Cases 1 through 9 show results from several patients with endometrial stromal tumors: cases 1, 4, 5, 7, 8, and 9 are positive, while cases 2, 3, and 6 are negative (photo adapted from a generous gift of Dr. Jeffrey Sklar, Yale University School of Medicine)

    - Currently, however, these techniques are most employed in research studies to discover a more limited number of candidate diagnostic markers for subsequent focused analysis
    - Comparative methods require careful and controversial selection of control samples
- RT-PCR (*see* Fig. 15.5) uses RNA to detect small molecular variations as with DNA PCR,

but principal differences include variable template expression and absence of introns
  - Distinction of molecular variants is one of the primary indications for RT-PCR
  - Fresh or frozen tissue is optimal; formalin-fixed tissues require a very small (e.g., <200 bp) RT-PCR product for most consistent results, but are often problematic regardless
  - RNA is very susceptible to degradation from ubiquitous RNases, and meticulous technique is required, as are prompt and thorough tissue preservation
  - RT-PCR is prone to contamination in the same way as is PCR, and the same precautions apply, with the addition of "no RT" controls
  - RT-PCR requires different primers for each chromosomal variant, and likely for different molecular variants also
  - "Real time" RT-PCR can be used to quantitate gene expression
- Southern blot hybridization enables detection of chromosomal rearrangements and molecular variants, as well as large intragenic deletions
  - Need sample frozen or fresh tissue
  - Technically challenging, slow, costly, usually radioactive; near obsolete
  - Can distinguish between molecular variants, potentially with a single assay design, but multiple probes and enzymes may be needed, depending on intron size and distance between breakpoints
  - Most useful for alterations spanning too great a distance for simple PCR, yet too small for FISH (e.g., large intragenic deletions)
- IHC is a surgical pathology technique that uses antibodies directed against proteins whose expression is an indication of underlying molecular pathology
  - Antibodies are applied directly to tumor sections on a glass slide, are detected with a chromogenic substrate, and enable rapid assessment of individual tumor cells by a trained pathologist
  - Two broad types of antibodies are used: polyclonal and monoclonal
    - Polyclonal antibodies are developed by inoculating an animal (most often a rabbit) with the target protein; the animal mounts an immune response and antibodies are purified from its serum
      - Typically, polyclonal antibodies have broad and variable specificity and strength, and vary considerably from one lot to another
    - Monoclonal antibodies are produced by fusing in vitro the splenocytes from an inoculated animal (typically mouse) with myeloma cells, forming "hybridoma" cells that are separated and grown individually in culture. The hybridoma cells secrete individual immunoglobulins, which can be harvested from culture
      - Typically, monoclonal antibodies are very specific to an individual antigenic epitope, and are very strong. They can be renewed in culture and, therefore, have less lot–lot variation
  - Most IHC antibodies are directed against a normal protein, and are used to detect abnormalities that change the amount of expression of either a normal protein or an abnormal fusion protein that has the normal antigenic epitope
  - Newer approaches at developing mutant protein-specific antibodies hold promise for distinguishing abnormal proteins from normal proteins. These are most useful in tumors with one or a limited number of molecular variants

Specific Sarcomas

Alveolar Rhabdomyosarcoma

- Basic pathology (*see* Fig. 15.6)
  - Malignant neoplasm of skeletal muscle, with characteristic histology
  - Nests of small, round, undifferentiated cells separated by thin fibrous septae
- Clinical features
  - Occurs primarily in 10–30-year-old patients
  - 20% of all rhabdomyosarcomas

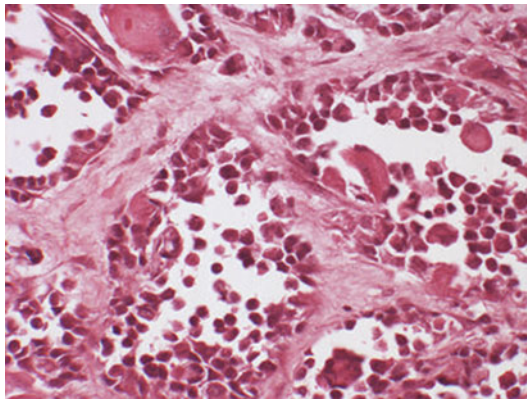

Fig. 15.6 Alveolar rhabdomyosarcoma, showing the characteristic pattern of tumor cell growth, with tumor cells adherent to the periphery of, and floating dyscohesively within, alveolar spaces separated by fibrous septae (slide courtesy of Dr. Christopher Fletcher, Brigham and Women's Hospital)

  - Presents most commonly in extremities and perineum
  - Prognosis is poor; worst of all rhabdomyosarcoma variants
  - Treatment is chemotherapy
- Molecular genetic pathology
  - t(2;13)(q35;q14) in approximately 70% of cases (*see* Fig. 15.2A)
    - *PAX3* gene (at 2q35) breakpoint in intron 7
    - *FOXO1* (FKHR) gene (at 13q14) breakpoint in intron 1
    - Fusion protein contains the N terminal DNA-binding domain of *PAX3* and the C terminal transcription activating domain of *FOXO1*, leading to oncogenesis through activation of growth and proliferation genes with *PAX3* binding sites, including *MITF* and *PDGFB*
  - t(1;13)(p36;q14) in approximately 10% of cases
    - *PAX7* gene (at 1p36) breakpoint in intron 7
    - *FOXO1A* gene (at 13q14) breakpoint in intron 1
  - Fusion gene organization and consequence are analogous to the *PAX3–FOXO1* fusion
    - *PAX7–FOXO1* is commonly amplified on double minute chromosomes
  - 20% of cases have neither t(2;13) nor t(1;13)
    - These cases represent a genetically heterogeneous subgroup, with some that have variant translocations involving related members of the *PAX* and/or *FOXO1* gene families (e.g., *PAX–NCOA1*, *PAX3–AFX*), and some that truly lack rearrangements or have other types of genetic abnormalities
- Molecular diagnostics
  - Test indications
    - Establish diagnosis
    - Prognosis: *PAX7–FOXO1* cases are associated with localized lesions and favorable prognosis
    - Minimal residual disease detection after therapy, bone marrow involvement
  - Additional technical considerations
    - FISH: commercial break apart probe is available for *FOXO1* but cannot distinguish between the two chromosomal variants, t(2;13) and t(1;13)(*PAX3–FOXO1* and *PAX7–FOXO1*)
    - RT-PCR: many different designs have been reported, including oligo dT/random/*FOXO1A*-specific primers for RT, one-step/two-step/nested amplification, and gel electrophoresis/Southern transfer probe hybridization/real time detection
      - Wild-type *FKHR* is constitutively expressed and can serve as control transcript
      - Breakpoints are restricted to intron 7 of *PAX3/PAX7* and intron 1 of *FOXO1*, but are variable within these large introns, precluding DNA analysis by PCR
      - Consensus *PAX* primers can be designed, enabling amplification of both chromosomal variants with one reaction
    - Southern blot: multiple probes/digests needed due to large introns (20 kb for *PAX* genes, 130 kb for *FKHR*) with varying breakpoints
  - Additional interpretive considerations
    - *PAX7–FOXO1A* is commonly amplified on double minute chromosomes in a subgroup of ARMS

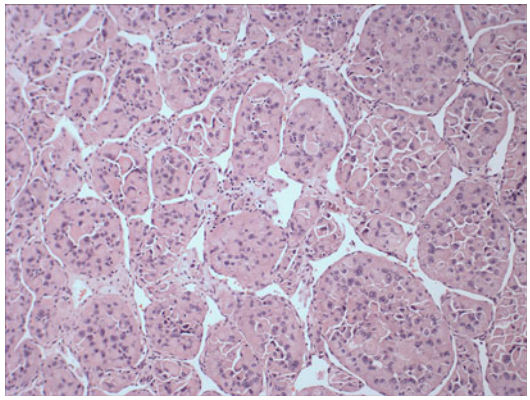

Fig. 15.7 Alveolar soft part sarcoma, showing the characteristic alveolar pattern of growth, with well-circumscribed round nests of uniform, eosinophilic, polygonal tumor cells. A reticulin stain would highlight the boundaries of the tumor cell nests, and a PAS stain would demonstrate cytoplasmic crystals

    - Fusion-negative cases have outcomes intermediate between those with *PAX7–FOXO1* and *PAX3–FOXO1*
    - Fusion transcript can be detected in samples in the absence of morphologic evidence of disease, suggesting a role in minimal residual disease testing

Alveolar Soft Part Sarcoma

- Basic pathology (*see* Fig. 15.7)
  - Mesenchymal tumor of uncertain histogenesis with distinctive morphology
    - Alveolar nests of tumor cells surrounded by reticulin framework
    - Uniform round cells with single nuclei
    - Periodic acid Schiff (PAS)-positive granular cytoplasmic crystals
    - Characteristic rectangular/rhomboid cytoplasmic crystals seen with electron microscope
- Clinical features
  - Often occurs in second or third decade, more frequently in females
  - Extremities (especially, thighs/buttocks) and orbit are most common sites, but also in sites with no skeletal muscle
  - Relatively indolent clinical course, with approximately 50% 10-year survival, but distant metastases are common and most patients die of the disease
  - Tumors are refractory to chemotherapy, and treatment is aggressive surgical excision
- Molecular genetic pathology
  - der(17)t(X;17)(p11.2;q25) in approximately 100% (*see* Fig. 15.2B)
    - *TFE3* gene (at Xp11.2) breakpoints in introns 1 and 2
    - *ASPSCR1* (*ASPL*) gene (at 17q25)
    - Fusion protein contains the N terminus of ASPSCR1 and the C terminus of TFE3, including TFE3 DNA-binding domain, under regulation of *ASPSCR1* promoter
- Molecular diagnostics
  - Test indications: establish diagnosis
  - Additional technical considerations
    - Karyotype: unbalanced translocation, der(17): the reciprocal translocation partner, der(X), is usually absent
    - FISH: no commercial probes are currently available
    - RT-PCR: not commonly performed, as significance of distinguishing molecular variants (intron 1 or intron 2 TFE3 breakpoints) is unclear
    - IHC: nuclear localization of TFE3 is sensitive and specific, and is the simplest and most widely employed diagnostic method
  - Additional interpretive considerations
    - A balanced translocation, t(X;17)(p11.2;q25), involving the same TFE3 and ASPSCR1 genes, is seen in a specific subset of renal adenocarcinomas, in pediatric and young adult patients

Angiomatoid Fibrous Histiocytoma

- Basic pathology
  - Tumors can resemble hemangioma, with nests of histiocyte-like cells, hemorrhagic spaces, and chronic inflammatory cells
  - Multiple nonendothelialized pseudovascular spaces with recent and old hemorrhage present

- Clinical features
  - Painless, slow growing, subcutaneous mass, primarily in the extremities
  - Typically affects children/teens
  - Symptoms may include fever, anemia, weight loss
  - Wide local excision is typically sufficient, as the tumor has a very low rate of metastasis
- Molecular genetic pathology
  - t(12;16)(q13;p11) associated with *ATF1–FUS* fusion
    - *FUS1* gene (at 16p11), exon 5
    - *ATF1* gene (at 12q13), exon 5
  - t(12;22)(q13;q12) associated with *EWSR1–ATFI* gene fusion
    - *EWSR1* gene (at 22q12), exon 7
    - *ATF1* gene (at 12q13), exon 5
  - t(2;22)(q34;q12) associated with *EWSR1–CREB1* gene fusion
    - *EWSR1* gene (at 22q12), exon 7
    - *CREB1* gene (at 2q34), exon 7
    - Although these fusions were not described initially in AFH, *EWSR1–CREB1* fusion may be the most common rearrangement in this tumor
  - Each EWSR1 fusion protein retains the bZIP domain mediating DNA-binding and dimerization of CREB1 or ATF1. The kinase inducible domain (KID), which is either excluded or truncated in different forms of EWSR1–ATF1, is not included in EWSR1–CREB1
- Molecular diagnostics
  - Test indications: establish diagnosis
  - Additional technical considerations
    - FISH: commercial break apart probe is available for *FUS* and *EWSR1*
      - FISH for *EWSR1* cannot distinguish between t(12;22); and t(2;22); clinical significance of this distinction is unclear
    - IHC:TFE3 but not MITF-M is overexpressed in the EWSR1–ATF1 positive tumors
  - Additional interpretive considerations
    - Both t(12;22) and t(2;22) have also been reported in clear cell sarcoma
    - The t(12;22) has been also reported in hyalinizing clear cell carcinoma of the salivary glands

Atypical Lipoma/Well-Differentiated Liposarcoma/Dedifferentiated Liposarcoma

- Basic pathology
  - Low grade neoplasm of adipose tissue with several morphologic subtypes, including lipoma-like (most common), sclerosing, and inflammatory
  - All have mature fat with variably-sized adipocytes and fibromyxoid stroma with spindle cells and focal cellular atypia
  - Lockhern cells have sharply outlined nuclear vacuoles
  - Mitoses and lipoblasts are uncommon
  - Dedifferentiated liposarcoma involves (typically) abrupt transition to high grade area with mitoses (>5 per 10 high-powered fields), nonlipogenic cells, and heterologous elements, including metaplastic bone
- Clinical features
  - Most common type of liposarcoma
  - Primarily affects adults, fourth to sixth decades
  - Typically involves lower limbs, retroperitoneum, paratesticular regions, mediastinum
  - May recur locally
    - Clear surgical margins are critical, especially for sclerosing type
    - Retroperitoneal liposarcomas are difficult to resect and often dedifferentiate
  - Dedifferentiation
    - ~10% of cases, more often from retroperitoneal or paratesticular sites
    - Confers aggressive behavior
      - Recurrence 40–80%
      - Metastasis 10–15%
      - Death 30–50%
- Molecular genetic pathology
  - Supernumerary ring and/or giant marker chromosome(s) in virtually all cases

    - Intratumor variability in size and number
    - Typically involve material from 12q13–q21
      - Oncogenes in these regions include *MDM2*, *CDK4*, *HMGA2*, *GLI*, *CHOP*
      - Material from other chromosomes also often involved
  - Coexisting other numerical/structural abnormalities in ~30% of cases
    - Mostly appear random, other than
      - Loss of 13q material
      - Telomeric associations involving 11p
  - A subset has gain of material at 12q15–q24 instead of rings/giant markers
    - Associated with minimal atypia
    - Distinct from 12q13–12q15 balanced translocations seen in simple lipoma
  - Similar rings and giant marker chromosomes are also seen in dedifferentiated liposarcoma, though a more complex karyotype is frequently observed
- Molecular diagnosis
  - Test indications: currently unclear
  - Technical considerations
    - Karyotype is typical method, but dedifferentiated liposarcoma areas grow in vitro very poorly
    - FISH: commercial break apart probe is available for *MDM2*
    - Rings/giant marker chromosomes are negative for centromeric probes

Clear Cell Sarcoma (Melanoma of Soft Parts)

- Basic pathology (*see* Fig. 15.8)
  - Mesenchymal neoplasm of uncertain histogenesis, with nests of tumor cells with melanocytic differentiation
  - Positive stain reactions for melanin, S100, HMB45, MITF, and melanosomes evident by electron microscopy
  - Separated by reticulin fibrous septae
  - Cells have uniform cytology, with clear or eosinophilic cytoplasm

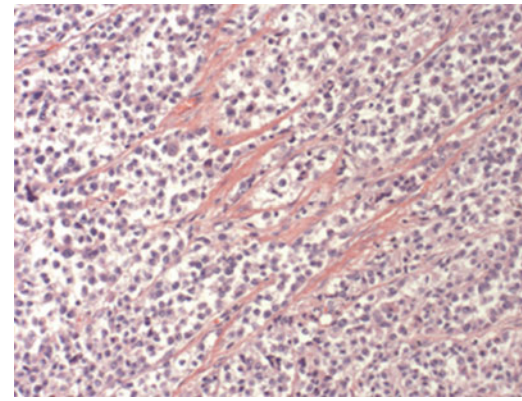

Fig. 15.8 Clear cell sarcoma (H&E stain), showing characteristic histologic appearance, with nests of tumor cells rimmed by thin fibrous septae. Tumor cells are polygonal with clear or eosinophilic cytoplasm and round-oval uniform nuclei with prominent nucleoli. Immunohistochemical stains for HMB-45 or S100 would demonstrate melanocytic differentiation

  - Melanomyctic markers may not be expressed in clear cell sarcomas that arise in the GI tract
- Clinical features
  - Mostly adolescents, young adults
  - Commonly involves extremities, especially foot and ankle from/near tendons, fascia, aponeuroses, but may arise from a wide range of anatomic sites, gastrointestinal (GI) included
  - Often painful
  - Progression is slow and gradual, but relentless, with a high propensity for regional or distant metastases
  - Little sensitivity to conventional multiagent chemotherapy, and treatment is usually radical resection
- Molecular genetic pathology
  - t(12;22)(q13;q12) in approximately 90% of cases (*see* Fig. 15.2C)
    - *EWSR1* gene (at 22q12) breakpoints in intron 7, 8, or 10
    - *ATF1* gene (at 12q13) breakpoints in intron 3 or 4
  - Molecular variants
    - Type 1: *EWSR1* exon 8—*ATF1* exon 4 (85%)
    - Type 2: *EWSR1* exon 10—*ATF1* exon 5
    - Type 3: *EWSR1* exon 7—*ATF1* exon 5

  - Fusion gene has N terminal transactivating domain of EWS and C terminal leucine zipper dimerization and DNA-binding domains of ATF1, under control of ubiquitously expressed *EWS* promoter, causing oncogenesis by increased activation of genes bearing *ATF1* sites
  - *EWSR1–ATF1* soft tissue clear cell sarcomas show consistent melanocytic differentiation, as well as expression of MITF-M transcript
  - t(2;22)(q34;q12) exclusively in tumors of GI tract
    - *EWSR1* gene (at 22q12)
    - *CREB1* gene (at 2q34)
    - *EWSR1–CREB1* tumors lack melanocytic markers
- Molecular diagnostics
  - Test indications: establish diagnosis
  - Additional technical considerations
    - FISH: a commercial break apart probe is available for *EWSR1*, but cannot distinguish between different molecular variants of *EWSR1–ATF1*, or the chromosomal variant, t(2;22)
    - RT-PCR: can distinguish between different molecular variants of *EWSR1–ATF1*, but clinical significance of this distinction is unclear
  - Additional interpretive considerations
    - GI tract clear cell sarcomas with either *EWSR1–ATF1* or *EWSR1–CREB1* lack melanocytic markers, in contrast to the *EWSR1–ATF1* soft tissue clear cell sarcoma
    - Both t(12;22) and t(2;22) have also been reported in angiomatoid fibrous histiocytoma
    - The t(12;22) has been also reported in hyalinizing clear cell carcinoma of the salivary gland

Dermatofibrosarcoma Protuberans

- Basic pathology (*see* Fig. 15.9)
  - Uncommon neoplasm of low to intermediate malignant potential

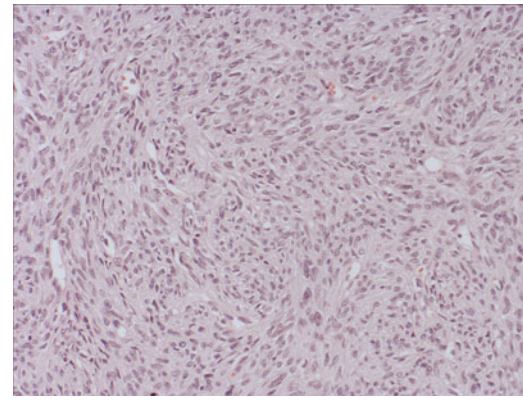

Fig. 15.9 Dermatofibrosarcoma protuberans (H&E stain). The tumor consists of a continuous sheet of spindled cells arranged in tight storiform whorls

  - Composed of noncircumscribed nodular lesions of S100−/CD34+ spindle cells arranged in tight storiform whorls
  - Involving dermis and subcutaneous zones of skin, usually with a thin zone of dermis between the tumor and epidermis
  - Pigmented (Bednar tumor) and myxoid variants exist
- Clinical features
  - Most commonly occurs in ages 30–50 years
  - Trunk and proximal extremities are most common sites
  - Initially grows slowly, with a later phase of rapid growth
  - Frequently recurs locally (50%), even after wide resection (12%), and rarely metastasizes (1–4%)
  - Wide surgical resection is usual treatment
    - A number of clinical studies have shown a high response rate to imatinib therapy in both locally advanced and metastatic lesions
- Molecular genetic pathology
  - Supernumerary ring chromosome, derived from a t(17;22)(q22;q13) in most cases
    - *COL1A1* gene (at 17q21.31–q22) has many different breakpoints, exons 29 and 32 being slightly more frequently involved
    - *PDGFB* gene (at 22q13) breakpoint is in intron 1

    - Fusion gene includes nearly the entire *PDGFB* sequence fused to a variable length of N terminal *COL1A1* sequence, under control of the *COL1A1* promoter, leading to oncogenesis by constitutive activation of the *PDGFB* growth signaling
    - The frequency of unbalanced cytogenetic abnormalities suggests a dosage effect or a low level of amplified expression of *PDGFB*
- Molecular diagnostics
  - Test indications: establish diagnosis
  - Additional technical considerations
    - Karyotype: rare variant translocations have been reported
    - FISH: no commercial probes are available, but whole-painted chromosome probes for chromosomes 17 and 22 are useful to identify the presence of chromosomal material of both chromosomes in ring/marker chromosomes
    - RT-PCR: multiple breakpoints in *COL1A1* can complicate analysis; most protocols involve nested RT-PCR, with multiple primers spaced throughout *COL1A1*
  - Additional interpretive considerations
    - The same *COL1A1–PDGFB* fusion has been reported in giant cell fibroblastoma of childhood, but in linear t(17;22), not supernumerary ring chromosomes

Desmoplastic Round Cell Tumor

- Basic pathology (*see* Fig. 15.10)
  - Aggressive, poorly differentiated tumor with characteristic histology
  - Infiltrating nests of small round cells with prominent desmoplasia
  - Evidence of multilineage differentiation including immunoreactivity for keratin, desmin, and neuron-specific enolase
  - Characteristically involves the peritoneum
- Clinical features
    - Mostly in children (boys) and young adults

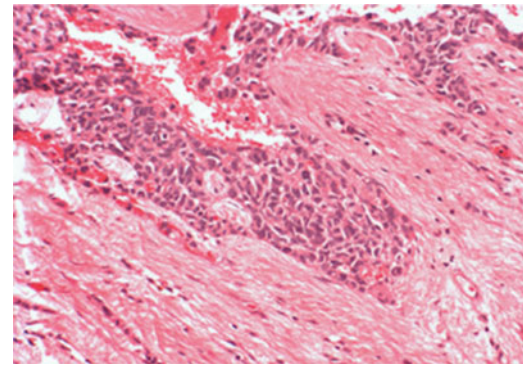

Fig. 15.10 Desmoplastic small round cell tumor (H&E stain). The tumor consists of sheets of small tumor cells with little cytoplasm, growing within dense desmoplastic stroma

    - Located almost exclusively on the peritoneal surfaces of the abdomen
    - Very aggressive tumor, with a very poor prognosis: 35% overall progression-free survival at 5 years
    - Treatment is surgery, followed by intensive chemotherapy and radiotherapy
- Molecular genetic pathology
  - t(11;22)(p13;q12) in approximately all cases (*see* Fig. 15.2D)
    - *EWSR1* gene (at 22q12) breakpoint usually in intron 7, but also 8 and 9
    - *WT1* gene (at 11p13) breakpoints in intron 7
    - Fusion protein includes N terminal transactivation domain of EWSR1 and the C terminal zinc finger DNA-binding domain of WT1, under control of the *EWSR1* promoter, leading to oncogenesis through overexpression and increased activation of WT1 DNA-binding domain
    - Molecular variants
      - *EWSR1* exon 7—*WT1* exon 8
      - *EWSR1* exon 8—*WT1* exon 8
      - *EWR1S* exon 9—*WT1* exon 8
- Molecular diagnostics
  - Test indications
    - Establish diagnosis, as differential diagnosis of small, round blue cell tumors of childhood is broad, and requires ancillary testing to narrow

  - Additional technical considerations
    - IHC: antibodies to the carboxy terminus of WT1 show overexpression in these tumors, while antibodies to the amino terminus of WT1 show absence of expression; IHC is sensitive, specific, and simple, and is the most commonly employed diagnostic method
    - FISH: commercial break apart probe is available for *EWSR1*, but cannot distinguish desmoplastic round cell tumor (*EWSR1–WT1*) from extraosseous Ewing sarcoma (*EWSR1–FLI1* and other *EWSR1* fusions), which is often another diagnostic consideration in these patients
    - RT-PCR: can distinguish molecular variants, but this distinction is currently of unknown clinical significance
    - Southern blot: enabled by the short length of *WT1* intron 7, which contains the breakpoints
  - Additional interpretive considerations: rare chromosomal variants must be further investigated by FISH, RT-PCR, and IHC

Endometrial Stromal Sarcomas

- Basic pathology (*see* Fig. 15.11)
  - Uncommon tumor of endometrial stroma, with benign, low grade and high grade variants
  - Grade is based primarily upon extent of infiltration of adjacent myometrium, cytologic pleomorphism, and mitotic activity
  - Cells resemble proliferative phase endometrial stroma, but displace uninvolved benign glandular elements
- Clinical features
  - Primarily affects middle-aged women
  - Patients present with vaginal bleeding, pelvic pain
  - Prognosis is grade-dependent
    - Benign stromal nodules are cured surgically
    - Low grade endometrial stromal sarcomas can recur after surgery (20%), often many years later, and rarely (10%) metastasize
    - High grade endometrial stromal sarcomas are aggressive, with frequent recurrence and metastasis

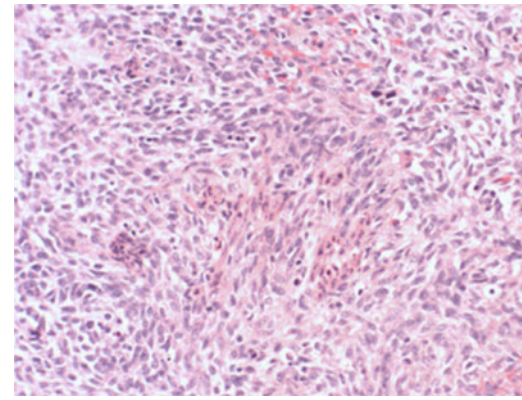

Fig. 15.11 Endometrial stromal sarcoma. The tumor consists of a proliferation of bland, small round cells with scant cytoplasm and smooth chromatin, resembling endometrial stroma

- Molecular genetic pathology
  - t(7;17)(p15;q21) in approximately 50–60% of cases (*see* Fig. 15.2E)
    - *JAZF1* gene (at 7p15)
    - *SUZ12* (*JJAZ1*) gene (at 17q21)
    - Fusion protein contains nearly all of SUZ12, a polycomb group transcriptional repressor, fused to the amino terminal region of JAZF1, under control of the *JAZF1* promoter. The mechanism by which this gene fusion induces neoplasia is still being actively investigated
  - t(6;7)(p21;p15) in approximately 20% of cases
    - *JAZF1* (at 7p15)
    - *PHF1* gene (at 6p21)
    - *PHF1*, like *SUZ12*, is homologous to a *Drosophila* zinc finger Polycomb gene
  - t(10;17)(q22;p13)
    - *FAM22A/B* genes (at 10q23.2 and 10q22.3)
    - *YWHAE* gene (at 17p13)
    - The 14–3–3 oncoprotein results from a t(10;17) genomic rearrangement, leading to fusion between 14–3–3ε (YWHAE) and either of two nearly

identical FAM22 family members (FAM22A or FAM22B)
    - The rearrangement results in an inframe fusion between *YWHAE* (exon 1–5) and one of the two highly homologous genes (*FAM22A* or *FAM22B*, exon 2–7)
- Molecular diagnostics (see Fig. 15.5)
  - Test indications
    - Establish diagnosis
  - Additional technical considerations
    - Karyotype: single cases with other chromosome abnormalities have been reported
    - FISH: no commercial probes are available for t(7;17) and t(10;17)
  - Additional interpretive considerations
    - The t(7p15)/*JAZF1* positive tumors appear to be more common in low grade endometrial stromal sarcomas of classic histology, but has been reported in cases with high grade histology, and even in occasional mixed tumors (adenosarcoma, carcinosarcoma)
    - The t(10;17) positive tumors appear to be histologically higher grade and clinically more aggressive than *JAZF1*-rearranged tumors. These tumors display high grade (but nonpleomorphic) round cell histology that is immunophenotypically undifferentiated and frequently includes an admixed low grade spindle cell component with fibrocollagenous/fibromyxoid stroma that is positive for ER, PR, and CD10 immunohistochemically
    - YWHAE rearrangement and JAZF1 rearrangement are mutually exclusive
    - The (10;17)(q22;p13) induced *YWHAE–FAM22* genetic fusion seen in endometrial stromal sarcoma is the identical recurrent translocation reported in clear cell sarcoma of the kidney

Epithelioid Hemangioendothelioma

- Basic pathology
  - Proliferation of round (epithelioid) cells that typically form cord-like structures embedded within an edematous, proteoglycan-rich, extracellular matrix
  - No specific biomarkers distinguish this tumor from other vascular lesions
- Clinical features
  - Wide age range
  - Affects both genders equally
  - Arises in soft tissue and bone, as well as visceral organs, especially liver and lungs
  - Two prognostic categories, classic and malignant, stratified by mitotic activity and size
  - Treatment is by surgical resection, although multifocal visceral disease may be treated with transplantation
- Molecular genetic pathology
  - t(1;3)(p36.3;q25) in ~85% of cases
    - *CAMTA1* gene (at 1p36.3) breakpoints in exon 8 or 9
    - *WWTR1* gene (at 3q25) breakpoint in exon 2, 3, or 4
    - Fusion protein contains the amino terminus of WWTR1 and the carboxy terminus of CAMTA1, under the transcriptional control of the *WWRT1* promoter
    - Molecular variants
      - Type 1: *WWTR1* exon 4—*CAMTA1* exon 8
      - Type 2: *WWTR1* exon 4—*CAMTA1* exon 9
      - Type 3: *WWTR1* exon 2—*CAMTA1* exon 9
      - Type 4: *WWTR1* exon 3—*CAMTA1* exon 9
      - Molecular diagnostics
  - Indications for molecular genetic testing: diagnosis
    - This gene fusion has not been detected in any of the morphological mimics of epithelioid hemangioendothelioma, such as hemangioendothelioma, epithelioid angiosarcoma, or epithelioid sarcoma-like hemangioendothelioma
  - Additional technical considerations
    - FISH: no commercial probes are available

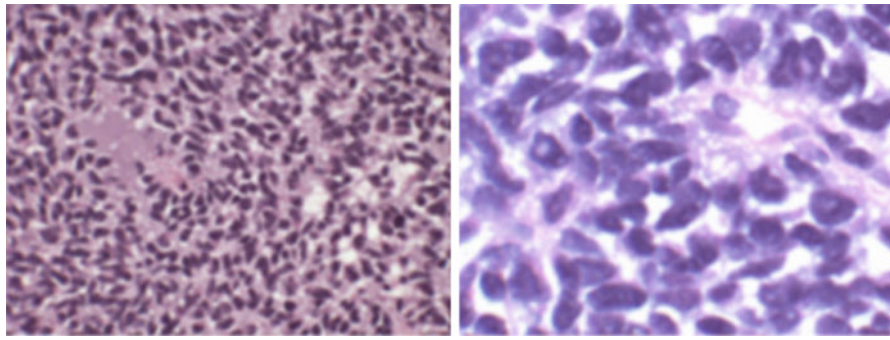

Fig. 15.12 Primitive neuroectodermal tumor. This member of the Ewing sarcoma family is an extraosseous tumor composed of sheets of small, uniform, round cells with modest eosinophilic cytoplasm, and occasional cytoplasmic glycogen vacuoles. Focal rosettes may be present

Ewing Sarcoma

- Basic pathology (*see* Fig. 15.12)
  - Small round blue cell tumor
  - Initially described in bone, but may also involve extraosseous sites
  - Tumors often have large areas of necrosis
  - The cells are small, uniform, and round
  - PAS-positive glycogen granules
  - Immunoreactivity for CD99/O13 antigen (*MIC2* gene product)
- Clinical features
  - Most common in children (5–20) and young adults (<30)
  - Usually presents with pain or swelling, but can also present with systemic symptoms
  - Usually involves diaphysis of long bones, originating in medullary cavity and eventually penetrating through cortex to soft tissues
  - Characteristic "onion skin" appearance of cortex on X-rays as tumor lifts periosteum and new bone is laid down
  - Frequently metastasizes
  - Controversy regarding extraosseous lesions, and whether they represent true Ewing sarcoma
    - Peripheral neuroepithelioma also called primitive neuroectodermal tumor, esthesioneuroblastoma (olfactory epithelium), and Askin tumor (chest wall)
  - Poor prognosis when treated with surgery and radiotherapy (5-year survival 5–8%), but multiagent chemotherapy has increased 5-year survival to approximately 75%
    - Molecular genetic pathology
  - t(11;22)(q24;q12) in 90–95% (*see* Fig. 15.2F)
    - *EWSR1* gene (at 22q12) has multiple breakpoints
    - *FLI1* gene (at 11q24) has multiple breakpoints
    - Fusion protein contains carboxy terminal DNA-binding transcriptional activation domain of FLI1 and the amino terminal transactivation domain of EWSR1, under the regulatory control of the ubiquitously expressed *EWSR1* promoter. This leads to oncogenesis through upregulation of genes with FLI1 sites
    - Molecular variants (*see* Fig. 15.13)
      - Type I: *EWSR1* exon 7—*FLI1* exon 6 (~60%)
      - Type II: *EWSR1* exon 7—*FLI1* exon 5 (~20%)
      - Many other variants, but the breakpoint is always downstream of *EWSR1* exon 7 and upstream of *FLI1* exon 9
  - Chromosomal variants are seen in 5–10% of cases, all involving *EWSR1* with different partner genes
    - t(21;22)(q12;q12) with *EWSR1–ERG* fusion

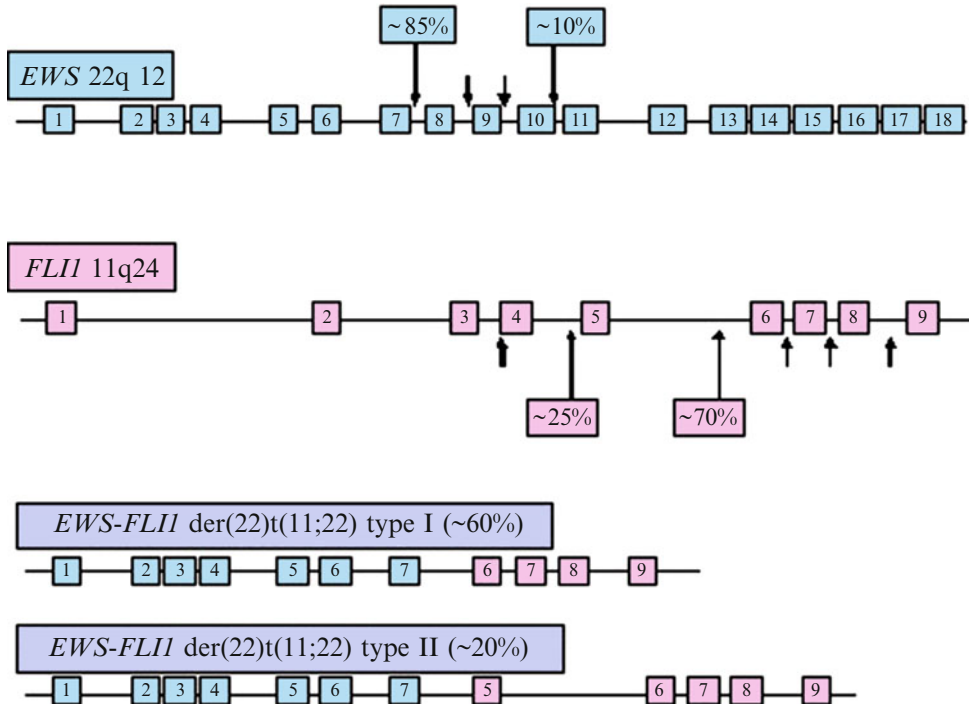

Fig. 15.13 Structure of the *EWSR1* and *FLI1* genes, and the most common *EWSR1-FLI1* fusions seen in Ewing sarcoma. The *upper figure* shows the structure of the *EWSR1* gene, with the most prevalent translocation breakpoints indicated by *long arrows* (intron 7 and intron 10), and less prevalent breakpoints indicated by *short arrows*. The *next figure* shows the *FLI1* gene in the same manner, with the most common breakpoints in intron 5 and intron 4. At the *bottom* are the two most common fusion types, type I (*EWSR1* intron 7—*FLI1* intron 5) and type II (EWSR1 intron 7—*FLI1* intron 4). Note that the figures are not drawn to scale

- t(7;22)(p22;q12) with *EWSR1–ETV1* fusion
- t(17;22)(q12;q12) with *EWSR1–E1AF* fusion
- t(2;22)(q33;q12) with *EWSR1–FEV* fusion
- inv(22)(q12q12) with *EWSR1–ZSG* fusion
  - These *EWSR1* gene fusions contain the N terminal portion of EWSR1 and the C terminal DNA-binding domain of an ETS transcriptional family member (e.g., ERG, ETV1, E1AF, FEV) and are, thus, analogous to the canonical *EWSR1–FLI1* fusions
- t(20;22)(q13;q12) with *EWSR1–NFATC2* fusion
  - The *NFATC2* gene involved in the t(20;22), is a transcription factor that is not a member of the ETS family. The inframe fusion gene contain the C terminal of EWSR1, encoded by the first 8 exons, and the N terminal of NFATc2, encoded by exons 3–10
- t(16;21)(p11;q22) with a *FUS–ERG* fusion
  - The FUS gene, involved in the t(16;21), belongs to the TET family of RNA-binding proteins, and shows considerable homology with *EWSR1*
  - *FUS* is the most frequent gene replacing *EWSR1* in other sarcoma translocations
  - A variant t(2;16) (q35;p11) associated with a *FUS–FEV* fusion gene has been recently reported, suggesting that the *FUS* rearrangement could be underreported

- Molecular diagnostics
  - Test indications
    - Establish a diagnosis: the differential diagnosis of small round blue cell tumors is broad and requires ancillary methods. This is especially useful when the tumor presents in an unusual site
    - Recent prospective randomized multi-institute trials described the absence of prognostic importance of type 1 EWS–FLI1 fusion
    - Minimal disease monitoring: RT-PCR may be used for staging patients for bone marrow involvement in the absence of radiologic or morphologic evidence. Moreover, some studies have looked at detecting circulating tumor cells with RT-PCR
  - Additional technical considerations
    - FISH: a commercial break apart probe is available for *EWSR1* and can detect all molecular variants, but cannot distinguish among chromosomal variants, and cannot distinguish Ewing sarcoma from other sarcomas with *EWSR1* translocation (e.g., clear cell sarcoma, intraabdominal desmoplastic round cell tumor, extraskeletal myxoid chondrosarcoma, myxoid liposarcoma)
  - RT-PCR: variability of breakpoints and diversity of molecular variants provides an assay design challenge
    - A single primer set to *EWSR1* exon 7 and *FLI1* exon 6 will detect approximately 80% of cases and enable size-based distinction of the type I and type II transcripts, but will not detect fusion transcripts with more distal *FLI1* breakpoints or, potentially, large fusion transcripts with more distal *EWSR1* breakpoints
    - A single primer set to *EWSR1* exon 7 and *FLI1* exon 9 could detect all fusion types, but transcripts with distal *EWSR1* breakpoints and/or proximal *FLI1* breakpoints may be too large for reliable detection by RT-PCR
    - Chromosomal variants may also be detected due to conservation of *EWSR1* breakpoints and high homology between genes partnered with *EWSR1*: consensus primers have been designed that anneal to *FLI1/ERG/FEV* and to *TEV1/E1AF*
    - Southern blot: *EWSR1* breakpoints occur over a relatively small area (~7 kb), enabling Southern blot detection of all fusion genes, but *Alu* repeat polymorphism in intron 6 in African Americans can complicate the analysis
  - Additional interpretive considerations
    - The same translocations are seen in primary osseous and in extraosseous Ewing sarcomas, but are characteristically absent in olfactory neuroblastomas (esthesioneuroblastomas), suggesting that these tumors may be unrelated to Ewing sarcomas
    - Therefore, we need to be aware of these cytogenetic variant Ewing sarcomas in order to modify their detection in clinical practice. A negative results generated by RT-PCR using specific fusion transcript or FISH for *EWSR1*, should no preclude the diagnosis of Ewing sarcoma in the contest of typical morphology and immunophenotype features. Moreover, cytogenetic analysis is still a gold standard to detect these rare translocations
    - A similar t(16;21) associated with *FUS–ERG* has been previously reported in a subgroup of AML

Gastrointestinal Stromal Tumor

- Basic pathology
  - Mesenchymal tumor of GI viscera
  - Composed most often of pure spindle cells, but epithelioid and mixed variants occur
  - Tumors are now believed to arise from the interstitial cells of Cajal present within the gut wall
  - Contain features of smooth muscle and neural tissues
- Clinical features
  - Most gastrointestinal stromal tumors (GISTs) arise in the stomach (60%) and

small intestine (25%), but they can occur anywhere in the GI tract as well as in omentum, retroperitoneum, and mesentery
  - GIST patients vary widely in age, but peak around 60 years
  - Prognosis is largely dependent on size and mitotic activity; patients with gastric GISTs tend to fare better than those with intestinal tumors
  - Surgery is the primary treatment, but recurrence and dissemination are inevitable for high risk lesions (>5 cm, >5 mitoses/50 high power fields). Some GISTs have been treated successfully with imatinib (Gleevec)
- Molecular genetic pathology
  - Unlike the other sarcomas in this chapter, GISTs are not characterized by a chromosomal pathology (i.e., translocations, inversions, etc.), but rather by molecular genetic (i.e., point mutations, insertions, deletions) pathology
  - *KIT* gene (at 4q12) is mutated in 80–85% of cases
    - Activating mutations include small inframe deletions and insertions, and point mutations
    - Most mutations are in exons 11 and 9, but mutations in exons 13 and 17 have also been described
  - A subset of GISTs has mutations in the *KIT*-related *PDGF* receptor-α (*PDGFRA*) gene (also at 4q12), in exons 18 and 12
- Molecular diagnostics
  - Test indications
    - Establish diagnosis
    - Prognosis: exon 9 mutations are associated with malignant GISTs
    - Theranosis: GISTs with exon 11 mutations are most likely to respond to imatinib (Gleevec)
    - Acquired mutations, especially in exons 13 and 17, may confer resistance to imatinib
  - Additional technical considerations
    - IHC is a simple, sensitive, and specific means of assessing overexpression of KIT (CD117), but variability between commercial antibody preparations and between different laboratory protocols has led to some inconsistency in published results, particularly with regard to specificity
      - IHC cannot distinguish between mutations that have different implications for therapy response, and is most useful as a screening tool to select cases for molecular analysis
    - PCR: DHPLC and high resolution melt curve analysis are quick and sensitive screening methods for detection of both deletions and point mutations that is free of some of the inconsistencies that affect IHC
    - Sanger sequencing is typically used for specific identification of each mutation detected by a screening method
  - Additional interpretive considerations
    - GISTs with *PDGFRA* mutations are negative by IHC, as are some GISTs with *KIT* mutations and others with acquired imatinib resistance

Infantile Fibrosarcoma

- Basic pathology
  - Spindle cell neoplasm of early childhood
  - Bundles of interdigitating cells that are immunoreactive for vimentin, but not smooth muscle, desmin, or S100
- Clinical features
  - One of the more common soft tissue sarcomas of childhood
  - Soft tissue mass, usually noted at, or soon after, birth
  - 70% in extremities, followed by head/neck, trunk
  - Excellent prognosis
  - Treatment is complete surgical excision
  - Sensitive to chemotherapy
- Molecular genetic pathology
  - t(12;15)(p13;q26) in approximately 95%
  - *ETV6* gene (at 12p13)
  - *NTRK3* gene (at 15q26)
  - Fusion protein contains the N terminal helix-loop-helix protein dimerization

domain of *ETV6* and the C terminal tyrosine kinase domain of *NTRK3*, presumably leading to oncogenesis through increased activation of *NTRK3* kinase and downstream signal transduction; the fusion protein has oncogenic in vitro activity

- Molecular diagnostics
  - Test indications
    - Establish diagnosis
    - Therapy selection: other spindle cell lesions of childhood may lack sensitivity to chemotherapy
  - Additional technical considerations
    - Karyotype: the t(12;15) is cryptic, and difficult to detect by standard GTG-banding
      - Polysomies of chromosomes 8, 11, 17, and/or 20 are common
    - FISH: a commercial break apart probe is available for *ETV6*
  - Additional interpretive considerations
    - The same t(12;15) has been reported in congenital mesoblastic nephroma
    - A renal lesion with similar histopathology and clinical features
    - In secretory carcinomas of the breast and of the salivary glands

Inflammatory Myofibroblastic Tumors

- Basic pathology
  - Proliferation of myofibroblastic spindle cells, usually with a prominent mixed inflammatory component
  - Lesion has many pseudonyms, reflective of its controversial nature
    - Inflammatory pseudotumor
    - Plasma cell granuloma
    - Pseudosarcomatous myofibroblastic proliferation
    - Postoperative spindle cell nodule
    - Atypical fibromyxoid tumor
  - Epithelioid inflammatory myofibroblastic sarcoma is a distinctive variant tumor
    - Epithelioid and round cell morphology
    - Nuclear membrane or perinuclear ALK immunostaining
    - Arising in intraabdominal locations
    - Aggressive clinical course and predilection for male patients
- Clinical features
  - Most often in children and young adults
  - Can involve both soft tissues and viscera
  - Patients often present with systemic symptoms (fever, weight loss) and anemia
  - Usually indolent course, especially in the lung; more aggressive course in the abdomen (e.g., epithelioid inflammatory myofibroblastic sarcoma)
  - Tumor-related mortality, approximately 10%, usually due to local destruction rather than metastasis
  - Aggressive behavior, with metastasis, has been described, often associated with morphologic change (round cell transformation)
  - Usual management is surgical excision
- Molecular genetic pathology
  - 2p23 rearrangement in approximately 50% of cases
    - *ALK* gene (at 2p23) is invariably fused by chromosomal translocation to a wide variety of partner genes
      - t(1;2)(q25;p23) with *TPM3–ALK* fusion
      - t(2;19)(p23;p13.1) with *TPM4–ALK* fusion
      - t(2;17)(p23;q23) with *CLTC–ALK* fusion
      - t(2;2)(p23;q13) with *RANBP2–ALK* fusion
      - inv(2)(p12q35) with *ATIC–ALK* fusion
      - t(2;11)(p23;p15) with *CARS–ALK* fusion
      - t(2;4)(p23;q21) with *SEC31L1–ALK* fusion
  - *ALK* gene encodes a membrane-associated protein with a cytoplasmic tyrosine kinase domain, and fusion proteins are believed to undergo homodimerization, which is predicted to trigger stimulus-independent activation of *ALK* tyrosine kinase domain
- Molecular diagnostics
  - Indications for molecular genetic testing

    - Establish a definitive diagnosis: the differential diagnosis is very broad, and ranges from self-limited benign reactive proliferations to aggressive malignancies
  - Additional technical considerations
    - Karyotype: 50% of these tumors do not have an *ALK* rearrangement
    - FISH: a commercial break apart probe is available for *ALK*
    - RT-PCR is very difficult, given the wide range of possible partner genes
    - IHC: staining for *ALK* is simplest, quickest, and most sensitive methodology
      - Diffuse cytoplasmic staining when the fusion involves the cytoplasmic proteins TMP3, TPM4, CARS, ATIC, and SEC31L1
      - Granular cytoplasmic staining with CLTC and nuclear membrane staining with RANBP2
      - A correlation between the nuclear membrane pattern and *RANBP2–ALK* fusion seems to be consistent in epithelioid inflammatory myofibroblastic sarcoma
  - Additional interpretive consideration
    - *ALK* rearrangements are also seen in anaplastic large cell lymphoma and in ~5% of lung adenocarcinomas, where they have been shown to confer a favorable response to treatment with crizotinib, a tyrosine kinase inhibitor
    - In 2011, the U.S. Food and Drug Administration approved a commercial ALK split apart FISH assay (Abbott Molecular) to be used to select lung cancer patients for treatment with crizotinib (Xalkori™, Pfizer)

Low Grade Fibromyxoid Sarcoma

- Basic pathology
  - Rare soft tissue neoplasm of low malignant potential with uncertain histogenesis
  - Contains a mixture of hypocellular areas with collagenous stroma and more cellular areas with myxoid stroma
  - Focal collagen rosettes are seen in a subset of cases
- Clinical features
  - Incidence is presumed to be low, but it is likely that these lesions have been underrecognized
  - Painless mass, typically in the lower extremities, especially thigh in young male adults
  - Treatment is wide local resection
  - Local recurrences in approximately 10%, and metastasis is seen in 5%
- Molecular genetic pathology
  - t(7;16)(q33–34;p11) in 95% (*see* Fig. 15.2G)
    - *FUS* gene (at 16p11) with breakpoints in exon 5, 6, and 7
    - *CREB3L2* (*BBF2H7*) gene (at 7q33–34) with breakpoints in exon 5 and 6
    - Rarely, ring chromosome assumed derived from chromosomes 7 and 16 has been also reported
    - Fusion protein contains carboxy terminal portion of CREB3L2, including B-ZIP DNA-binding domain and amino terminus of FUS, containing transactivation domain, under regulation of ubiquitously expressed *FUS* promoter. Mechanism of oncogenesis is likely through dysregulation of CREB3L2 transcriptional targets
    - Molecular variants are related to the different *FUS* and *CREB3L2* breakpoints
  - Chromosomal variant; t(11;16)(p11;p11) in 5% of the cases
    - *FUS* gene (at 16p11) with breakpoint in exon 5
    - *CREB3L1* gene at (11p11) with breakpoint in exon 6
- Molecular diagnostics
  - Indications for molecular genetic testing
    - Establish a definitive diagnosis
  - Additional technical considerations
    - Karyotype: t(7;16) is cryptic and can easily be missed by G-banded chromosome analysis
    - FISH (*see* Fig. 15.3): a commercial break apart probe is available for *FUS*
    - RT-PCR: relative proximity of breakpoints facilitates RT-PCR analysis

- Additional interpretive consideration
  - The t(7;16)/*FUS–CREB3L2* has been reported in hyalinizing spindle cell tumors with giant rosettes (so-called unusual variant of low grade fibromyxoid sarcoma) and sclerosing epithelioid fibrosarcoma

Mesenchymal Chondrosarcoma

- Basic pathology
  - Biphasic sarcoma, with small round/slightly spindled cells and islands of chondroid matrix
  - Differential diagnosis can be challenging in samples with minimal chondroid matrix, and includes other "small round blue cell tumors"
- Clinical features
  - Affects young patients, between 10 and 20 of age
  - Late distal recurrences
  - Poor outcome
- Molecular genetic pathology
  - *HEY1–NCOA2* fusion by genome-wide screen of expression data
    - *HEY1* gene (at 8q21.1) with breakpoint in exon 4
    - *NCOA2* gene (at 8q13.3)/with breakpoint in exon 13
    - The *HEY1–NCOA2* fusion replaces the C terminal portion of the HEY1 by the NOAC2 AD1/CIS and AD2 domain, while retaining the Hey1 bHLH DNA-binding/dimerizations domain
    - These genes are only ~10 Mb apart, and this fusion can be the results of a cryptic interstitial deletion or paracentric inversion between the 8q13.3 and 8q21.1 region
- Molecular diagnostics
  - Indications for molecular genetic testing
    - Diagnosis
      - The noncartilage components of this sarcoma are often predominant, and such a lesion can be confused with other small cell neoplasms
      - The 8q rearrangement is cryptic and can easily be missed by G-banded chromosome analysis
      - FISH: no commercial probes are available

Myoepithelioma, Soft Tissue

- Basic pathology
  - Myoepithelial tumors represent a family of lesions with variable terminology, based on anatomical location
    - Pleomorphic adenoma of salivary glands
    - Benign mixed tumor in the skin, and myoepithelial tumor/parachordoma in the soft tissue
  - Often have uniform rounded cell morphology and clear cytoplasm in deep-seated soft tissue
  - Criteria for confirming the diagnosis include coreactivity for EMA ± cytokeratin AE1/AE3 and S100 ± GFAP
- Clinical features
  - >50% of cases occur in children or young adults
  - Most common in the extremities, followed by head and neck
- Molecular genetic pathology
  - *EWSR1*(22q12) rearrangement in approximately 50% of cases
    - t(6;22)(p21;q12)
      - *EWSR1* gene (at 22q12)
      - *POUF5F1* gene (at 6p21)
      - This translocation is identified in a subset of deep-seated tumors of extremities, in children or young adults with distinct clear cell morphology
    - t(1;22)(q23;q12)
      - *EWSR1* gene (at 22q12)
      - *PBX1* gene (at 1q23)
      - This translocation is identified in a subset of tumors with a deceptively bland appearance, composed mainly of spindle cells embedded in a fibrotic stroma, resembling, in areas, desmoid-type fibromatosis

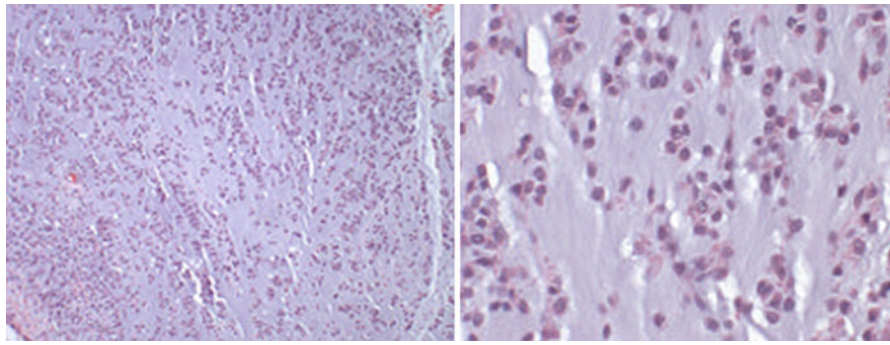

Fig. 15.14 Extraskeletal myxoid chondrosarcoma. Low-power image on the *left* shows ribbons and cords of tumor cells in a myxoid stroma. High-power image on the *right* shows cytologic features of the cancer cells, including small, dark nuclei vacuolated cytoplasm, and bubbly myxoid stroma

    - t(19;22)(q13;q12)
    - *EWSR1* gene (at 22q12)
    - *ZNF444* gene (at 19q13)
    - Very rare translocation, less 2% of the cases
  - EWSR1 negative myoepithelial tumors are more often benign, superficially located, and show ductal differentiation, suggesting the possibility of a distinct subgroup
- Molecular diagnostics
  - Indications for molecular genetic testing
    - *EWSR1* rearrangement is a common event in myoepithelial tumors arising outside the salivary glands
  - Additional technical considerations
    - FISH: a commercial break apart probe is available for *EWSR1* and can detect all molecular variants, but cannot distinguish among chromosomal variants, and cannot distinguish soft tissue myoepitheliomas from other cancers with *EWSR1* translocation
    - RT-PCR: variability of breakpoints and diversity of molecular variants provides an assay design challenge
  - Additional interpretive considerations
    - t(6;22)/*EWSR1–POU5F1* has been reported in three cases of hidradenoma of the skin and one case of mucoepidermoid carcinoma of salivary gland

Myxoid Chondrosarcoma, Extraskeletal

- Basic pathology (*see* Fig. 15.14)
  - Rare soft tissue tumor of characteristic histology and disputed histogenesis
  - The name may be a misnomer, as it is clearly a different lesion from skeletal chondrosarcoma
  - Well-circumscribed, lobular mass with gelatinous or mucoid cut surface
  - Microscopically, it is composed of multiple lobules with myxoid stroma and polygonal, stellate, or spindled tumor cells with cytoplasm that may be vacuolated, mimicking signet ring cells or physaliferous cells
    - Myxoid areas characteristically have columns, cords, or strands of tumor cells, while hypercellular areas can have many different patterns of growth, including solid sheets with minimal or no myxoid matrix
    - Cells are immunoreactive for vimentin, S100, and epithelial membrane antigen
    - Differentiated chondrocytes are rare
    - Electron microscopy shows evidence of cartilaginous differentiation
- Clinical features
  - This rare tumor is most common in middle age adults, and is very rare in children (5% of patients are younger than age 20); males are more frequently affected than females

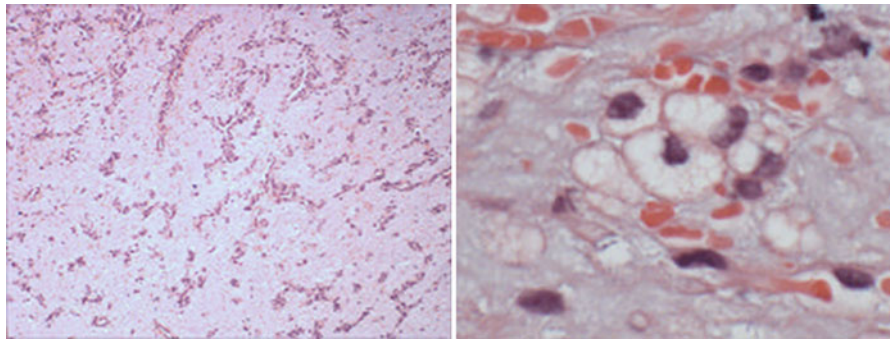

Fig. 15.15 Myxoid liposarcoma. Low-power image on the *left* shows characteristic pattern of delicate arborizing capillaries that vaguely resemble chicken wire. High-power image on the *right* shows characteristic lipoblasts with scalloped nuclei indented by circular globules of optically clear cytoplasmic fat

  - The lesion is most common in extremities (85%), particularly lower (75%), and is usually located in deep soft tissues, where it presents as a slow growing mass
  - Usual treatment is wide surgical excision, with adjuvant chemotherapy and/or radiotherapy in case of lymph nodes or metastasis
  - Prognosis is poor, with mean survival of 20 months, and a high rate of metastasis, especially to lungs
- Molecular genetic pathology
  - t(9;22)(q22;q12) in approximately one-third of cases (*see* Fig. 15.2H)
    - *EWSR1* gene (at 22q12) breakpoints in introns 7, 11, 12
    - *NR4A3* (*CHN*, *TEC*, *NOR1*) gene (at 9q22)
    - Fusion contains nearly entire *NR4A3* steroid/thyroid hormone nuclear receptor protein juxtaposed to amino terminal portion of *EWSR1*, under regulatory control of the constitutively expressed *EWS* promoter
      - The exact mechanism of oncogenesis is unclear
  - Molecular variants
    - Type 1: *EWSR1* exon 12—NR4A3 exon 3
    - Type 2: *EWSR1* exon 7—NR4A3 exon 2
    - *EWSR1* exon 11—*NR4A3* exon 1
  - Chromosomal variants
    - t(9;17)(q22;q11) with *TAF15* (*TAF2N*, *TAFI168*, *RBP56*)–*NR4A3* fusion
    - t(9;15)(q22;q21) with *TCF12* (*HTF4*)–*NR4A3* fusion
    - t(3;9)(q11;q22) with *TFG–NR4A3* fusion
- Molecular diagnostics
  - Test indications: establish diagnosis, as morphology in hypercellular lesions is not distinctive
  - Additional technical considerations
    - FISH: commercial break apart probe is available for *EWSR1*, can detect t(9;22) but not other chromosomal variants
    - RT-PCR: different primers for type 1 and type 2 fusion transcripts may be indicated due to large distance between *EWSR1* breakpoints in these two molecular variants

Myxoid Liposarcoma

- Basic pathology (*see* Fig. 15.15)
  - Most common malignant soft tissue tumor in adults
  - Arising from adipose tissue
  - Tumor consists of hypocellular myxoid tissue with a rich capillary network in a classic "chicken wire" pattern
  - Characteristic lipoblasts, mononuclear cells, or multinuclear cells with cytoplasmic lipid vacuoles that push aside and indent the nucleus
  - May be difficult to identify

- Clinical features
  - Primarily affects adults (median age, 55–60)
  - Lower extremities most often affected
  - Usual treatment is surgical excision
  - Five-year survival is good for pure myxoid liposarcoma (70%), but not for round cell liposarcoma (18%)
  - Metastasis is usually to lung
- Molecular genetic pathology
  - t(12;16)(q13;p11) in 90% of cases (*see* Fig. 15.2I)
    - *FUS* gene (at 16p11) breakpoints in introns 5, 7, and 8
    - *DDIT3* (*CHOP*, *GADD153*) gene (at 12q13) breakpoint in intron 1 or exon 2
    - Oncogenicity of FUS–CHOP fusion protein has been shown in vitro and in animal models
    - Molecular variants
      - Type I: *FUS* exon 7—*DDIT3* exon 2 (20%)
      - Type II: *FUS* exon 5—*DDIT3* exon 2 (70%)
      - Type III: *FUS* exon 8—*DDIT3* exon 2 (10%)
  - Chromosomal variant: t(12;22)(q13;q12) with *EWSR1–DDIT3* fusion
- Molecular diagnostics
  - Indication for testing
    - Establish diagnosis
  - Additional technical considerations
    - FISH: commercial break apart probe is available for *DDIT3*, *FUS*, and *EWS*. The *CHOP* (*DDIT3*) probe is most useful for myxoid liposarcoma, as it enables detection of both t(12;16) and t(12;22)
    - RT-PCR: a single primer set (*FUS* exon 5 and *DDIT3* exon 3) can amplify all three molecular variants, which can then be distinguished by size
  - Additional interpretive considerations
    - Round cell liposarcoma, a high grade liposarcoma, also contains the same t(12;16), suggesting that these lesions may be high grade variants of myxoid liposarcoma, rather than a separate category
    - Some areas in myxoid liposarcoma may resemble lipoblastoma, a benign lesion of childhood
      - In very rare cases, myxoid liposarcoma may occur in children, and in these cases molecular diagnosis may be particularly useful
      - Lipoblastomas carry a rearrangement of 8q12, involving the *PLAG1* gene
    - Balanced translocations involving 12q13–q15 are also seen in benign lipomas, but the gene involved is usually *HGMA2* (*HMGIC*), not *DDIT3*

Myxoinflammatory Fibroblastic Sarcoma

- Basic pathology
  - Multinodular architecture with alternating myxoid and cellular areas
  - Prominent inflammatory infiltrate containing a variable number of mononucleated and multinucleated Reed–Sternberg-like tumor cells
  - Prominent viral inclusion-like nucleoli and mucin-containing pseudolipoblasts
- Clinical features
  - Rare low grade sarcoma
  - Subcutaneous tissues of digital extremities
  - Repeated local recurrences, sometimes requiring amputation, but rare metastases
- Molecular genetic pathology
  - der(10)t(1;10)(p22;q24), typically unbalanced
    - *TGFBR3* gene (at 1p22)
    - *MGEA5* gene (at 10q24)
    - Both candidate genes for the gene fusion are transcribed in opposite directions and, thus, are unable to form a fusion transcript
    - TGFBR3 and MGEA5 have losses of 5′ sequence
    - The der(10) chromosome contains the residual 3′ sequences from TGFBR3 and MGEA5
  - Additional chromosome aberrations involving chromosome 3 (e.g., ring or marker chromosome), with amplification of 3p11.1–12.1, containing *VGLL3* gene
  - Overexpression of *FGF8* on 10q was identified by microarray analysis, suggesting that this translocation may alter

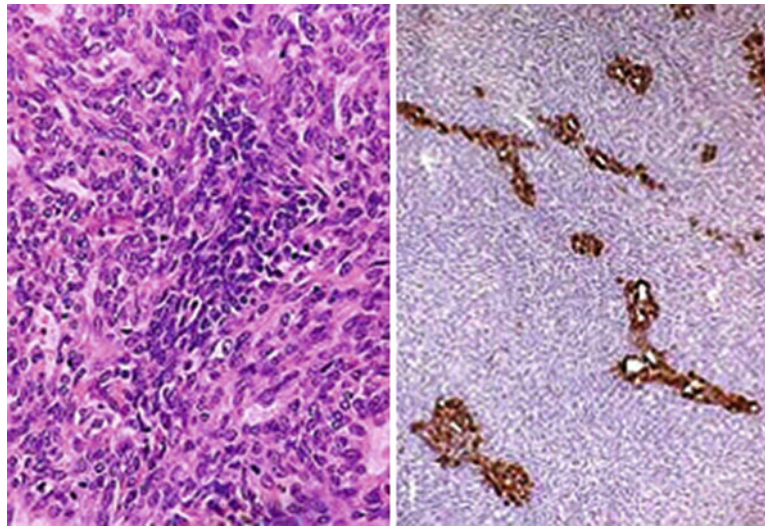

Fig. 15.16 Synovial sarcoma. High-power view on the *left* shows predominantly spindle-shaped tumor cells with focal epithelial patterns, including a rudimentary glandular structure in the lower right corner. The image on the *right* shows a low-power view of a biphasic synovial sarcoma stained with polyclonal antibodies directed against a mixture of cytokeratins, demonstrating epithelial elements (*brown*) growing within the spindle cell population (*blue*)

gene transcription away from the breakpoint

- Molecular diagnostics
  - Indications for molecular genetic testing: Currently unclear
  - Additional technical considerations
    - Cytogenetic analysis is best to detect der(10)t(1;10)
    - FISH: no commercial probes are available
    - RT-PCR: Expression levels of both genes do not appear altered
  - Additional interpretive considerations
    - The same genetic alteration, der(10)t(1;10), has been reported in hemosiderotic fibrolipomatous tumor, a predominantly fatty lesion arising with predilection in the subcutaneous tissues of the ankle of middle-aged women
    - The coexistence of mixed tumors, with both pathologic features, either synchronously or metachronously in primary lesions or a subsequent recurrence, suggests either different morphologic variants or different level of progression of a single entity

Synovial Sarcoma

- Basic pathology (*see* Fig. 15.16)
  - Malignant soft tissue tumor of uncertain histogenesis
  - Features of both mesenchymal and epithelial differentiation
  - The name is a misnomer, as the tumor is unrelated to synovium
  - Although classic synovial sarcoma is a biphasic tumor with both spindled and epithelial morphology, monophasic variants are equally common
  - Most monophasic synovial sarcomas are spindle cell type
  - Both cell types express both epithelial (e.g., keratin, epithelial membrane antigen, carcinoembryonic antigen [CEA]) and mesenchymal (e.g., vimentin) markers by IHC
- Clinical features
  - Fourth most common sarcoma, with overall incidence of 2.75/100,000
  - Primarily affects adolescents and young adults, and males are more often affected than females
  - Usually presents in para-articular regions of extremities, especially knees and ankles

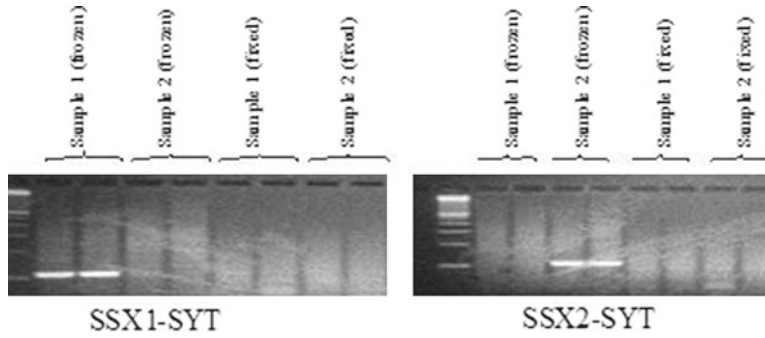

Fig. 15.17 RT-PCR of synovial sarcomas. The two gels show RT-PCR analysis of two cases (1 and 2), using paired reactions for the *SSX1-SS18* and *SSX2-SS18* fusion transcripts, from both frozen and formalin-fixed paraffin-embedded tissues. All samples were tested in duplicate. The frozen samples each showed RT-PCR amplification of fusion transcripts with one set of primers, indicating which variant of translocation was present, but amplification failed for paraffin-embedded tissues from the same cases. It is not uncommon for fixed tissue sections to show poor amplification in RT-PCR assays, presumably due to degradation of RNA

  - Disease course ranges from very aggressive to very indolent, with 5-year survival 45–60%
  - Favorable prognostic factors include young age, small tumor, low mitotic activity
  - Lymph node metastasis more common than in other sarcomas (10–15% of cases)
  - Complete surgical excision is usual treatment; postoperative radiotherapy and adjuvant chemotherapy may enable limb-sparing surgery and limit metastasis
- Molecular genetic pathology
  - t(X;18)(p11.2;q11.2) in approximately 90% (*see* Fig. 15.2J)
    - *SS18* (*SYT*) gene (at 18q11.2)
    - *SSX1* or *SSX2* genes (both at Xp11.2)
    - Fusion protein contains carboxy terminal portion of SSX1 or SSX2, both containing Kruppel-type zinc finger DNA-binding transcriptional repression domains, and amino terminal portion of SYT, containing a novel QPGY transactivation domain, under control of the TATA-less CpG rich SS18 promoter
      - Mechanism of oncogenesis remains under investigation
    - Molecular variants involve the fusion of either *SSX1* or *SSX2* to *SS18*
      - These genes have extreme (>90%) homology to one another
      - Rare cases of *SSX4–SYT* fusions have also been reported
  - Chromosomal variant: a single case of t(X;20)(p11;q13), with an *SSX1–SS19L1* fusion being reported
- Molecular diagnostics
  - Indications for molecular genetic testing
    - Establish diagnosis: classic biphasic lesions are generally straightforward to diagnose, but monophasic and poorly differentiated lesions may require cytogenetic and/or molecular tests
    - Prognosis: this is controversial, as early studies suggested that *SS18–SSX1* fusions have significantly worse prognosis (survival ~40% vs. ~80% for *SS18–SSX2*), but more recent data has shown that there is no association between fusion type and outcome when a three-part histologic grading scheme is used
  - Additional technical considerations
    - Karyotype: rare variant translocation or marker chromosomes can occur
    - FISH: commercial break apart probe for *SS18* is available, but cannot distinguish *SS18–SSX1* from *SS18–SSX2*
    - RT-PCR is required to distinguish *SS18–SSX1* from *SS18–SSX2* transcripts (*see* Fig. 15.17)

- Additional interpretive considerations
  - Biphasic tumors have, almost exclusively, *SS18–SSX1* fusions
  - Monophasic tumors can have either fusion, but *SS18–SSX2* fusions are more likely
  - Any synovial tumor with abnormal karyotype without a t(X;18) needs to be further investigated by FISH and molecular techniques
  - Cases have been reported of tumors with coexisting *SS18–SSX1* and *SS18–SSX2* transcripts detected by RT-PCR

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Molecular Pathology of the Central Nervous System

16

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Contents

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Tumors of the Central Nervous System

Overview

- While morphologic and immunohistochemical evaluations remain the gold standard in the diagnosis of central nervous system (CNS) neoplasms, molecular techniques and cytogenetic analysis are serving an expanding role in supplementing the classification of the more difficult and challenging cases
- The recent findings that certain genetic alterations may influence the survival or therapeutic responsiveness of some CNS neoplasms, add a whole new dimension to the significance of ancillary molecular testing in these tumors
- More importantly, the discovery of new candidate genes in CNS tumors may allow for molecular-targeted therapy (so-called gene therapy), which in theory is more specific to tumor cells and less toxic to normal cells
- Among the most commonly utilized techniques in the genetic characterization of CNS neoplasms, are fluorescence in situ hybridization (FISH), loss of heterozygosity (LOH), and comparative genomic hybridization (CGH)

L. Cheng and J.N. Eble (eds.), *Molecular Surgical Pathology*,
DOI 10.1007/978-1-4614-4900-3_16,

- Gene expression profiling of CNS tumors by cDNA macroarrays provides genetic fingerprinting, which promises to serve both a diagnostic and therapeutic role

Glial Tumors

Astrocytomas

- General molecular concepts in diffuse (fibrillary) astrocytomas
  - Definition
    - A group of diffusely infiltrative gliomas characterized by astrocytic features and variable expression of glial fibrillary acidic protein (GFAP). They are the most common (~40%) among primary CNS neoplasms and encompass a heterogeneous group of neoplasms (Table 16.1)
    - Diffuse astrocytomas, particularly glioblastoma, have been the most studied human gliomas in the past two decades
    - Glioma tumorigenesis is a highly complex process that involves activation of oncogenes and inactivation of tumor suppressor genes
  - Genetic susceptibility
    - Several inherited tumor syndromes impose an increased susceptibility to developing astrocytomas; although rare, these provided a first clue to understanding the role of specific genes, their associated pathways and to testing them in animal models. Familial cancer syndromes implicated in the pathogenesis of gliomas include
      - Li–Fraumeni syndrome and *TP53* germline mutations: chromosome 17p13
      - Turcot syndrome
        - Mismatch repair (MMR) associated (type I): 3q21.3 (*MLH1*), 2p16 (*MSH2* and *MSH6/GTBP*), 5q11-q13 (*MSH3*), 2q32 (*PMS1*), or 7p22 (*PMS2*)
        - Familial adenomatous polyposis (FAP)-associated (type 2): 5q21 (adenomatous polyposis coli [*APC*])

Table 16.1 Diffuse astrocytomas and their corresponding WHO grade

Fibrillary astrocytoma (low grade: grade II or anaplastic: grade III)
Gemistocytic astrocytoma (grade II or III)
Protoplasmic astrocytoma (grade II)
Glioblastoma (grade IV)
Gliosarcoma (grade IV)
Gliomatosis cerebri (grade III)

      - Tuberous sclerosis (TS [*TSC1*: 9q34; *TSC2*: 16p13.3])
      - Neurofibromatosis type 1 (*NF1*): chromosome 17q11.2
      - Neurofibromatosis type 2 (*NF2*): chromosome 22q12
      - Retinoblastoma (*RB*): chromosome 13q14
      - Multiple enchondromatosis (Maffucci/Ollier disease)
  - Genetic alterations and pathways implicated in the pathogenesis of diffuse astrocytomas
    - Tumor suppressor genes
      - *TP53/MDM2/p14^{ARF}* pathway
        - *TP53* gene
          - Maps to chromosome 17p13.1
          - *TP53* is a transcriptional transactivator, which has various regulatory functions involving cell cycle, cell differentiation, apoptosis, angiogenesis, and DNA repair. DNA damage activates p53
          - *TP53* inactivation appears to be an early event in astrocytoma tumorigenesis and later progression toward secondary glioblastoma. Thus, the frequency of *TP53* mutations does not significantly increase during malignant progression
        - Loss of normal p53 function may result from altered expression of any of the *TP53*, *MDM2*, and *p14^{ARF}* genes (see below)
          - *TP53* mutations are a genetic hallmark of secondary glioblastoma (>65%) and observed in >60% of grade II

astrocytomas. The gemistocytic variant of astrocytoma has the highest frequency of *TP53* mutations
- ✦ *TP53* mutations are observed in ~25% of primary (de novo) glioblastomas
- ✦ The underlying mechanisms for *TP53* mutations in primary vs. secondary glioblastomas appear different
- ✦ *TP53* mutations correlate with younger age and a giant cell phenotype in glioblastomas
- ✦ Activated p53 induces the overexpression of p21 (cell cycle regulator), which causes growth arrest
- ✦ The immunohistochemical expression of p53 does not necessarily imply the presence of a mutation (74% concordance)

▪ *MDM2* gene
- ✦ Mouse double minute (*MDM2*) gene maps to chromosome 12q 14.3–q15
- ✦ *MDM2* encodes a transcription factor that binds to mutant and wild-type p53 proteins, inhibits the activity of wild-type p53 and promotes its degradation, providing an alternative mechanism for escaping p53-regulated cell growth
- ✦ Under normal circumstances, *MDM2* gene transcription is induced by wild-type p53 creating an autoregulatory feedback loop that regulates the activity of p53 protein and the expression of MDM2
- ✦ *MDM2* amplification and immunohistochemical overexpression in primary glioblastomas is observed at a rate of 10% and 50%, respectively
- ✦ MDM2 overexpression has been found to be a negative prognostic indicator in some studies
- ✦ *MDM4* gene shows similar characteristics to *MDM2* but maps to chromosome 1q32

▪ $p14^{ARF}$ gene
- ✦ Similar to *CDKN2A* and *CDKN2B* genes, $p14^{ARF}$ maps to chromosome 9p21
- ✦ $p14^{ARF}$ encodes a protein that directly binds to MDM2 and inhibits *MDM2*-mediated p53 degradation
- ✦ $p14^{ARF}$ homozygous deletion or hypermethylation deregulates p53 function in the absence of *TP53* mutation
- ✦ Conversely, $p14^{ARF}$ expression is negatively regulated by p53
- ✦ $p14^{ARF}$ homozygous deletion and hypermethylation can be found in low grade diffuse astrocytoma (one-third) and glioblastoma (50% primary and 75% secondary)

◆ $p16^{INK4a}$/*CDK4/RB1* pathway
- ▪ The members of this signaling pathway control the cell cycle progression from G1 to S phase. Therefore, any altered expression involving these genes will result in loss of cell cycle control
- ▪ *RB* gene (*RB1*) maps to chromosome 13q14. The RB protein (retinoblastoma or pRB1) acts as a nuclear phosphoprotein involved in cell cycle regulation (prevents uncontrolled cell proliferation). It is phosphorylated by the CDK4/cyclin D1 complex
- ▪ *CDKN2A* and *CDKN2B* alterations (*see* below) may inhibit the pRB1 phosphorylation resulting in uncontrolled cell proliferation
- ▪ *RB1* hypermethylation was seen more frequently in secondary

glioblastoma (43%) than in primary glioblastoma (14%). It was not detected in low grade or anaplastic astrocytoma (late event)

- *p16*^{INK4a} (*CDKN2A*) and *p15* (*CDKN2B*) genes map to chromosome 9p21 and encode p16 and p15, respectively
- p16/p15 are proteins that act as negative regulators of the cell cycle by inhibiting cyclin-dependent kinases (CDK [CDK4/CDK6])/cyclin D complexes and their ability to phosphorylate the pRB1
- Subsequently, homozygous deletion of *CDKN2A* results in uncontrolled cell proliferation through loss of p16^{INK4a} function
- *CDKN2A* homozygous deletion and hypermethylation can be found in anaplastic astrocytoma and glioblastoma
- *RB1* and *CDNK2A* alterations in primary gliomas are inversely correlated
- CDK4 and CDK6 are CDK that promote G1 to S phase progression. The *CDK4* gene, which maps to chromosome 12q13–14, is amplified in approximately 15% of high grade gliomas, usually in the absence of p16^{INK4a} deletion. *CDK6* maps to chromosome 7q21–q22 and appears to have similar qualities to *CDK4*
- *CDK4/CDK6* amplification, cyclin D1 overexpression, and/or *RB1* show similar consequences to *CDKN2A/CDNK2B* mutations and appear to be mutually exclusive. Gene inactivation in the *CDNK2A/CDK4/RB1* pathway occurs at an overall frequency of 40–50% in both primary and secondary glioblastomas
- The *p16^{INK4a}/CDK4/RB1* pathway may provide for a candidate target gene therapy strategies

- *PI3K/PTEN/AKT* pathway
  - Phosphatase and tensin homology (*PTEN*), also known as mutated in multiple advanced cancers (*MMAC1*), or TGFβ-regulated and epithelial cell enriched phosphatase (*TEP1*) gene is mapped to chromosome 10q23.3, a genomic region frequently lost in glioblastoma
  - *PTEN* encodes a lipid phosphatase that catalyzes dephosphorylation of phosphatidylinositol-3,4,5-triphosphate (PIP3), negatively regulating the activity of phosphatidylinositol 3-kinase (PI3K) and thereby inhibiting cell proliferation, essentially acting as a gatekeeper of the PI3K pathway
    - ✦ PI3K converts PIP2 to PIP3 which in turn activates downstream effector molecules such as AKT and the mammalian target of rapamycin (mTOR) which are essential in regulating cell migration and invasion as well as cell proliferation and survival
    - ✦ PI3K is recruited to the cell membrane upon binding of growth factors to EGFR and other growth factor receptors
  - The PI3K pathway may represent a common therapeutic target to most glioblastomas due to its central position in the signaling cascade affecting proliferation, apoptosis, and migration
  - *PTEN* mutations have been implicated in glioma formation and progression. They are observed in 15–40% of glioblastomas, particularly primary glioblastoma, but homozygous deletions are very rare
  - AKT is a downstream protein serine/threonine kinase (PKB) in the *RTK/PTEN/PI3K* pathway and is

the principal PIP3 target. *RTK/PTEN/PI3K* pathway leads to activated AKT and phospho-AKT levels which help glioma cells grow uncontrolled, evade apoptosis, and enhance tumor invasion
- *PTEN* alterations also characterize oligodendroglioma progression and have been identified in meningiomas and a number of extracranial neoplasms

- Deleted in malignant brain tumors 1 (*DMBT1*) gene
  - Maps to chromosome 10q25.3–26.1
  - Homozygously deleted in (~30%) of glioblastomas
  - Usually codeleted with *PTEN* in glioblastomas
- Chromosome 10
  - In addition to *PTEN*, and *DMBT1*, the long arm of chromosome 10 (10q) is believed to harbor at least one more tumor suppressor locus
  - Overall, LOH on chromosome 10 regions or complete loss of chromosome 10 are perhaps the most common (60–95%) genetic alterations in glioblastomas. LOH 10q occur at similar frequencies in primary and secondary glioblastomas while LOH 10p occurs almost exclusively in primary glioblastoma. LOH 10 is far less common in lower grade astrocytomas

- Protooncogenes
  - Epidermal growth factor receptor (*EGFR/ERBB1/HER1*)
    - EGFR is a transmembrane tyrosine kinase receptor encoded by a gene mapped to chromosome 7p11
      - Under normal conditions, EGFR binds to one of its extracellular ligands, of which TGF and TGFA are the most common. Ligand binding to EGFR induces receptor phosphorylation and homodimer formation which in turn activate a complex downstream signal transduction cascades leading to DNA synthesis and cell proliferation
      - *EGFR*-amplified cells appear to facilitate tumor proliferation, migration, infiltration/invasion, resistance to apoptosis, and tumor neovascularization
      - *EGFR* gene is the most commonly amplified gene in astrocytic tumors, including glioblastoma where it is amplified in 40–60% of primary glioblastoma, but rarely in secondary glioblastoma
      - Amplification of *EGFR* gene in glioblastoma leads to downstream activation of PI3K/PKB/mTOR/rpS6
      - Genomic *EGFR* amplification is typically accompanied by overexpression at the protein level, which can also be assessed immunohistochemically. EGFR overexpression, however, has about 70–90% correlation with amplification status
      - *EGFR* amplification correlates with older age and a small cell phenotype in glioblastomas
      - In addition, in cases where the wild-type receptor (EGFRwt) has undergone genomic amplification, several mutant forms are detected as *EGFR* gene amplification induces structural alterations producing several truncated, tumor-specific, variants of EGFR; the most common of which is delta EGFR (EGFRvIII), present in up to 50% of amplified glioblastomas, followed by

EGFRc958, observed in about 20% of amplified cases, often in association with EGFRvIII
- The above results in several possible homo- and heterodimerization products involving EGFRwt and its mutant forms, complicating the process and potentially evading EGFR targeted therapies
- Mutant EGFR increases tumor cell proliferation and has an antiapoptotic effect. Subsequently, overexpression of mutant EGFR in glioma cells confers resistance to chemotherapeutic agents
- To reverse its antiapoptotic effect, EGFR may be therapeutically targeted using two different EGFR inhibitors: monoclonal antibodies-MoAbs (examples include cetuximab, matuzumab, and panitumumab) and small molecule inhibitors of EGFR tyrosine kinase activity, such as gefitinib, erlotinib and lapatinib. The mechanism of receptor inhibition differs between the two types of drugs. MoAbs interfere with EGFR activation by blocking the extracellular ligand-binding domain. Protein kinase inhibitors (PKI) block the intracellular tyrosine kinase-mediated signaling pathways. Interestingly, anti-EGFR therapies, which act as antidimerization agents, were found to effectively block EGFRwt dimerization and activation but did not equally impair EGFRvIII homodimers, EGFRwt–EGFRvIII or EGFRvIII–EGFRc958 heterodimers. Therefore, independent of mutant EGFR dimerization status, EGFR amplification or expression in GBMs have limited use as prognostic factors for response to anti-EGFR therapeutics
  - It appears that there is a quantitative level and regional variability of EGFRwt–EGFRvIII dimers within GBM patient samples that may be of potential biologic or clinical relevance
  - *EGFR* point mutations are infrequent (3–5%) in glioblastomas
  - *EGFR* amplification is usually accompanied by $p16^{INK4a}$ deletions but shows inverse relationship with *TP53* and *PTEN* mutations

- Platelet-derived growth factor (*PDGF*)
  - Platelet-derived growth factor receptor (*PDGFR*) is a tyrosine kinase receptor encoded by a gene that maps to chromosome 4q12
  - *PDGF* has three known ligands and two cell surface receptor kinases (*PDGFRA* and *PDGFRB*)
  - *PDGF* is expressed by astrocytic tumor cells while its tyrosine kinase receptor *PDGFRB* is expressed on endothelial cells
  - *PDGF* ligands and receptors are expressed almost equally among various grades of astrocytoma and are therefore implicated in the early stages of astrocytoma formation
  - *PDGFRA* gene amplification is only detected in a small subset of glioblastomas
- Vascular endothelial growth factor (*VEGF*)
  - *VEGF* family is comprised of a group of growth factors (*VEGF* A–D) that exert their angiogenic and lymphangiogenic effects

through the activation of three tyrosine kinase receptors, *VEGFR1*, *VEGFR2*, and *VEGFR3*, which are normally expressed by endothelial cells, monocytes/macrophages, and hematopoietic precursors
- *VEGF* is the most important regulator of vascular functions in glioma-induced angiogenesis
- In glioblastoma, *VEGF* is expressed by astrocytic tumor cells while its tyrosine kinase receptors 1 and 2 are expressed on endothelial cells
- In addition to inducing angiogenesis, *VEGF* and its receptors (*VEGFR*) cause vascular permeability and may also be responsible for breakdown of the blood–brain barrier and peritumoral edema in glioblastoma
- *VEGF* is upregulated in perinecrotic pseudopalisading cells of glioblastoma
- *VEGF* production can be stimulated by hypoxia

◆ *CDK4* and *CDK6* (see above)
◆ *CCND1* and *CCND3*
- Cyclin D1 and cyclin D3 map to chromosomes 11q13 and 6p21, respectively
- Similar to *CDK4/CDK6*, they are cell cycle regulators that promote G1 to S phase progression
- *CCND1/CCND3* amplification/overexpression is identified in primary glioblastoma

◦ Promoter hypermethylation
◆ Recently, several genes that show promoter hypermethylation in astrocytic gliomas, particularly glioblastomas, have been identified. These include:
- Cell cycle regulatory genes (*CDKN2A*, *CDKN2B*, *RB1*, $p14^{ARF}$, and *TP53*)
- Apoptosis-associated genes such as *APAF1*
- *MGMT* gene, which encodes the constitutively expressed DNA repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT) that protects cells against alkylating agents. Virtually all glioblastoma patients are currently treated with the orally administered alkylating agent temozolomide (TMZ). TMZ acts to methylate primarily the O^6 position of the nucleotide guanine, resulting in cell death. However, since cells have an inherent DNA repair mechanism that can counter the effects of TMZ, MGMT will irreversibly transfer a methyl group from the O^6 position of the modified guanine to a cysteine residue of the MGMT protein, mitigating against the cytotoxic effects of chemotherapy
- *MGMT* promoter methylation is frequently present in glioblastoma (45–75%). Its presence has been linked to longer survival of glioblastoma patients treated with TMZ, giving it a predictive value. Nevertheless, *MGMT* promoter methylation status has been shown to have a prognostic advantage in elderly patients with glioblastoma irrespective of TMZ therapy. Furthermore, glioblastoma patients with unmethylated *MGMT* seem to benefit from alkylating agent administration and hence TMZ therapy is not currently withheld based on *MGMT* methylation status

◆ Similar to *MGMT*, promoter methylation of the *RB1*, $p14^{ARF}$, *TIMP3*, and *TP53* genes are common in glioblastoma, with higher frequency in secondary than primary glioblastoma
◆ *RASSF1A* tumor suppressor gene
◆ *TFP12* and *SLIT2* genes, whose proteins inhibit invasion and migration

- Approximately 40% of glioblastomas demonstrate hypermethylation and transcriptional downregulation of the carboxyl-terminal modulator protein (*CTMP*) gene, which encodes an inhibitor of protein kinase B/Akt
- Current data suggest that aberrant methylation of genes is more prevalent than genetic alterations, in particular in low grade astrocytomas

- Other genes
  - *IDH*
    - Recently, a genome-wide survey identified somatic mutations in the *IDH1* (isocitrate dehydrogenase-1) gene at a high frequency in younger patients with secondary glioblastomas and subsequently in lower grade diffuse gliomas
    - *IDH1* gene is located on chromosome 2p33. The IDH1 protein, physiologically located in the cytoplasm and peroxisomes, catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate, resulting in the production of reduced nicotinamide adenine dinucleotide phosphate (NADPH). NADPH is an important intracellular antioxidant
    - *IDH1* mutations are an early event in gliomagenesis. They occur in a high proportion (60–100%) of diffuse gliomas of World Health Organization (WHO) grade II and III and in secondary glioblastomas (>80%) but are rare (<5%) in primary glioblastomas. IDH1 is highly expressed in oligodendrogliomas and oligoastrocytomas (>80%) but rare in pediatric and brain stem gliomas
    - Mutations in the *IDH2* gene are much less common (2–5%) than *IDH1*. *IDH1* and *IDH2* mutations are mutually exclusive
    - *IDH1* mutations at codon 132 are by far the most common accounting for over 90% of the *IDH1* mutations. These are characterized by a base-pair exchange of guanine to adenine (G395A) resulting in a substitution of the amino acid arginine by histidine (R132H). Rarely, other types of *IDH1* mutations including R132C (4%), R132L (1%), R132S (2%), and R132G (2%) are found in diffuse gliomas
    - *IDH1* mutation status may be assessed using DNA sequencing (classical Sanger sequencing being most commonly used). However, the availability of a robust, highly specific immunohistochemical monoclonal antibody against the mutated IDH1–R132H protein, which can be performed on formalin-fixed, paraffin-embedded (FFPE) samples, makes quick testing highly feasible for every day surgical neuropathology practice. While the yield is higher with frozen samples, genetic analysis of FFPE tissues is highly effective given the very short DNA segment involved
    - As expected with a cytoplasmic protein, anti-IDH1–R132H immunoreactivity is distinctly cytoplasmic, but often accompanied by a weaker nuclear immunoreactivity
    - IDH1–R132H immunohistochemistry is exceptionally useful in the differential diagnosis of diffuse gliomas and reactive gliosis and in differentiating oligodendrogliomas and diffuse astrocytomas from other glial tumors that may enter the differential diagnosis particularly localized gliomas, ependymomas and those with clear cell features. Tumor heterogeneity does not seem to be a significant issue, though reports

of variable immunoreactivity patterns in oligoastrocytomas exist, with the oligodendroglial component showing more consistent IDH1 staining. Gemistocytic astrocytes reportedly show weaker immunoreactivity
- A cautionary note is that while anti-IDH1–R132H immunohistochemistry seems to be highly specific, this testing detects approximately 90% of the known *IDH* mutations and a negative stain should not be interpreted as evidence of *IDH*-wild type and does not exclude a diffuse glioma. Furthermore, *IDH1* mutations are only detectable in 50–80% of diffuse astrocytomas
- *IDH1* mutations correlate with younger age at diagnosis, the presence of *TP53* mutation, combined Ip/I9q deletion, *MGMT* promoter hypermethylation and inversely correlate with loss of chromosome 10, *EGFR* amplification, polysomy of chromosome 7, and the presence of necrosis but not with the Ki67 proliferation index
- Early studies suggest improved survival times for diffuse gliomas with *IDH1* mutations, independent of other prognostic variables. One study showed improved outcome for *IDH1* and *IDH2* mutated tumors, with median overall survival of 31 vs. 15 months for glioblastoma lacking mutations and 65 vs. 20 months for anaplastic astrocytoma. The observation that anaplastic astrocytomas lacking *IDH* mutations have been shown to have shorter survival times lead some to argue that these are probably underdiagnosed glioblastomas. It has also been suggested that anaplastic oligodendrogliomas lacking *IDH* mutations might also lack combined 1p/19q deletions. However, the complete role of *IDH* mutations as prognostic indicators is still being defined
- The role of *IDH* mutations as a predictive marker requires additional studies. Targeted therapies are not yet available
- Deleted in colorectal cancer (*DCC*)
- Located on chromosome 18q21; encodes a cell surface receptor
- Induces apoptosis and G2 to M cell cycle arrest in tumor cells
- The immunohistochemical loss of *DCC* expression increases during glioma progression (late event in secondary glioblastoma)
- Less frequently implicated in primary glioblastoma

Low Grade Fibrillary Astrocytoma

- Definition
  - A well-differentiated (WHO grade II), diffusely infiltrative glial neoplasm comprised of fibrillary neoplastic astrocytes
- Clinical features
  - Children and young adults
  - Occurs anywhere in the white matter of cerebrum, cerebellum, brainstem, or spinal cord
  - Radiographically appears as a poorly defined, noncontrast-enhancing solid lesion
- Pathologic features
  - Low to moderate cellularity
  - Ill-defined infiltrative borders
  - Nuclear atypia is usually mild to moderate
  - Mitoses are rare or absent and vascular proliferation and necrosis are universally lacking
- Genetic findings
  - High frequency of *IDH* mutations (60–100%) and immunohistochemical expression, see above
  - *TP53* mutations (>60% of cases); clinical outcome does not appear to be influenced by *TP53* mutational status, although some

studies suggest a shorter progression interval for those with *TP53* mutation
- LOH on 17p with complete absence of a wild-type gene is seen in most cases with *TP53* mutation
- $BRAF^{V600E}$ mutations are absent
- Copy number alterations
  - Gains on chromosome 7, usually as trisomy/polysomy, and 8q amplification, constitute the most common chromosomal abnormalities (>50% of cases) detected by CGH
  - Gains on 7q, 5p, 9, and 19p and losses on 19q, 1p, and Xp were most frequently identified by CGH in one study
  - Losses of chromosome 6, 10p, 13q, 22q, and sex chromosome in small percentage of cases
- *PDGFRA* overexpression; (not amplification) preferentially in tumors with LOH on 17p
- $p14^{ARF}$ and *MGMT* promotor methylation in approximately 30% and 50% of cases, respectively
- Reported in association with inherited multiple enchondromatosis type 1 (Ollier disease)

Gemistocytic Astrocytoma

- Definition
  - A diffusely infiltrative astrocytoma in which gemistocytic astrocytes comprise at least 20% of the tumor cells
- Clinical features
  - Similar to diffuse fibrillary astrocytoma
- Pathologic features
  - Usually WHO grade II; grade III if showing signs of anaplasia
  - Gemistocytic astrocytes are characterized by large, glassy, eosinophilic cytoplasm with an arborizing network of randomly oriented processes. The nuclei are eccentrically placed with small nucleoli
- Genetic findings
  - *TP53* mutations are more common (>80% of cases) than the typical grade II astrocytoma
  - Otherwise, similar alterations to WHO grade II diffuse astrocytoma

Protoplasmic Astrocytoma

- Definition
  - A superficially located, diffuse astrocytoma of low cellularity characterized by prominent microcyst formation (WHO grade II)
- Clinical features
  - Superficial location
  - Otherwise, similar to diffuse fibrillary astrocytoma
- Pathologic features
  - Protoplasmic astrocytes are small cells with little cytoplasm and scant GFAP immunoreactivity
  - Mucoid degeneration
  - Microcyst formation
- Genetic findings
  - Little information exists on the molecular genetics of protoplasmic astrocytoma; however, it is believed that the molecular events are comparable with those seen in other low grade diffuse astrocytomas

Anaplastic Astrocytoma

- Definition
  - A diffuse astrocytoma exhibiting cellular atypia and mitotic activity (WHO grade III)
- Clinical features
  - Predominantly adults; older than patients with low grade astrocytomas and younger than those with glioblastomas
  - Preferentially involves the cerebral hemispheres
  - May involve brainstem and thalamus in children
  - Male:female ratio of 1.8:1
  - Often demonstrates focal or patchy radiographic enhancement
- Pathologic features
  - Wide spectrum of histologic appearance that features the presence of one or more of the following in focal or diffuse patterns
    - Increased cellularity
    - Cytologic atypia
    - Mitotic activity (more than 1 mitosis in entire biopsy)
    - High proliferative activity (>4%)
  - Necrosis and vascular proliferation are absent

- Genetic findings
  - Similar to WHO grade II diffuse astrocytoma, there is high frequency of *IDH* and *TP53* mutations, LOH 17p, and chromosome 7 gains
  - Deletions of *p16* (*CDKN2A*) (30%), $p14^{ARF}$, and *p15* (*CDKN2B*) (all on chromosome 9p)
  - *CDK4* amplification and overexpression (10%), preferentially in tumors without *CDKN2A* deletion or mutation
  - *RB1* alterations (25%) in tumors lacking *CDK4* and *CDKN2A* abnormalities
  - *PTEN/MMAC1* mutations are less frequent (18–23%), than glioblastoma. *PTEN* mutation implies a poor prognosis
  - *EGFR* amplification is rare (10%) compared to glioblastoma
  - Deletions on chromosome 6 (30%), 10q (30–60%), 11p (30%), 19q (40%), and 22q (30%)
  - $BRAF^{V600E}$ mutations are virtually absent

Glioblastoma

- Definition
  - A highly malignant (WHO grade IV), poorly differentiated, diffuse astrocytoma that may be primary (de novo) or secondary (from a lower grade glial neoplasm)
- Clinical features
  - Mostly adults in their sixth, seventh, or eighth decades
  - Preferentially involves the cerebral hemispheres
  - Male:female ratio of 1.5:1
  - Typically demonstrates ring-like enhancement radiographically
- Pathologic features (Fig. 16.1)
  - Highly cellular
  - High degree of cytologic and nuclear anaplasia
  - Highly mitotic, including atypical forms
  - High proliferative activity
  - Vascular proliferation, sometimes glomeruloid
  - Geographic and palisading necrosis
- Genetic findings
  - Glioblastoma is as genetically heterogeneous as it is phenotypically. Much of the genetic heterogeneity can be attributed to the two distinct pathways through which glioblastoma evolves; primary 95% vs. secondary 5%. It is now well accepted that de novo (primary) glioblastoma shows different genetic alterations from secondary glioblastoma
  - Nevertheless, the functional consequences of the different genetic alterations are similar since they result in alterations of the same pathways (*TP53*, *RB1 PTEN/PI3K/AKT*, and mitogen-*PTEN*-activated protein kinase)
  - Recently, the Cancer Genome Atlas Network (TCGA) subdivided glioblastomas into *classical*, *mesenchymal* and *proneural* subtypes, using gene expression-based molecular classification. Each group shows a different aberration and gene expression, which may predict therapy efficacy. The *proneural* subtype was associated with younger age, *PDGFRA* abnormalities, *IDH1* and *TP53* mutation and resistance to TMZ and radiation therapy. The *classical* GBM with *EGFR* abnormalities showed the best response to therapy, while the *mesenchymal* subtype, characterized by high expression of *CHI3L1* and *MET* and *NF1* mutation/deletion, reported only a partial response to treatment

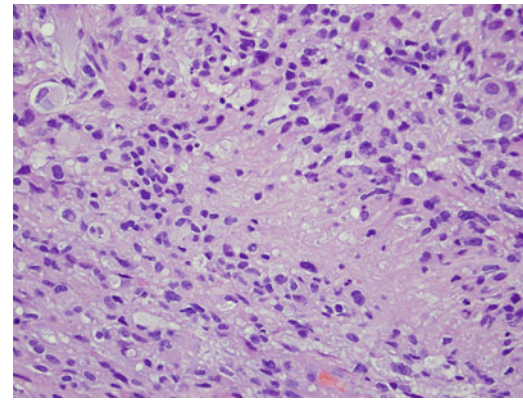

Fig. 16.1 Glioblastoma: a hypercellular glial neoplasm with large hyperchromatic irregular nuclei. Note the high degree of nuclear pleomorphism and the area of palisading necrosis toward the center

- The fact that glioblastomas share many genetic alterations, where each individual tumor has its own unique pattern of genetic changes represents a considerable barrier to the development of effective therapeutic intervention
- Being the most malignant of all astrocytic neoplasms, glioblastomas harbor the most genetic alterations, as they are thought to accumulate sequential changes along the path to malignancy
- LOH on chromosome 10 occurs at a high frequency in all types of glioblastomas, regardless of age (adult vs. pediatric) and regardless of their evolution pathway (primary vs. secondary)
- Immunohistochemical detection of p53 protein occurs at a higher frequency than *TP53* mutations in both primary and secondary glioblastomas
- High grade gliomas demonstrate loss of p27 (cell cycle regulator) expression (expressed in 44% of grade II astrocytomas compared with only 2% of glioblastomas)
- *Primary glioblastoma* (older age of onset and an aggressive clinical course)
  - Higher frequency of:
    - *EGFR* amplification and immunohistochemical overexpression (–40% and 60%, respectively)
    - Homozygous deletion of *p16* (*CDKN2A*) (~40%) and $p14^{ARF}$
    - *CDK4* amplification
    - *MDM2/MDM4* amplification: *MDM2* gene amplification and immunohistochemical overexpression in approximately 10% and 50%, respectively
    - *RB1* mutation/homozygous deletion
    - 10q loss/monosomy 10 (70%)
    - *PTEN* mutation (largely restricted to primary glioblastoma)
  - *IDH* mutations are very rare or absent, as are $BRAF^{V600E}$ mutations
  - In primary glioblastoma, LOH 10 usually manifests as loss of the entire chromosome (LOH 10p and 10q) with 10q loss being especially associated with the small cell phenotype of glioblastoma
  - Nearly all glioblastomas with *EGFR* amplification show simultaneous loss of chromosome 10 (LOH 10p and 10q)
  - *TP53* mutations are less frequent (10–30%) than secondary glioblastoma. However, the p53 pathway is altered in more than two-thirds of primary glioblastomas, due to either *TP53* mutation, $p14^{ARF}$ alteration, or *MDM2/MDM4* amplification
  - *TP53* mutations, *p16* (*CDKN2A*) deletion, *EGFR* amplification, and *PTEN* mutations are inversely associated with each other, except for a positive correlation between *p16* (*CDKN2A*) deletion and *EGFR* amplification
  - *MDM2* overexpression/amplification and *TP53* inactivation are mutually exclusive events as MDM2 protein binds to p53 and inhibits its activity
  - *p16* (*CDKN2A*) deletions and *RB1* alterations are also mutually exclusive
  - LOH 19q is rare (<10%) but has been implicated in malignant progression of astrocytic lesions. In addition, chromosome, 19 alteration is a feature shared by all three types of diffuse gliomas (astrocytomas, oligodendrogliomas and ependymomas)
  - Gain of chromosome 7
  - LOH 17
  - Chromosome 3 alterations
- Secondary glioblastoma (younger age of onset and a more protracted clinical course)
  - *TP53*, and *IDH* mutations and LOH 10q are among the most common genetic abnormalities (65%, 85%, and 63%, respectively)
  - *EGFR* gene amplification is absent or exceedingly rare in secondary glioblastoma but its protein may be detected immunohistochemically in a minority of cases
  - In secondary glioblastoma, LOH 10 is usually limited to the long arm of chromosome 10 (LOH 10q; >60%)

    - *p16* (*CDKN2A*) deletions, amplification of *EGFR*, *MDM2* or *MDM4* and *PTEN* mutations are rare
    - Loss of *RB* function and *CDK4* amplification are common alterations
    - *PDGFRA* gene amplification is much less frequent than lower grade astrocytomas (<10%) but more common than primary glioblastoma
    - LOH 19q and 13q are common (>50%)
    - Loss of immunohistochemical expression of DCC is common (~50)
  - LOH 1p is equally detectable in primary and secondary glioblastoma (12–15%) while LOH 22q is significantly more frequent in secondary glioblastoma (82%)
  - While the vast majority of pediatric glioblastomas arise de novo, their genetic alterations more closely resemble those seen in adult secondary glioblastomas, albeit with less frequency of *TP53* mutations and LOH 17q. *TP53* mutations are even less frequent in the very young (<3 years) compared to older children. Microsatellite instability (MSI) is more common than in adult glioblastoma and typically associated with shorter survival. Unlike adult primary glioblastomas, they show a low rate of *EGFR* amplification, and *PTEN* and *CDKN2* deletions as well as absence of *MDM2* amplification. Copy number abnormalities include gain of 1q, 3q, and 16p and loss of 8q and 17p
  - The *small cell glioblastoma* phenotype typically shows *EGFR* amplification, $p16^{INK4a}$ (*CDKN2A*) homozygous deletion, *PTEN* mutations and LOH 10q
  - *Giant cell glioblastoma* is a rare variant of glioblastoma clinically characterized by well-circumscribed, superficially located cortical lesions in patients of a slightly younger age group than is typical for glioblastoma. Histologically, they show predominance of giant bizarre-shaped and multinucleated astrocytes embedded in a reticulin-rich stroma. Their genetic alterations are a mixture of what is found in primary and secondary glioblastomas. They demonstrate high frequency of *TP53* (~90%) and *PTEN* (~30%) mutations, but generally lack *EGFR* amplification and $p16^{INK4a}$ and $p14^{ARF}$ deletions
  - *Gliosarcoma* is another uncommon variant of glioblastoma characterized by a distinctly biphasic pattern of glial and mesenchymal areas. Its genetic alterations are very similar to those of primary glioblastoma except for less frequent or absent *EGFR* amplification and overexpression. It has been shown that both gliosarcoma components are monoclonal
- Possible prognostic implications
  - The prognostic significance of *EGFR* amplification and overexpression status in glioblastoma is highly controversial with some studies reporting significantly shorter survival periods, others showing no significant correlation while some reports claiming a favorable clinical outcome. EGF tyrosine inhibitors and anti-*EGFR* targeted immunotherapy are available with mixed results
  - Early studies suggest improved survival times for glioblastoma patients with *IDH1* mutations, independent of other prognostic variables (see above)
  - The prognostic value of *TP53* mutations remains an unsettled issue
  - Most studies point to 10q loss/monosomy 10 as an independent predictor of shorter patient survival
  - *MDM2* amplifications correlated with poor outcome in both univariate and multivariate analysis
  - *MGMT* promoter methylation has been proven to be an independent and stronger prognostic factor, better than age, stage and tumor grade, and predicting responsiveness to alkylating agents, including TMZ
  - Losses involving p16, 19q, and p27 are alterations that all have shown promise as potential prognostic markers in adult astrocytoma

Pilocytic Astrocytoma

- Definition
  - A relatively benign (WHO grade I), slowly growing form of localized astrocytoma that has a propensity to involve children and young adults and occurs predominantly in the infratentorium
- Clinical features
  - May occur at any age; however, it is most common in children and young adults (first and second decades)
  - Pilocytic astrocytoma is by far the most common glioma in children
  - Has been reported throughout the CNS, but occurs at a disproportionally higher frequency in the posterior fossa. Other preferred sites include the optic nerve and chiasm, thalamus/hypothalamus, and brainstem
  - Clinical presentation is largely dependent on site of involvement and includes signs of increased intracranial pressure, visual disturbances, and cerebellar symptoms
  - Radiographically appears as well-circumscribed, contrast-enhancing lesions. Those outside the brainstem and thalamic/hypothalamic axis frequently show cyst formation. A classic cerebellar pilocytic astrocytoma shows a large solitary cyst with an enhancing mural nodule
- Pathologic features
  - Biphasic pattern showing alternating compact and loose areas (Fig. 16.2)
  - The compact areas are made up of bundles of spindled astrocytic cells characterized by elongated bland nuclei and bipolar wispy cytoplasm. These piloid cells show intense GFAP reactivity. Rosenthal fibers are usually abundant in these areas
  - The loose hypocellular areas may show prominent microcyst formation and deposition of eosinophilic granular bodies. The tumor cells have bland round to oval nuclei, pale cytoplasm, and short cobweb-like processes. GFAP is weakly immunoreactive
  - Oligodendroglial-like areas may be seen
  - Pilocytic astrocytomas are vascular tumors that may show vascular proliferation, including glomeruloid pattern. Unlike diffuse astrocytomas, the presence of vascular proliferation bears no prognostic significance. The same holds true for necrosis
  - Mitoses are rare but degenerative nuclear atypia may be prominent
- Genetic findings
  - Pilocytic astrocytoma is the most common CNS tumor as part of the NF1 complex. Optic nerve involvement is classic. Most *NF1-associated pilocytic astrocytomas* appear genetically distinct from sporadic tumors and carry allelic losses at the *NF1* tumor suppressor gene locus at 17q11.2 resulting in constitutive RAS activation and downstream hyperactivation of the mTOR pathway. This subset of tumors show reduced expression of neurofibromin protein, the *NF1* gene product and aldehyde dehydrogenase 1 family member L1 (ALDH1HL). The latter was also underexpressed in clinically aggressive pilocytic astrocytomas and in those with atypical histologic features. A clinical diagnosis of NF1 was present in 28% of anaplastic pilocytic astrocytomas in one study
  - *Sporadic pilocytic astrocytomas*, on the other hand, rarely demonstrate allelic losses

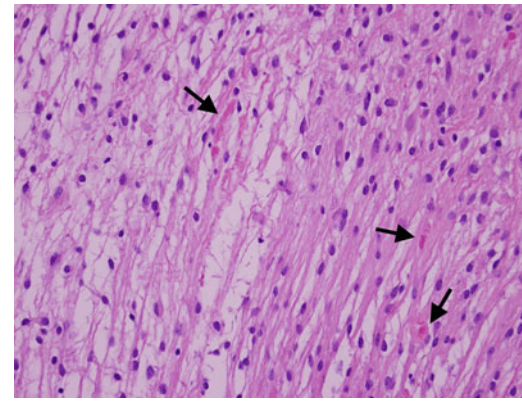

Fig. 16.2 Pilocytic astrocytoma: the characteristic biphasic pattern of relatively compact eosinophilic component made up of bipolar cells with elongated nuclei (*right side*) and loose hypocellular component with more stellate appearing tumor cells (*left side*). Note the scattered Rosenthal fibers (*arrows*)

at the *NF1* locus. In fact, neither *NF1* mutations nor loss of *NF1* mRNA expression were found in sporadic pilocytic astrocytomas, arguing against an important role of *NF1* in the tumorigenesis of sporadic pilocytic astrocytomas. However, sporadic pilocytic astrocytomas may activate RAS through other mechanisms. Interestingly, sporadic pilocytic astrocytomas show immunohistochemical overexpression of neurofibromin

- Consistent genetic abnormalities in sporadic pilocytic astrocytomas have only recently been identified. BRAF aberrations, most commonly in the form of tandem duplication at chromosome 7q34 containing a *BRAF–KIAA1549* gene fusion occur in 66–70% of the cases. These findings implicate aberrant activation of the MAPK pathway due to gene duplication or mutation of BRAF as a molecular mechanism of pathogenesis in low grade astrocytomas and suggest inhibition of the MAPK pathway as a potential treatment
- On the other hand, $BRAF^{V600E}$ missense mutations are infrequent (9%) but are strongly associated with extracerebellar location
- A variety of nonspecific gain and loss of genetic material from a number of chromosomes, including chromosomes 5, 6, 7, 8, 11, 12, 15, 17, 19, 20, and 22 have been reported. Gains of 5 and 7 are most frequent
- Molecular cytogenetic studies demonstrated heterozygous PTEN/10q and homozygous p16 deletions in 6/19 (32%) and 3/15 (20%) cases of anaplastic pilocytic astrocytoma, respectively, but not in conventional tumors or histologically benign recurrences. Therefore, activation of the *PI3K/AKT* in addition to *MAPK/ERK* signaling pathways may underlie biological aggressiveness in pilocytic astrocytoma
- *TP53* mutations and aberrant *PDGF* signaling are usually absent but TP53 protein immunohistochemical expression is occasionally present
- *IDH* mutations are lacking as is *EGFR* amplification
- Pilocytic astrocytomas show extremely high expression of APOD and galectin-3, unlike diffuse astrocytomas

Pleomorphic Xanthoastrocytoma

- Definition
  - Pleomorphic xanthoastrocytoma (PXA) is a rare form of localized, typically noninfiltrative astrocytoma of somewhat favorable outcome that occurs in superficial cortical locations in children and young adults. Most PXAs are thought to conform to WHO grade II tumors; however, grade III PXAs are not uncommon
- Clinical features
  - Children and young adults (first to third decades)
  - Almost invariably cerebral, predominantly involving the superficial temporal lobe with frequent meningeal involvement
  - Most patients present with seizures
  - Frequently shows radiographic cyst formation with an enhancing mural nodule
- Pathologic features
  - Grossly, the xanthomatous change may impart a yellowish discoloration
  - Relatively discreet tumor of moderate cellularity
  - Morphologically variable showing areas of spindled, reticulin-rich, and intensely GFAP-positive astrocytic cells admixed with areas comprised of large polygonal, lipid-rich astrocytes
  - Some of the tumor cells are characteristically highly pleomorphic with large bizarre, multinucleated nuclei and nuclear pseudoinclusions (Fig. 16.3). Mitoses are typically infrequent but when increased (>5/10 high power fields [hpf]) signify an anaplastic change (WHO grade III)
  - Eosinophilic granular bodies and perivascular lymphocytes are present
- Genetic findings
  - Recently, PXAs have been shown to harbor the highest frequency of $BRAF^{V600E}$ missense mutations within CNS tumors.

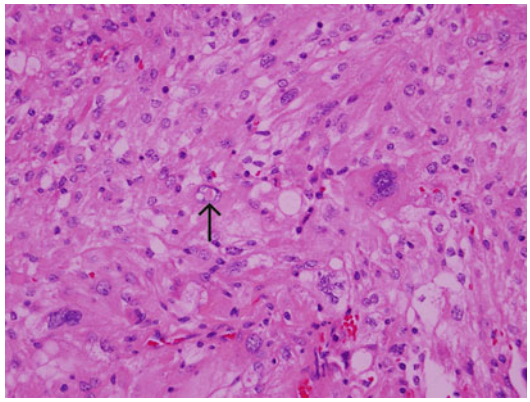

Fig. 16.3 Pleomorphic xanthoastrocytoma: large bizarre-shaped astrocytes, one with nuclear pseudoinclusion (*arrow*), are scattered in a background of low grade astrocytes

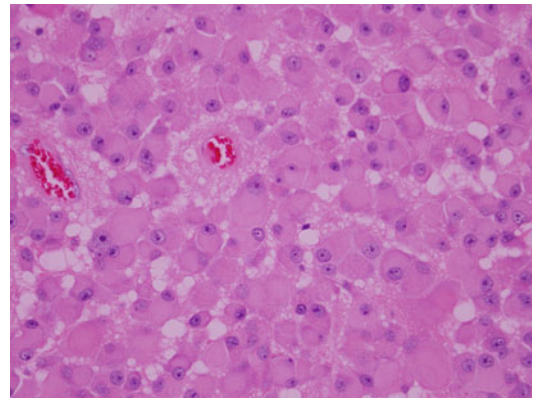

Fig. 16.4 Subependymal giant cell astrocytoma (SEGA): Sheets of loosely arranged, relatively uniform, round/epithelioid, tumor cells exhibiting large round vesicular nuclei with nucleoli and granular eosinophilic cytoplasm

Approximately two-thirds of PXAs, conventional and anaplastic, are involved
- Loss of chromosome 9, usually corresponding to 9p21.3 locus that includes the *CDKN2A/p14^{ARF}/CDKN2B* gene complex, is found in approximately 50% of PXAs
- *TP53* mutations have been reported in a small subset of PXAs (<10%)
- Unlike diffuse astrocytomas, alterations of the *EGFR*, *CDK4*, and *MDM2* genes, *PTEN* mutations, or LOH 10q have not been found in PXA
- Chromosome 1q abnormalities, gains of chromosomes 3 and 7, LOH 17, and LOH 22q have occasionally been reported

Subependymal Giant Cell Astrocytoma

- Definition
  - A benign (WHO grade I), well-circumscribed, intraventricular neoplasm of children and young adults that is almost always present in association with tuberous sclerosis (TS)
- Clinical features
  - Predilection for children and young adults
  - Preferentially located in the region of the foramen of Monroe
  - Most patients present with signs and symptoms of obstructive hydrocephalus
  - Almost exclusively associated with TS
  - Radiographically appear as well-circumscribed, contrast-enhancing, intraventricular masses, often with calcification
- Pathologic features
  - Well-circumscribed, sharply demarcated from adjacent parenchyma
  - Comprised of fascicles of large spindled and epithelioid cells with special perivascular arrangements/pseudorosettes
  - Tumor cells are uniquely characterized by large round vesicular nuclei with nucleoli resembling neurons and brightly eosinophilic astrocytic cytoplasm (Fig. 16.4)
  - GFAP immunoreactivity is usually present but variable. Neuronal markers may be positive
- Genetic findings
  - Most common CNS tumor of the TS complex (6–16%)
  - Lack of *RB* gene protein expression and focal p53 immunopositivity have been shown in approximately 60% of cases
  - Occasional reports showed LOH and allelic mutation of *TSC2*
  - In a study of eight subependymal giant cell astrocytomas (SEGAs), six tumors demonstrated expression of *TSC1* gene product (hamartin) or *TSC2* gene product (tuberin) but not both, suggesting that these tumors arose from either mutation of *TSC1* or *TSC2* genes. Paradoxically, two additional cases

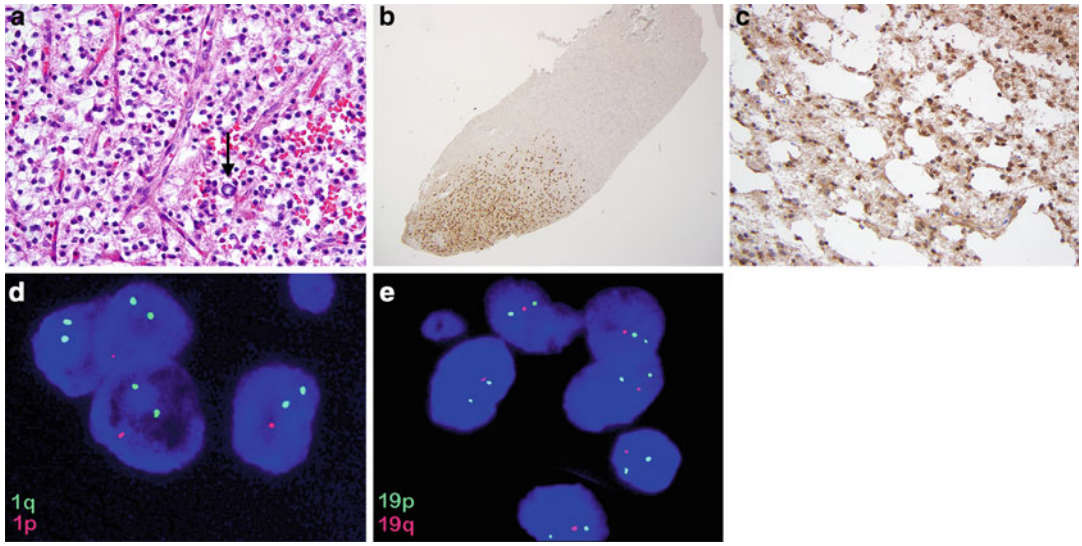

Fig. 16.5 Oligodendroglioma: (**a**) this WHO grade II oligodendroglioma shows a moderately cellular tumor comprised of uniform cells with the characteristic "fried-egg" appearance and the round nuclei. Note the branching capillaries (chicken-wire) and focal calcification (*arrow*). (**b**) IDH1-R132H immunohistochemistry can be of exceptional value in identifying the infiltrative edge of oligodendroglioma as in this example. (**c**) While IDH1-R132H staining pattern is cytoplasmic, a variable nuclear immunoreactivity is usually detectable. (**d**, **e**) Dual color FISH assays showing loss of 1p and 19q, respectively

showed expression of both gene products reflecting inactivation by alternative means

Oligodendroglioma

- Definition
  - A common form of infiltrative glioma of intermediate differentiation that shows predilection for the cerebral hemispheres of young adults and are thought to arise from oligodendroglial cells. Oligodendrogliomas may be WHO grade II or grade III (anaplastic oligodendroglioma). The existence of "grade IV" oligodendrogliomas is controversial and currently not recognized by the WHO
- Clinical features
  - Predominantly young adults
  - Rare in children and the very old
  - The subcortical white matter of the cerebral hemispheres (mostly frontal) is preferentially affected
  - Exceptionally rare in the cerebellum, brainstem, or spinal cord
  - Patients may present with focal neurological deficits, including seizure disorder, or more generalized symptoms as a result of increased intracranial pressure
  - Radiographically appear as fairly demarcated lesions in the cortex or subcortical white matter with no significant enhancement or peritumoral edema. Calcification is frequent
  - Radiographic enhancement and prominent peritumoral edema usually indicate anaplastic change
- Pathologic features (Fig. 16.5a)
  - Microscopically infiltrative, moderately cellular tumor with frequent calcification
  - Cortical invasion is essentially a constant feature, often producing the so-called secondary structures of Scherer
    - Perineuronal satellitosis
    - Perivascular condensation
    - Subpial accumulation
  - A rich network of thin-walled, branching capillaries (chicken-wire vasculature)
  - The uniform tumor cells are characterized by round to oval nuclear morphology with

smooth contour and indistinct chromatin pattern. Mitoses may be scattered. The cells may acquire perinuclear clearing secondary to a formalin-fixation artifact (fried-egg appearance)
  - Anaplastic features include hypercellularity, prominent mitotic activity (≥6/10 hpf), prominent microvascular proliferation, and increased proliferative index (>5%)
  - Typically, oligodendrogliomas are not reactive for GFAP; however, they occasionally may contain two types of GFAP-positive tumor cells
    - Those resembling small gemistocytes (minigemistocytes)
    - Gliofibrillary oligodendrocytes, which are otherwise histologically identical to oligodendroglioma cells
  - The vast majority of oligodendrogliomas are immunoreactive for anti-IDH1–R132H (Fig. 16.5b, c)
- Genetic findings (Fig. 16.5d, e)
  - LOH 19q is the most common genetic alteration (50–80% of all oligodendroglial tumors). The loss usually involves the entire arm due to an unbalanced t(1;19)(q10;p10) translocation. Partial deletions are rare
  - LOH 1p is the second most common genetic alteration. The loss also involves the entire chromosomal arm due to an unbalanced t(1;19)(q10;p10) translocation. Partial deletions are rare
  - LOH 1p is almost always associated with LOH 19q
  - Codeletion of chromosomal arms 1p and 19q as identified by LOH and FISH, is found in 50–90% of oligodendrogliomas and constitutes a unique "genetic signature"
  - 1p/19q codeletion is associated with enhanced survival and favorable response to chemotherapy and/or radiation therapy. 1p loss alone also shows a similar effect
  - The mechanism through which 1p/19q status influences therapeutic sensitivity in oligodendrogliomas is unknown. It remains unclear whether the 1p or 19q chromosomal arm harbors relevant tumor suppressor genes or any oligodendroglioma-specific genes for that matter
  - 1p/19q codeletion has been shown by some to correlate with chemoresponsiveness, irrespective of tumor morphology
  - Commercial laboratory testing for 1p/19q codeletion is readily available using FISH, LOH, and quantitative microsatellite analysis, though dual color FISH has emerged as the method of choice in many laboratories
  - 1p/19q codeletion has been reported in a small percentage of astrocytic tumors (<1%)
  - In pediatric oligodendrogliomas, codeletion 1p/19q is found at a much lower frequency (27%) compared with their adult counterparts. Deletions of *p16* (*CDKN2A*) and 10q were reported in 45% and 18% of cases, respectively. Interestingly, these molecular alterations including 1p/19q status have not been shown to correlate with biological behavior
  - Recently, *IDH* mutations have been found to be highly expressed in oligodendrogliomas of WHO grade II and III. They strongly correlate with 1p/19q codeletion status, found in up to 100% of 1p/19q codeleted oligodendrogliomas. Oligodendrogliomas lacking *IDH* mutations often show intact 1p/19q
  - Somatic mutations and insertions/deletions in the *CIC* gene on chromosome 19q13.2 have been found in 20/29 (69%) of oligodendrogliomas showing combined 1p/19q deletions and *IDH1/IDH2* mutations. In contrast, only 1/60 (2%) oligodendrogliomas and oligoastrocytomas without 1p/19q deletions showed *CIC* mutations. These findings suggest a functional interaction between *CIC* mutation, *IDH1/2* mutation, and 1p/19q codeletion
  - Unlike astrocytic neoplasms, loss of 17p, *TP53* mutation and p53 expression are uncommon (~10–20%). Their presence is mutually exclusive to 1p/19q deletion and shows no prognostic significance
  - EGFR immunohistochemical expression, not amplification, is common (~50%) in grades II and III. However, both *EGFR*

amplifications and 10q deletions are said to be extremely uncommon in pure oligodendrogliomas
- *PTEN* alterations have been implicated in oligodendroglioma progression to anaplasia and worsened survival primarily in those with intact 1p and 19q
- *MGMT* promoter hypermethylation and reduced expression is particularly common among 1p/19q-deleted oligodendrogliomas. Aberrant promoter methylation of a number of tumor suppressor genes (*CDKN2A*, *CDKN2B*, *RB1*, $p14^{ARF}$, *TP53*, *ESR1* (estrogen receptor 1) and *DAPK1* (death-associated protein kinase 1) is also common
- *PDGF* and its receptors are expressed in the vast majority of cases
- Overexpression of VEGF and decreased expression of p27 are seen in a subset of oligodendrogliomas, being inversely associated with tumor grade. However, their prognostic significance remains unclear
- Anaplastic oligodendrogliomas demonstrate a higher frequency of multiple chromosomal deletions including gains on 7 and 15q and losses on 4q, 6, 9p, 10q, 11, 13q, 18, and 22q
- Chromosomes 9p and 10 abnormalities, including *CDKN2A* gene (encoding $p16^{NK4A}$ and $p14^{ARF}$) deletions, are more frequent in anaplastic oligodendroglioma (~30% and 10%, respectively) suggesting a role in oligodendroglial tumor progression paralleling that observed in malignant astrocytic tumors. The presence of these deletions is predictive of shortened survival. $p16^{NK4A}$ deletions occur in oligodendrogliomas, irrespective of their 1p/19q status

Oligoastrocytoma

- Definition
  - An infiltrative glial neoplasm that is comprised of two types of cells morphologically resembling those seen in oligodendroglioma and diffuse astrocytoma. The concept of oligoastrocytoma has been widely endorsed by most neuropathologists over the past two decades. Grading of oligoastrocytoma is similar to that of oligodendrogliomas, except that the 2007 WHO classification of CNS tumors now recognizes a grade IV variant (also designated glioblastoma with oligodendroglial features). The diagnosis of the latter requires identification of necrosis
- Clinical features
  - Indistinguishable from those of pure oligodendrogliomas and pure diffuse astrocytoma, though their preferential involvement of the cerebral hemispheres is more closely aligned with oligodendrogliomas
  - Extremely rare in the brainstem, cerebellum, and spinal cord
- Pathologic features
  - According to the WHO, oligoastrocytomas are divided into biphasic (compact) and intermingled (diffuse) variants
  - The intermingled (diffuse) variant is the most frequent and shows both components intimately admixed. This variant often contains nuclei intermediate between those of oligodendroglioma and diffuse astrocytoma
  - The biphasic (compact) variant shows two distinct components displaying oligodendroglial and astrocytic differentiation
  - Anaplastic features include frequent mitotic activity, nuclear pleomorphism, microvascular proliferation, and high proliferative index
  - Similar to oligodendroglioma, oligoastrocytomas show high level of IDH1 immunohistochemical expression, particularly in the oligodendroglial component
- Genetic findings
  - Oligoastrocytomas are monoclonal neoplasms arising from a single progenitor cell; i.e., showing the same genetic alterations throughout the tumor regardless of morphologic component. Nevertheless, oligoastrocytomas are genetically heterogeneous and may assume genetic features similar to those of either oligodendrogliomas or diffuse astrocytomas

- 10–50% of oligoastrocytomas show codeletion of 1p/19q
- Loss of 19q alone is particularly common in oligoastrocytomas, often associated with a favorable outcome
- 30% of oligoastrocytomas showed genetic alterations common to astrocytic tumors (*TP53* mutations/LOH 17p, *EGFR* gene amplification, chromosome 10 abnormalities, *p16* deletions, and so on) and these patients had significantly shortened survival

Ependymoma

- Definition
  - A relatively well-circumscribed glial neoplasm arising from the ependymal cells lining the ventricles and the spinal canal. Except for the myxopapillary variant (WHO grade I), ependymomas are either WHO grade II or grade III (anaplastic ependymoma)
- Clinical features
  - May occur at any age but shows two age peaks; 0–16 years for infratentorial ependymomas and 30–40 years for spinal cord ependymomas
  - Third most common brain tumor in children
  - May occur at any site, including occasionally outside the ventricular system; most common in posterior fossa (children) and spinal cord (adults)
  - Most common glioma of the spinal cord
  - Presentation highly dependent on primary location. Obstructive hydrocephalus is frequent for intraventricular ependymomas
  - Radiographically appear as well-circumscribed, variably contrast-enhancing lesions. Cystic change and syrinx are common in supratentorial and spinal locations, respectively. Spinal examples are intraaxial, sausage-shaped lesions
- Pathologic features (Fig. 16.6)
  - Sharp demarcation from adjacent parenchyma

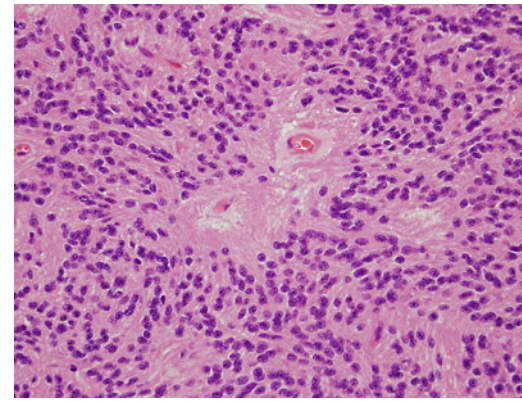

Fig. 16.6 Ependymoma: this WHO grade II ependymoma shows a moderately cellular tumor consisting of monomorphic population of cells with oval nuclei. Note the characteristic perivascular pseudorosettes toward the center of the image

  - Moderately cellular tumors made up of sheets of cells interrupted by perivascular pseudorosettes and occasionally true ependymal rosettes/canals
  - Ependymal cells show round to oval nuclei with small nuclei and rare mitoses
  - GFAP is usually positive, but highly variable, in processes converging on blood vessels. Epithelial membrane antigen (EMA) positivity is seen in a dot-like pattern and/or small intracytoplasmic lumina
  - Characteristic ultrastructure: intracytoplasmic lumina, cilia/microvilli, long intercellular junctions, and intermediate filaments
  - In addition to conventional ependymoma (WHO grade II), several histologic variants exist
    - Myxopapillary ependymoma (WHO grade I): Almost exclusively seated in the area of the filum terminale in young adults, this variant is characterized by prominent, hyalinized papillae embedded in a mucoid background
    - Cellular ependymoma (WHO grade II)
    - Papillary ependymoma (WHO grade II)
    - Tanycytic ependymoma (WHO grade II)
    - Clear cell ependymoma (WHO grade III)
  - Anaplastic features include hypercellularity, increased mitotic activity, and microvascular proliferation

- Genetic findings
  - Ependymomas appear to be genetically distinct from other gliomas as they lack genetic alterations of astrocytomas and oligodendrogliomas, except for chromosome 19 alteration
  - There is increasing evidence that spinal and intracranial ependymomas represent two distinct tumor subsets, both clinically and genetically
  - About 40% of ependymomas show no detectable genetic alterations
  - Spinal ependymomas are a manifestation of NF2 syndrome with *NF2* gene mutations (22q12) almost exclusively found in spinal ependymomas
  - Overall, 30% of adult ependymomas show chromosome 22 abnormalities (deletions, translocations, monosomy), showing an association with a spinal location, often showing concomitant *NF2* mutation. Intracranial examples with 22q deletions lack *NF2* mutation
  - Uncommon chromosomal aberrations include gain of 1q, 5q, 7, and 9 and losses involving chromosomes 6q, 9, 10, 13, 16, and 17p in pediatric intracranial ependymomas and gain of chromosome 7 in spinal ependymomas
  - Deletions involving *4.1B*, (*DAL-1*) and/or monosomy 18 have been detected in up to 67% of clear cell ependymomas. The latter may also demonstrate gains of chromosome 1q, and loss of chromosomes 9, 3, and 22q but lack deletions of 1p, 19q, and *NF2*. Loss of *4.1B* correlates with pediatric, intracranial and anaplastic ependymomas
  - Gain of 1q correlates with intracranial location and ERBB2 and ERBB4 coexpression, found mostly in pediatric ependymomas, correlates with higher Ki67 proliferation indices and worse outcome
  - Chromosome 1q gain, loss of 9 and 13, and deletion of *4.1G* appear to correlate with progression to anaplasia
  - *IDH* and $BRAF^{V600E}$ mutations are lacking

Subependymoma

- Definition
  - A benign (WHO grade I), well-circumscribed intra- or subventricular glial neoplasm comprised of sparsely cellular clusters of ependymal-like cells embedded in a fibrillary matrix with microcyst formation. Subependymomas are WHO grade I lesions
- Clinical features
  - May occur at any age; most common in adults
  - The fourth ventricle, followed by the lateral ventricles, is the most frequent site. Uncommon in the spinal cord
  - Usually asymptomatic and incidentally detected
  - Radiographically appear as sharply demarcated, nonenhancing, nodular intraventricular masses. May occasionally calcify and hemorrhage
- Pathologic features
  - Sharp demarcation from underlying parenchyma
  - Tumor is made up of microscopic islands and clusters of ependymal-like cells embedded in a background rich in fibrillary matrix
  - Prominent microcyst formation
  - Bland, round to oval nuclei with no significant nuclear pleomorphism and absent or rare mitoses
  - Examples of mixed ependymoma/subependymoma exist
- Genetic findings
  - Consistent genetic alterations have not been identified

Astroblastoma

- Astroblastoma is a rare glial tumor of uncertain origin
- Usually manifests in children and young adults as a well-circumscribed, contrast-enhancing solid or cystic hemispheric mass
- Histologically, it combines ependymal features (perivascular pseudorosettes) with astrocytic differentiation (GFAP-positive cells with

broad, nontapering processes). Vascular hyalinization is prominent
- No WHO grade has been assigned to astroblastoma yet, but they may be qualified as low or high grade
- Consistent genetic alterations have not been identified

Chordoid Glioma of the Third Ventricle

- This is a rare, low grade (WHO grade II) glioma of the third ventricle of adults that is histologically characterized by cords and clusters of epithelioid, GFAP-positive cells embedded in a mucin-rich background containing lymphoplasmacytic infiltrate
- Chromosomal losses at 11q13 and 9p21 have been reported. *EGFR* amplifications and *TP53* mutations are absent

Angiocentric Glioma

- Angiocentric glioma (WHO Grade I) is a rare, slow growing, newly described entity identified in children and young adults
  - Patients usually present with seizures
- Histologically, angiocentric glioma is characterized by uniform, spindle-shaped, cells with oval to elongated nuclei and speckled chromatin, and pink, tapering cytoplasm
  - Tumor cells are intimately associated with vessels; often oriented parallel to the vessels and may form streaming arrays in perivascular space with either single or multilayered cells
  - Mitoses are generally absent with a low MIB1 proliferation index
  - Tumor cells are strongly GFAP positive and show dot-like EMA staining pattern, like ependymoma
- Consistent genetic alterations have not been identified

Mixed Glioneuronal Neoplasms

Ganglion Cell Tumors

- These include gangliogliomas and gangliocytomas

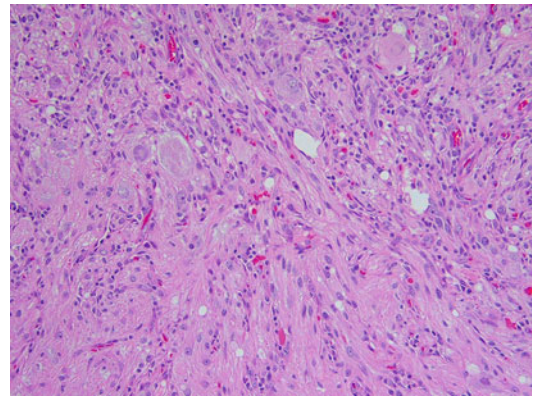

Fig. 16.7 Ganglioglioma: large dysmorphic neurons are scattered in a background of haphazardly-arranged low grade astrocytes. Note that the tumor is peppered with perivascular lymphocytes

- Gangliogliomas (WHO grade I or II) is the prototypical mixed glioneuronal neoplasm in which a well-circumscribed neoplastic glial component (pilocytic or fibrillary astrocytoma) contains dysmorphic ganglion cells either in clusters or individual cells (Fig. 16.7)
- Gangliocytoma (WHO grade I) occurs when the tumor is made up predominantly of clusters of large dysmorphic ganglion cells in a background of nonneoplastic glial elements
- Recently, approximately 20% of gangliogliomas have been shown to harbor $BRAF^{V600E}$ missense mutations, second only to PXAs in frequency
  - Gains of chromosome 7 were the most recorded

Dysembryoplastic Neuroepithelial Tumor

- Dysembryoplastic neuroepithelial tumor (DNET) is a benign (WHO grade I) intracortical glioneuronal neoplasm that preferentially affects children and young adults often with a protracted history of seizure disorder
- Consistent genetic alterations have not been identified

Desmoplastic Infantile Astrocytoma/Ganglioglioma (DIG)

- Desmoplastic infantile astrocytoma and desmoplastic infantile ganglioglioma are benign

(WHO grade I) glial neoplasms of infants <2 years of age
- They typically appear as radiographically large, complex, solid, and cystic hemispheric masses with contrast enhancement and midline shift
  - Both are characterized by inconspicuous glial elements embedded in a remarkably desmoplastic stroma resembling mesenchymal neoplasms
- Neuronal elements are additionally seen in DIG
- Consistent genetic alterations have not been identified

Papillary Glioneuronal Tumor
- This is a rare, benign (WHO grade I) newly described entity
  - Predominantly identified in young adults with seizure disorder
- Characterized by a well-circumscribed, solid to cystic, contrast-enhancing mass in the cerebral hemispheres (temporal)
  - Shows an obvious papillary/pseudopapillary architecture at low power and is made up of two distinct cell types surrounding a central fibrovascular core
  - An innermost layer of small, cuboidal GFAP-positive, cells with eosinophilic cytoplasm and round nuclei and an outer layer of larger clear, synaptophysin-positive, cells with neurocytic or ganglioid appearance
  - Mitoses are rare or absent and the Ki67 labeling index is very low
- Consistent genetic alterations have not been identified

Rosette-Forming Glioneuronal Tumor of the Fourth Ventricle
- This is another rare, benign (WHO grade I) newly described entity
  - Found exclusively in the posterior fossa of adults
  - Often occupying a large portion of the fourth ventricle
- Radiographically, the tumor is a circumscribed, solid mass with heterogeneous enhancement
- Characterized by a biphasic histology with clearly defined and spatially separate neurocytic and glial components
  - The neurocytic component shows a homogeneous population of small, clear cells in rosettes with large central spaces or papillae around central vessels and delicate processes forming a neuropil matrix adjacent to tumor cells and extending to central vessels
  - The glial component consists of solid pattern of highly fibrillary cells resembling pilocytic astrocytoma but may have an oligodendroglioma-like appearance with microcysts
  - Both neurocytic and glial components are histologically low grade with rare mitoses and low Ki67 labeling index
- Consistent genetic alterations have not been identified

Nonglial Tumors

Neuronal Tumors

Central Neurocytoma
- A low grade (WHO grade II), well-circumscribed neuronal neoplasm of young adults, preferentially situated in the lateral ventricles in the region of the foramen of Monroe
- Histologically, neurocytoma is comprised of nests of highly uniform round cells resembling those of oligodendroglioma
  - Tumor cells, which are embedded in a delicate fibrillar background, are characterized by a finely speckled chromatin pattern and infrequent mitoses
  - A network of fine capillaries and calcifications are frequent findings
  - Tumor cells are intensely immunoreactive for synaptophysin and NeuN but usually negative for GFAP and chromogranin
- No consistent genetic alterations reported; however, gains on chromosomes 2p, 7, 10q, 18q, and 13q were recorded in about 20% of cases. 1p/19q deletions are lacking

Paraganglioma

- Paragangliomas of the CNS are histologically identical to their systemic counterparts
  - In the CNS, the cauda equina is by far the most common location
  - Rare examples may manifest in the cerebellopontine angle, the sellar region, and pineal area
  - Those of the carotid body may show intracranial extension
- Genetic findings
  - Paragangliomas may occur in the setting of von Hippel–Lindau (VHL) syndrome and multiple endocrine neoplasia (MEN) types 2A and 2B
  - LOH 11q, LOH 3p, and LOH 1p have been reported

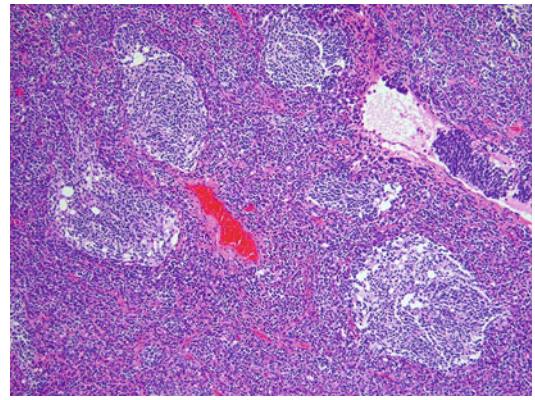

Fig. 16.8 Medulloblastoma: this nodular medulloblastoma shows neutrophil-rich islands embedded in a highly cellular, desmoplastic background. The cells within the nodules are better differentiated than the hyperchromatic and mitotic, carrot-shaped nuclei of the internodal areas

Embryonal Neoplasms

Medulloblastoma

- Definition
  - Medulloblastoma is a highly malignant (WHO grade IV), though exquisitely radiosensitive, primitive neuroectodermal cerebellar tumor with a tendency to spread along the cerebral spinal fluid (CSF) pathway
- Clinical features
  - Most commonly occurs in children, with 70% of cases occurring in individuals younger than 16 years and the peak age of presentation being 7 years
  - Also seen in young adults (ages 21–40)
  - Slight male predominance
  - By definition are located in the posterior fossa; majority arise in the vermis; hemispheric involvement increases with age
  - Clinical presentation includes cerebellar signs and symptoms and manifestations of CSF flow obstruction
  - Radiographically, classic medulloblastomas appear as central, homogeneously contrast-enhancing, solid cerebellar lesions
  - Leptomeningeal involvement is common
- Histopathologic variants
  - Classic medulloblastomas
    - Made up of sheets of back-to-back, relatively uniform small cells with intensely hyperchromatic nuclei and scant cytoplasm
    - Nuclei are round to oval or carrot-shaped and show abundant apoptotic bodies and frequent mitoses
    - Most often undifferentiated or show neuronal differentiation, but may occasionally demonstrate glial differentiation
  - Desmoplastic/nodular medulloblastoma (Fig. 16.8)
    - Characterized by a highly distinctive biphasic pattern of fibrillar, reticulin-free "pale" nodules embedded in a highly cellular, desmoplastic, and reticulin-rich background
    - Tumor cells within the nodules tend to be larger, more round, and far less anaplastic looking compared with the tightly packed and highly mitotic cells of the internodal areas
    - Nodules are typically intensely synaptophysin-positive but show a much lower proliferative activity than do the desmoplastic areas
  - Medulloblastoma with extensive nodularity
    - Usually seen in infants and is characterized by an expanded lobular architecture with large areas of neuropil-like tissue

harboring small round cells often in a streaming pattern, and markedly reduced internodular areas
  - Large cell/anaplastic medulloblastoma
    - A rare variant characterized by sheets and lobules of large, round, markedly pleomorphic cells with prominent nucleoli, frequent, often atypical, mitoses, and apoptosis
    - Nuclear molding and cell–cell wrapping are often seen
  - Medulloblastoma with myogenic differentiation
    - Any of the above variants that show skeletal muscle differentiation
  - Medulloblastoma with melanotic differentiation
    - Any of the above variants that demonstrate melanocytic differentiation
- Molecular variants
  - Gene expression profiling using genome wide microarray techniques have identified several molecular subgroups of medulloblastoma, based on characteristic gene expression profiles
  - Wingless (*WNT*) signaling pathway activation
    - 20% of medulloblastomas
    - Older children
    - Classic histologic subtype
    - Good prognosis
    - Activating mutations in the *WNT* pathway effector *CTNNB1*
    - Associated with monosomy 6
    - Immunohistochemistry: nuclear translocation of β-catenin
    - Cells of origin: lower rhombic lip and dorsal brainstem, therefore usually central location within the fourth ventricle
  - Sonic hedgehog (*SHH*) pathway activation
    - 10% of medulloblastomas
    - Children less than 3 years or older teenagers and adults
    - Desmoplastic/nodular or, less often, large cell/anaplastic histologic subtypes
    - Intermediate prognosis
    - Inactivating mutations in *PTCH1* seen in a subset of these patients
    - Associated with loss of chromosome 9
    - Cells of origin: granule cell precursors (external granular layer), therefore usually hemispheric locations
  - Non-WNT/SHH
    - 70% of medulloblastomas do not fall into the above two categories and are not associated with a particular cell signaling pathway; various studies have separated this group into anywhere from two to four subgroups
    - *MYC* amplification/overexpression
    - Isochromosome 17q
    - Large cell/anaplastic and other histologic subtypes
    - High levels of photoreceptor differentiation transcripts
    - Intermediate to poor prognosis
- General comments
  - Chromosome 17p deletion is the most frequent (30–60%) genetic alteration encountered in medulloblastoma. Isochromosome 17q (resulting from duplication of the long arm) is the most common mechanism through which 17p loss occurs. Interestingly, medulloblastoma shares a similar breakpoint to that observed in i(17q) of leukemia
  - Candidate tumor suppressor genes on 17p include *TP53*, *HIC1*, and *KCTD11*. *KCTD11* is involved in the *SHH* signaling pathway
  - Chromosome 17p loss by FISH has been detected almost exclusively in the context of large cell/anaplastic morphology
  - Abnormalities of 17p, particularly those arising in the absence of isochromosome 17q, have been linked to a more clinically aggressive behavior in some studies
  - Although some studies have linked the *TP53–ARF* tumor suppressor pathway to large cell/anaplastic medulloblastoma, the *TP53* gene, also located on chromosome 17p, does not appear to play a major role in the pathogenesis of sporadic medulloblastoma
  - Immunohistochemical overexpression of p53 has been shown to correlate with poor survival

  - *MYC* and *MYCN* gene amplifications occur with higher frequency in large cell medulloblastoma and therefore are associated with poor prognosis
  - Nuclear β-catenin is identified in 15–20% of medulloblastomas and usually indicates activation of the *WNT* signaling pathway and a better patient prognosis
  - Chromosome 1q gain is associated with poor prognosis whereas chromosome 6 and TRK-C expression indicate better prognosis
  - ERBB2 immunohistochemical overexpression, not gene amplification, is detected in up to 80% of medulloblastomas. High level expression (>50%) has been consistently associated with shortened patient survival
  - Chromosome 10q deletions, including *PTEN* (10q23) and *DMBT1* (10q25) tumor suppressor genes, have been reported in up to 40% of medulloblastomas. 10q loss also appears to correlate with large cell/anaplastic morphology
  - Chromosome 1q abnormalities, gain of chromosome 7, and chromosome 11 loss may also be seen
- Hereditary syndromes associated with medulloblastoma
  - Li–Fraumeni syndrome; *TP53* (17p13.1)
  - Nevoid basal cell carcinoma syndrome (Gorlin syndrome); *PTCH* (9q22.3)
    - Associated with *SHH* activation pathway
    - Medulloblastoma is of the desmoplastic (nodular) variant
    - Inactivating mutations of the *PTCH* gene have also been identified in a small subset of sporadic desmoplastic medulloblastoma
  - Turcot syndrome; *APC* (5q21–q22) and β-catenin mutations
    - Associated with *WNT* activation pathway
  - Rubenstein–Taybi syndrome; *CBP* (16p13.3)
  - Coffin–Siris syndrome
- Prognosis
  - High risk patients
    - Those who are 3 years old or younger
    - Those with metastases at presentation
    - Those with subtotal surgical resection
  - Standard risk patients
    - Those greater than 3 years of age, up to age 22 years
  - Overall survival rate of 70–80% for standard-risk patients, but only 40–60% in high-risk patients
  - Survivors often suffer long-term therapy-related effects, including neurocognitive sequelae, endocrine deficits, and second malignancies
  - Understanding the molecular basis of medulloblastoma is important in refining patient stratification in order to employ risk-adapted adjuvant therapies, and in the development of novel molecular targeted therapies
  - Small molecule inhibitors of *SHH* pathway are already being used to treat this subset of patients

Central Nervous System/Supratentorial Primitive Neuroectodermal Tumor (PNET)

- Highly undifferentiated, malignant (WHO grade IV), embryonal tumors of the cerebrum that do not conform to any of the currently defined histologic entities are given this designation. Several variants exist, including:
  - Ependymoblastoma
    - Affects neonates and young children
    - Histologically characterized by formation of a distinctive form of pseudostratified rosettes known as "ependymoblastic rosettes"
  - Neuroblastoma and ganglioneuroblastoma
    - Supratentorial embryonal tumors that show unequivocal neuroblastic differentiation (neuroblastic/Homer Wright rosettes, fibrillar background, and immunohistochemical evidence of neural differentiation) may be termed "neuroblastomas"
    - The additional presence of mature ganglionic cells defines "ganglioneuroblastoma"
  - CNS medulloepithelioma
    - Manifests immature tubular, trabecular or sometimes papillary arrangements of

primitive neuroepithelial cells resembling the embryonic neural tube
- Because of the heterogeneity of this group of tumors, studies of genetic abnormalities are limited

Atypical Teratoid Rhabdoid Tumor (AT/RT)

- Definition
  - A highly malignant (WHO grade IV) embryonal CNS tumor of infants and very young children showing rhabdoid features
- Clinical features
  - Vast majority of patients are under 2 years of age
  - Over 95% of cases are intracranial, and the majority is located in the posterior fossa
  - Spinal examples are rare
  - Slight male predominance
  - Clinical and radiographic features are similar to those of medulloblastoma and other CNS embryonal tumors
- Pathologic features
  - Architecturally complex, made up of a mixture of large, somewhat rhabdoid cells with reniform nuclei, prominent nucleoli and abundant eosinophilic cytoplasm, and smaller cells resembling those of medulloblastoma
  - Tight fascicles of small spindle cells giving the tumor a mesenchymal appearance may be present
  - Some tumor cells may be artificially vacuolated
  - Mitoses, necrosis, and calcifications are common
  - Complex immunohistochemical profile, often expressing EMA, GFAP, actin, and cytokeratins
- Genetic findings
  - Similar to renal and other extrarenal rhabdoid tumors, over 90% of AT/RTs demonstrate loss of all or part of chromosome 22, particularly involving 22q11.2
  - The *INI1* (*hSNF5/SMARCB1/BAF47*), a putative suppressor gene, was mapped to the 22q11.2 region. *INI1* deletions and/or mutation have been detected in the majority of AT/RT cases
  - Nearly all AT/RTs demonstrate absence of the nuclear immunohistochemical expression of *INII/BAF47* protein
  - FISH for monosomy 22, 22q deletion or the *INII* gene are commonly utilized as adjunct molecular studies in the diagnosis of AT/RTs and other pediatric embryonal tumors
  - Germline *INII* mutations have been detected in a minority of cases, representing a rhabdoid tumor predisposition syndrome

Meningeal Neoplasms

Meningioma

- Definition
  - A very common, relatively benign (usually WHO grade I), slowly growing, well-circumscribed tumor of the meninges and dura, thought to arise from the arachnoidal cap cells
- Clinical features
  - Primarily adults, with a peak incidence in the sixth and seventh decades
  - Exceedingly rare in children and the very old, but higher incidence of atypical forms
  - Female predominance; more pronounced for spinal meningiomas
  - May occur at any location within the CNS; most common over the cerebral convexities
  - Clinical presentation highly dependent on location; may be incidental finding
  - Radiographically appear as well-circumscribed, isointense, homogeneously contrast-enhancing, dural-based masses with a "dural tail" sign in the majority of the cases
- Pathologic features (Fig. 16.9)
  - Grossly appear as discrete, firm or rubbery masses with broad dural attachment and a characteristically lobular cut surface
  - Typical meningiomas are made up of tight whorls, lobules, or bundles of uniform, bland spindled cells with oval nuclei characterized by a delicate chromatin pattern with occasional central clearing, and infrequent mitoses

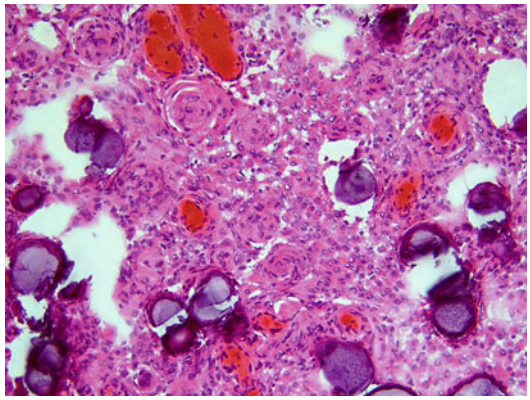

Fig. 16.9 Meningioma: a classic meningioma shows tight whorls and sheets of uniform spindled cells with bland vesicular nuclei. Calcifications in the form of psammoma bodies are abundant

- Calcification in the form of psammoma bodies is common
- Meningiomas are almost always EMA-positive
- Histological variants that do not influence the clinical outcome (WHO grade I)
  - Meningothelial (syncytial)
  - Transitional
  - Fibrous
  - Microcystic
  - Secretory
  - Psammomatous
  - Angiomatous
  - Lymphoplasmacyte-rich
  - Metaplastic
- Histologic variants with less favorable outcome
  - Clear cell (WHO grade II)
  - Chordoid (WHO grade II)
  - Papillary (WHO grade III)
  - Rhabdoid (WHO grade III)
- Atypical meningioma (WHO grade II) is characterized by a mitotic index ≥4/10 hpf, brain invasion, or at least three of the following softer criteria: loss of whorl formation, hypercellularity, high proliferative index (>4%), small cell change, macronucleoli, spontaneous necrosis, and loss of progesterone receptor expression
- Malignant (anaplastic) meningioma (WHO grade III) is characterized by a mitotic index ≥20/10 hpf or frank anaplasia (sarcoma, carcinoma, or melanoma-like histology)

• Genetic findings
  - Chromosome 22 loss; usually in the form of monosomy 22 is the most frequent chromosomal abnormality, encountered in about 40–70% of meningiomas
  - Allelic losses on chromosome 22 point to *NF2* as the major tumor suppressor gene in meningiomas
  - *NF2* gene mutations are common (~70% of sporadic meningiomas), and involve fibrous and transitional meningiomas most frequently. *NF2* gene mutations are much less frequent (~25%) in meningothelial meningiomas
  - *NF2* gene mutations are as frequent in atypical/anaplastic meningiomas as they are in grade I meningiomas, suggesting a role for *NF2* inactivation in the formation, rather than the progression of meningioma
  - Meningiomas are one of the most common neoplasms to arise in the setting of NF2 and often multiple
  - Meningiomas with *NF2* gene mutations show loss of expression of its protein product merlin (schwannomin)
  - In addition to merlin, two other membrane-associated proteins of the protein 4.1 family have been implicated in meningioma tumorigenesis: protein 4.1B (DAL-1) and protein 4.1R
  - Tumor suppressor in lung cancer 1 (*TSLC1*) has also been implicated in meningioma tumorigenesis
  - In general, meningiomas tend to acquire more chromosomal abnormalities, typically deletions of chromosomal regions other than the frequent 22q loss, as they progress from benign to WHO grades II and III
  - Atypical and anaplastic meningiomas show additional allelic losses involving chromosomes 1p, 6q, 9q, 10q, 14q, 17p, and 18q, with deletions of 1p, 10q, and 14q being most frequent. 1p deletions, the most common structural alteration, and the combined deletion of 1p/14q have been shown to

correlate with increased risk of recurrence and shorter progression-free survival
  - Atypical and anaplastic meningiomas show chromosomal gains involving 1q, 9q, 12q, 15q, 17q, and 20q
  - Losses of *CDKN2A/p16* region on chromosome 9p21 are seen in the majority of anaplastic meningiomas and seem to indicate poor outcome compared with anaplastic meningiomas lacking 9p21 deletions. Therefore, FISH analysis of 9p21 may be of prognostic significance in anaplastic meningioma
  - *TP53* gene alterations are very infrequent but immunohistochemical expression of p53 protein may be detected in a small percentage of higher grade meningiomas
  - *PTEN* mutations are occasionally encountered
  - Clonality studies suggest that multiple meningiomas are usually monoclonal
  - FISH studies for 22q12 (*NF2*), 18q11.3 (*4.1B*), 1p36 (*4.1R*), and 14q status may be useful in supporting the diagnosis of anaplastic meningioma or ruling out other malignancies

Hemangiopericytoma

- Similar to meningiomas, hemangiopericytomas of the CNS are usually dural-based. They have a tendency to occur at a slightly younger age and show a slight male predominance. Histologically, hemangiopericytomas of the CNS are indistinguishable from those occurring in other locations
- Hemangiopericytomas are aggressive neoplasms with potential for recurrence and distant metastases. They may be WHO grade II or III
- No consistent genetic alterations reported

Solitary Fibrous Tumor

- Clinically and radiographically, solitary fibrous tumors of the CNS are indistinguishable from meningiomas. Histologically, they are no different from those arising in somatic soft tissues
- No consistent genetic alterations reported

Choroid Plexus Tumors

- Definition
  - These are intraventricular, papillary epithelial neoplasms derived from the choroid plexus epithelium. They may be benign (papilloma; WHO grade I) or malignant (carcinoma; WHO grade III)
- Clinical features
  - Most common in children, may be congenital
  - Overall, papillomas are much more common than carcinomas; however, carcinomas occur with higher frequency in children
  - Most common in the lateral and third ventricles in children and fourth ventricle in adults
  - Patients present with signs and symptoms of increased intracranial pressure
  - Radiographically appear as solid, homogeneous, contrast-enhancing, intraventricular masses, often accompanied by hydrocephalus
- Pathologic features
  - Choroid plexus papillomas (CPP) are composed of delicate papillary fronds covered by a layer of well-differentiated columnar epithelium characterized by uniform oval to round basal nuclei and absent or rare mitoses
  - Choroid plexus carcinomas (CPC) on the other hand may lack conspicuous papillary areas and be entirely solid. Their cells typically show high grade features with frequent mitoses and necrosis
  - Choroid plexus tumors are generally positive for S100, synaptophysin, and keratin
- Genetic findings
  - *VHL* allele loss may be observed in CPPs associated with VHL disease
  - CPP rarely occurs in association with Aicardi syndrome (psychomotor retardation, infantile spasm, corpus callosum agenesis, chorioretinal abnormalities)
  - CPP may also occur in the setting of Li–Fraumeni syndrome in which case *TP53* germline mutations have been detected

- CPP demonstrates hyperploidy with gains on chromosomes 5, 7, 8, 9, 12, 15, 17, 18, 20, and 21 and losses on chromosomes 10 and 22q
- CPC exhibits gains on chromosomes 1, 4, 8q, 9p, 12, 14q, 20q, and 21 and LOH 1p, 1q, 3p, 5q, 9q, 10q, 13q, 18q, and 22q
- CGH analysis suggests that pediatric and adult CPPs are genetically distinct
- Notch signaling pathway activation has been linked to a subset of CPPs
- Immunohistochemical expression for p53 is observed in most CPCs and rare CPPs but usually lack corresponding *TP53* mutations
- Despite reports of 22q loss and *INI1* alterations in CPCs, INI1 protein expression is retained, including in those with "rhabdoid morphology"
- PDGF overexpression and amplification is frequent and may provide a target for future therapy

Suprasellar and Sellar Tumors

Craniopharyngioma

- Craniopharyngioma is a relatively benign (WHO grade I) epithelial neoplasm of the sellar region that is thought to arise from Rathke pouch remnants
  - Two histological subtypes are recognized
    - Adamantinomatous
    - Papillary
  - The former is more common in children and is usually partially cystic while the latter occurs predominantly in adults in the region of the third ventricle
  - Compared with their papillary counterpart, adamantinomatous craniopharyngiomas are additionally characterized by the presence of wet keratin, calcifications, and cholesterol clefts (Fig. 16.10)
- No consistent genetic alterations reported

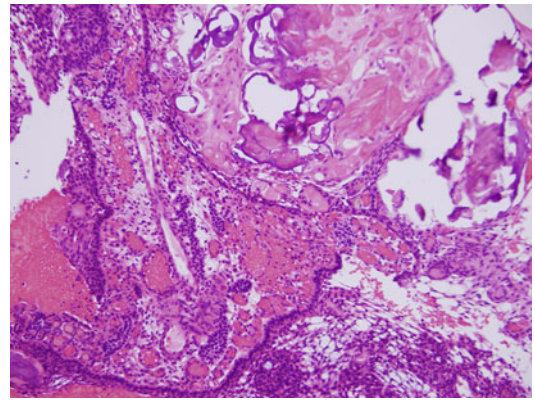

Fig. 16.10 Craniopharyngioma: adamantinomatous craniopharyngioma is characterized by palisaded squamous epithelium, wet keratin, and calcification

Pineal Parenchymal Tumors

- Pineal parenchymal tumors encompass a rare group of pineal region tumors that are thought to arise from pineocytes. These have a wide histological spectrum
  - Pineocytomas are relatively benign (WHO grade II) tumors of adults characterized by sheets or lobules of well-differentiated cells forming the distinctive "pineocytomatous" rosettes. The tumor cells have round nuclei with open chromatin pattern
  - Pineal parenchymal tumors of intermediate differentiation generally lack the "pineocytomatous" rosette formation and show higher degree of cellularity and atypia than pineocytomas. However, they are less cellular and less primitive looking than pineoblastoma
  - Pineoblastomas are highly malignant (WHO grade IV) tumors of children characterized by primitive embryonal morphology with rosettes of either the neuroblastic (Homer Wright) or the retinoblastic (Flexner–Wintersteiner) type
  - Papillary tumor of the pineal region (WHO grades II–III) has recently been described
- Apart from the rare pineoblastoma associated with familial bilateral RBs in the so-called "trilateral retinoblastoma syndrome" no consistent genetic alterations have been reported

Germ Cell Tumors

- Definition
  - Germ cell tumors of the CNS are rare, preferentially midline tumors of children and young adults that show similar characteristics to those arising in the gonads
- Clinical features
  - Children and young adolescents are most affected
  - Midline intracranial structures are most commonly involved (vast majority occur in the pineal and suprasellar regions), though they have been reported throughout the CNS
  - Pineal region tumors show male predominance
  - Pineal region tumors usually present with signs and symptoms of increased intracranial pressure while those of the suprasellar region may manifest due to visual symptoms or disturbances along the hypothalamic–hypophyseal axis (e.g., diabetes insipidus)
- Pathologic features
  - Morphologically identical to their gonadal and other extragonadal counterparts
  - Germinomas are by far the most common subtype (Fig. 16.11a)
- Genetic findings
  - Similar to testicular germ cell tumors, most CNS germinomas show overrepresentation of chromosome 12p, often manifesting as isochromosome 12p (Fig. 16.11b)
  - Patients with Klinefelter syndrome and Down syndrome appear to be more susceptible to develop germ cell tumors, including those of the CNS, than the average individual

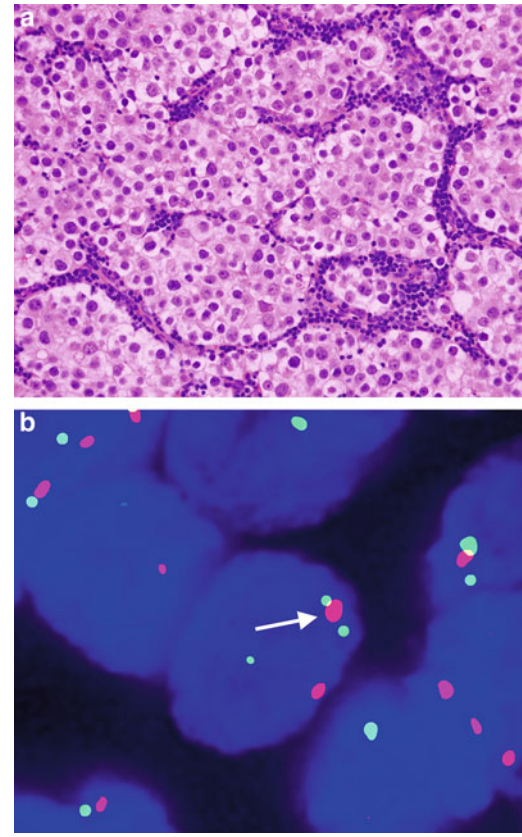

Fig. 16.11 Germinoma: (**a**) nests of polygonal tumor cells characterized by clear cytoplasm and large round nuclei with prominent nucleoli are decorated by thin fibrous septa-rich in lymphocytes. (**b**) Isochromosome 12p is a common finding in CNS germinoma (two *green* 12p signals closely juxtaposed to one *red* centromeric probe signal, *see arrow*)

Hemangioblastoma

- Definition
  - A benign (WHO grade I), richly vascular tumor of uncertain histogenesis
- Clinical features
  - Sporadic cases occur in adults while those associated with VHL tend to involve younger patients
  - Sporadic cases are largely limited to the cerebellum but those associated with VHL syndrome may be multiple and additionally manifest in the brainstem and spinal cord
  - Most show the characteristic radiographic appearance of a cyst with a contrast-enhancing mural nodule. "Flow voids" may be encountered
- Pathologic features
  - These are discrete neoplasms made up of a variable mixture of small capillaries and large vacuolated or lipidized stromal cells
  - The stromal cells' nuclei may show hyperchromasia and nuclear pleomorphism but mitoses are infrequent

  - Often cystic but occasionally solid
  - Mast cells may be a diagnostically helpful finding
- Genetic findings
  - Approximately 25% of hemangioblastomas occur in the setting of VHL syndrome
  - A minority of sporadic hemangioblastomas show mutations or deletions of the *VHL* gene
  - EGFR, VEGF, and VEGF receptors are expressed at high levels in the stromal cells

Schwannoma

- Definition
  - A benign (WHO grade I) slowly growing tumor that occurs throughout the peripheral nervous system but intracranially involves the vestibular division of the eighth cranial nerve. Only schwannomas of the CNS are discussed next
- Clinical features
  - Bilateral vestibular schwannomas are the hallmark of *NF2*
  - The vestibular division of the eighth cranial nerve is the most common intracranial location. Rare intracerebral and intramedullary examples have been reported
  - Patients may present with tinnitus, hearing difficulties, or facial paresthesias
  - Radiographically appear as well-circumscribed, often cystic, homogenously contrast-enhancing masses
- Pathologic features
  - Encapsulated tumors of moderate to low cellularity
  - Biphasic architecture
    - Antoni A areas: compact, elongated cells arranged in alternating fascicles, sometimes forming distinctive nuclear palisades (Verocay bodies)
    - Antoni B areas: loosely textured less cellular areas with more stellate looking cells
  - Thick hyalinized blood vessels with hemosiderin-laden macrophages
  - Aggregates of lipid-laden cells
  - Degenerative atypia in the form of nuclear pleomorphism is common but mitoses are infrequent
  - Prominent pericellular reticulin
  - Intensely and diffusely immunoreactive for S100
  - Histologic variants include cellular, plexiform, and melanotic schwannomas
- Genetic findings
  - The vast majority of schwannomas are sporadic
  - Schwannomas may arise in the setting of *NF2* or schwannomatosis (multiple peripheral schwannomas)
  - About 50% of psammomatous melanotic schwannomas are found in patients with Carney complex (autosomal dominant disorder with lentiginous facial pigmentation, cardiac myxoma, endocrine overactivity, and calcifying Sertoli cell tumors)
  - Inactivating mutations of the *NF2* gene are identified in about 60% of schwannomas
  - Some schwannomas may show loss of chromosome 22q in the absence of detectable *NF2* gene mutations
  - Loss of the immunohistochemical expression of the *NF2* gene product (merlin/schwannomin) is identified in the vast majority of schwannomas regardless of *NF2* gene mutations

Molecular Pathology of Neurodegenerative Diseases

Overview

- The ravages of advanced age affect both mind and body, and perhaps nowhere do we recognize this more so than with the degenerative diseases of the nervous system
- Combined now with the gross and microscopic changes that have been known for >50 years for many of the neurodegenerative ailments are a vast and rapidly expanding array of genetic and molecular data that have transformed how we look at and classify these diseases

- Many of these disorders have both sporadic and familial forms, providing clues to the pathogenetic basis of these diseases
- Some of these diseases have unique populations of cells affected and unique cytopathic changes, though overlapping neuropathological features are also common
- Neuropathological examination at autopsy is still the gold standard for diagnosing most neurodegenerative diseases
- Investigation of new biomarkers of neurodegenerative disease hopes to recognize these diseases early to allow for earlier treatments
- Described in this section are some of the more prevalent and better studied, although unfortunately, still not fully understood or treatable disorders with an emphasis on the essential molecular pathological changes involved

General Molecular/Cellular Mechanisms of Neurodegeneration

- Protein aggregation and transport dysfunction
  - Misfolding and aggregation of proteins is a hallmark of many neurodegenerative diseases, though it is still not known in many cases whether these phenomena are central to the pathogenesis, secondary injury, or perhaps even protective to the cell
  - Aggregates that form are multimeric but often have a predominant protein and tend to affect certain neuronal or glial cells in different diseases (Table 16.2)
  - Aggregates can form intracellularly in cytosol, nucleus, or neuritic processes with diverse cellular pathological consequences often including cell death
  - Soluble oligomers and multimers released in the extracellular interstitial spaces may be also be toxic theoretically causing regional pathology
  - Molecular motors within neuritic processes consist of a network of cytoskeletal elements known as kinesins and dyneins
  - When aggregates are found in neuritic processes the major pathological defect is to these molecular motors and retrograde and anterograde transport, essential processes that allow the movement of organelles and molecules crucial to cell function along the processes
  - The histological finding when neuritic transport is disrupted is spheroid formation (also termed axonal swellings or axonal bulbs)
- Mitochondria dysfunction
  - Mitochondria are the energy producing (in the form of oxidative phosphorylation and ATP production) organelles of all cells in the nervous system
  - Mitochondrial proteins are encoded by both nuclear DNA and mitochondrial DNA. Mutations in genes from both sources become more numerous with age and have been suggested to play a role in organelle dysfunction and neurodegenerative diseases
  - Damaged mitochondria leads to increased accumulation of oxidative molecules including reactive oxygen species, which can injure other organelles and induce apoptosis and/or necrosis
  - High levels of oxidants can also be very deleterious to mitochondria inducing a state called mitochondrial permeability transition, with an uncoupling of oxidative phosphorylation often leading to cell death
- Neuroinflammation
  - An emerging field of inquiry thought to play a part in many neurodegenerative diseases; though unknown whether it serves both a primary and secondary role
  - Astrocytes and microglia serve as the local resident cells in the CNS-mediating inflammation
  - Circulating immune cells (B cells, T cells, and monocytes/macrophages) and autoantibodies may also play a yet to be defined role in neurodegenerative processes; their importance in multiple sclerosis, infectious disease, and trauma are better established
  - The major cytokines and chemokines involved include: tumor necrosis factor-α, granulocyte macrophage colony stimulating factor (GM-CSF), interleukin (IL)-1α,

Table 16.2 Neurodegenerative diseases and their associated protein aggregate pathology

Neurologic disease	Primary cell type affected	Primary anatomical region affected	Protein aggregation
Alzheimer disease	Neurons	Neocortex, hippocampus, entorhinal cortex	Neurofibrillary (tau) tangles and amyloid plaques
Parkinson disease	Neurons	Substantia nigra, nucleus basalis, locus ceruleus	Lewy bodies (SNCA)
Lewy body disease	Neurons	Substantia nigra, neocortex	Cortical Lewy bodies (SNCA)
Amyotrophic lateral sclerosis	Upper and lower motor neurons	Motor cortex, lower motor neurons anterior horn of spinal cord	Bunina bodies, skein-like aggregates, hyaline bodies (TDP-43, ubiquitin)
Corticobasal degeneration	Neurons and glia	Basal ganglia and cerebral cortex	Cytoplasmic inclusions (tau)
Multisystem atrophy	Glia and neurons (less)	Brainstem, midbrain, cerebellum, striatum	Cytoplasmic inclusions (SNCA)
Prion diseases	Neurons	Variable and diffuse depending on subtype	Amyloid and prion plaques
Frontotemporal dementias	Neurons	Hippocampus, frontal and temporal cortices	Tau tangles/aggregates (Pick bodies in Pick disease)
Huntington disease	Neurons	Caudate, putamen, and cerebral cortex	Polyglutamine tract inclusions in neurites and nuclei
Progressive supranuclear palsy	Neurons and glia	Globus pallidus, midbrain, pons, subthalamic nucleus	Globose neurofibrillary tangles (neurons) (tau) Coiled bodies (oligodendrocytes) (tau) Tufted and thorn (astrocytes) (tau)

IL-1β, IL-2, IL-4, IL-6, interferon-γ, IL-10, IL-12, IL-18, tumor growth factor-β, macrophage inflammatory protein (MIP-1), macrophage chemotactic protein (MCP-1)
  - Inflammatory mediators may alter the blood–brain barrier, synapse function, apoptosis, edema, protein aggregation, mitochondrial function, and cell-to-cell communication (glial and/or neuronal)
- Survival vs. apoptosis factors
  - Neurons are continuously signaled throughout life by autocrine, paracrine, and endocrine factors to varying degrees, which promote survival and prevent programmed cell death apoptosis
  - Polypeptide factors known as neurotrophins influence survival, differentiation, proliferation, and apoptosis of both neuronal and glial cells
  - Examples include: nerve growth factor, brain-derived neurotrophic factor, glial-derived neurotrophic factor, ciliary neurotrophic factor, insulin-like growth factor
  - Secreted neurotrophins can be taken up at nerve terminals and retrogradely transported to the cell body to exert their effect or act as secreted ligands with action on specific cell surface receptors, which activate second messenger cascades
  - Pathological conditions such as hypoxia/ischemia, electrolyte abnormalities, trauma, neuroinflammation, toxic exposures, and genetic abnormalities can all induce apoptosis through a variety of pathways in the central and peripheral nervous systems (CNS/PNS)

Alzheimer Disease (AD)

- Clinical/epidemiology
  - AD is the most common of the neurodegenerative diseases with an increasing incidence every decade of life
  - Slight female over male prevalence

  - Worldwide disease affecting all races
  - The prevalence roughly doubles every 5 years, starting from a level of 1% for the 60–64-year-old population and reaching 40% or more for some 85–89-year-old cohorts
  - Disturbances in recent memory formation, difficulties carrying out activities of daily living, language impairment, deficits in spatial ability and orientation, and alterations in mood and behavior are the clinical hallmarks of AD
  - AD progresses with much variability in individuals with an average of 1–3 years of early symptoms before diagnosis, 1–3 years from diagnosis until need for more intensive care from family or nursing home, and then 1–3 years before death
- Gross and histological neuropathology
  - Brains show variable cerebral atrophy with hippocampi and adjacent temporal cortices showing consistent diminution in size
  - Frontal and parietal lobes also often show diffuse atrophy with narrowed gyri and widened sulci, and occipital lobes are less affected
  - Coronal slices invariably display thinned cortical gray matter strips and enlarged ventricular system underscoring the widespread neuronal loss
  - The microscopic hallmarks of this disease are amyloid plaques, neurofibrillary tangles, neuronal loss, and reactive glial changes (Fig. 16.12)
  - AD plaques can be visualized with Congo red stain and are composed of Aβ protein deposits forming amyloid surrounded by degenerating neuritic processes
  - The majority of AD brains also show amyloid angiopathy
  - Neurofibrillary tangles are intracellular inclusions of a variety of shapes composed of several proteins with the microtubule-associated protein tau being the predominant; they are best seen with silver stains such as Bodian or Bielschowski or immunohistochemistry against tau
- Genes associated with sporadic and familial AD (Table 16.3)
  - Approximately 75% of AD cases are thought to be sporadic, 25% hereditary with the latter group showing either early or late onsets
  - Mutations in presenilin ([PSEN], *PSEN1*, *PSEN2*), or amyloid precursor protein (*APP*) genes account for the majority of early onset

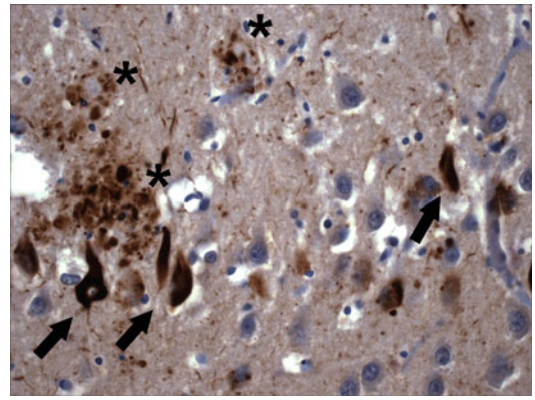

Fig. 16.12 Histological hallmarks of Alzheimer disease. Immunohistochemistry against tau protein highlights neurofibrillary tangles (*arrows*) and dystrophic neurites within Alzheimer type plaques (*asterisks*)

Table 16.3 Genes associated with Alzheimer disease

Gene	Chromosome	Protein	Disease onset; transmission
APP	Chrom 21q21	Amyloid β-(A4) precursor protein	Early onset; autosomal dominant
PSEN1	Chrom 14q24.3	Presenilin 1	Early onset; autosomal dominant
PSEN2	Chrom 1q31–q42	Presenilin 2	Early onset; autosomal dominant
APOE (risk factor gene)	Chrom 19q13.2	Apolipoprotein E	Late onset; sporadic increased risk of AD (ε4 allele) decreased risk AD (ε2 allele)

familial AD, but there exists a wide spectrum of mutations in these genes as well as variable clinical and pathologic presentations
- Individuals with trisomy 21 who survive beyond 45 years of age nearly all develop AD changes
- A genetic susceptibility gene for the apolipoprotein E (APOE) protein has been identified in sporadic AD
- APOE has three alleles: APOE ε2, APOE ε3, and APOE ε4. Individuals that produce the ε4 form are at a greater risk for developing AD, while those that have the ε2 have decreased risk
- Polymorphisms in the *APOE* isoforms may also confer variable susceptibility
- Genetic testing for the APOE alleles is not generally done outside of the research setting, as the beneficial/detrimental effects are not absolute
- Investigators are actively looking for other risk factor-associated genes using linkage analysis

• Possible molecular mechanisms of pathogenesis of AD
- Controversy over the relative importance of the plaques vs. tangles in the pathogenesis of AD has existed for many years in the neuroscience research community of "tauists and βAPPtists"
- Plaques are extracellular deposits of fibrils and amorphous aggregates of amyloid β peptide (Fig. 16.13)
- Neurofibrillary tangles are intracellular fibrillar aggregates of tau that exhibit hyperphosphorylation and oxidative changes

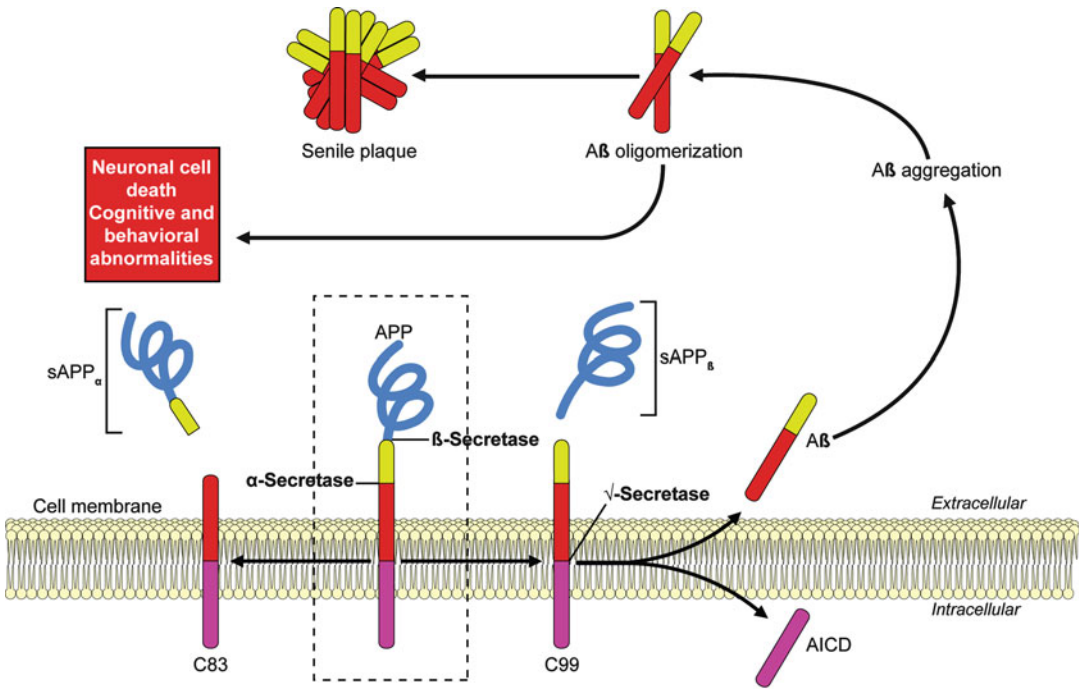

Fig. 16.13 APP processing and Aβ accumulation. Mature APP (*center*, inside dashed box) is metabolized by two competing pathways, the α-secretase pathway that generates sAPPα and C83 and the β-secretase pathway that generates sAPPβ and C99. Some β-secretase cleavage is displaced by ten amino acid residues and generates sAPPβ′ and C89. All carboxyterminal fragments (C83, C99) are substrates for γ-secretase, generating the APP intracellular domain, and respectively, Aβ, among others. Aβ aggregates into small multimers (dimers, trimers, and so on) known as oligomers. Oligomers appear to be the most potent neurotoxins, while the end stage senile plaque is relatively inert (adapted from Gandy 2005)

  - Mutations in *APP*, *PSEN1*, or *PSEN2* lead to accumulations of atypical Aβ peptide, which makes up the major protein component of amyloid plaques
  - Tau mutations have been identified in frontotemporal dementias (*see* below in the section on "Tauopathies"), a group of disorders that are thought to exist in a spectrum with AD
  - The "taucentric" and "amyloidocentric" viewpoints of AD neurodegeneration may converge at the level of PSEN
    - *PSEN* mutations lead to both tau tangles and amyloid plaques
    - PSEN is a core component of the γ-secretase responsible for the accurate cleavage of the Aβ peptide
  - Aβ aggregates in the form of oligomers and fibrils in the interstitial space may be toxic to neurons
  - Aβ aggregates may also form in vessels called amyloid angiopathy
  - Prior head trauma predisposes to AD, with the proposed mechanism being a "sensitization" of glia and the neuroimmune response, though the exact molecular triggers are not known
- Diagnosis
  - An increased number of plaques and tangles combined with the appropriate clinical course are used to make the postmortem diagnosis of AD
  - Most pathologists will follow diagnostic guidelines of the NIA–Reagan and Consortium to Establish a Registry (NIA–CERAD) for AD criteria
  - New slightly modified criteria from the NIA–Reagan/CERAD criteria are have been proposed in 2012 and recommend an "ABC" staging protocol for the neuropathologic changes of AD, based on three morphologic characteristics of the disease: A is for amyloid, B is for Braak neurofibrillary tangle staging protocol, and C is for the Consortium to Establish a Registry for AD neuritic plaque scoring system
  - Premortem diagnosis of sporadic AD relies on clinical history combined with clinical, cognitive, and laboratory examinations to help rule in AD and rule out other forms of dementias
  - New clinical diagnostic criteria recognize early stages of disease with a preclinical onset stage recognized by positive biomarkers ("signature" biomarkers still be researched) and a mild cognitive impairment (MCI) stage that precede AD
  - Genetic analysis in familial AD with appropriate genetic counseling is available for *PSEN1*, but because of the small number of families with mutations in *PSEN2* and *APP*, testing for these genes is currently only done in research labs
  - Imaging modalities to look for hydrocephalus and generalized cortical and hippocampal atrophy may complement the clinical diagnosis
  - "Functional" imaging studies such as positron emission tomography (PET), functional magnetic resonance imaging, and radioligands used for identifying AD pathology are under investigation

Parkinson Disease (PD)

- Clinical/epidemiology
  - PD affects approximately 1% of population over 65
  - Disease onset for sporadic PD varies from 20 to 80 years of age but is most common from 55 to 65; familial forms may occur much early
  - Found throughout the world but with variable prevalence in different races and countries
  - Slightly higher prevalence in males over females
  - Hallmark clinical findings are bradykinesia, resting tremor, rigidity, and postural instability
  - Some patients also have overlapping symptoms (and pathology) with AD
  - Autonomic, cognitive, and psychiatric disturbances affect some patients
- Gross and histologic neuropathology
  - Pallor of substantia nigra and locus ceruleus grossly

  - Lewy bodies (LB): round and eosinophilic cytoplasmic inclusions surrounded by a pale halo, found within the neurons of the substantia nigra (Fig. 16.14), locus ceruleus, nucleus basalis of Meynert, medulla, and thalamus (less frequently in numerous other nuclei)
  - Lewy neurites (LN): dystrophic neurites found in similar distribution but also in amygdale and hippocampus
  - LB and LN immunostain positively with antibodies against α-synuclein (SNCA) and ubiquitin
  - Loss of dopaminergic nigrostriatal neurons and noradrenergic neurons in the substantia nigra and locus ceruleus, respectively, leads to the clinical manifestations of PD
- Genes associated with PD
  - Monogenic linkages to PD have only been discovered in the last 10 years, though recognition of familial genetic component has been known for much longer
  - A diverse set of ten or more genes is now known to lead to dopaminergic neuron degeneration and PD or related disorders (Table 16.4)
  - *SNCA* was the first gene identified with mutations associated with familial PD; polymorphisms in the promoter region may be associated with increased risk of sporadic PD
  - Ten percent of all early onset familial PD cases are due to a wide variety of mutations in the *PARK2* gene, which encodes for a protein important in the ubiquitination pathway
- Possible molecular mechanisms of pathogenesis of PD
  - Mitochondrial dysfunction and oxidative stress are thought to be important contributors to neuronal death in PD
  - Complex I deficits are found in mitochondria from sporadic and familial PD patients
  - Pesticides and toxins such as 1-methyl-4-phenyl-1,2,3,6-tetrohydropyridine (MPTP) can affect complex I function and have been implicated in PD

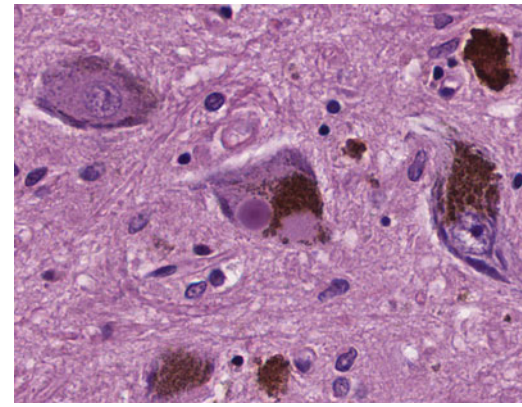

Fig. 16.14 Neuromelanin-containing neurons of the substantia nigra with center neuron displaying a Lewy body

  - MPTP is a contaminant in synthetic opiate production that was accidentally injected by a group of individuals who then developed PD-like syndrome
    - SNCA
      - The physiologic role of SNCA is unknown, but it is associated with lipid rafts, which may be important in synaptic vesicles and synapse formation and function
      - SNCA fibrils are important components of LB and LN, and mutations in the gene promote increased fibrillar aggregations
      - Overexpression of normal SNCA in sporadic AD may occur and increase LB/LN formation
      - Oxidative damage may play a role in the aggregation of SNCA in sporadic PD, and mutant SNCA can interact with mitochondria to increase sensitivity to mitochondrial toxins
      - SNCA may interact with tau or amyloidogenic proteins to increase aggregation
      - The important role for SNCA in both forms of familial PD as well as all sporadic PD place PD as the most prominent diseases known as "synucleinopathies"
      - Dementia with LB also called diffuse Lewy body disease is also a

Table 16.4 Genes associated with Parkinson disease

PARK locus	Gene	Map position	Clinical phenotype	Pathology
PARK1/4	*SNCA*	4q21	Parkinsonism with common dementia	Lewy bodies
PARK2	*PARK2*	6q25–q27	Early-onset, slowly progressing parkinsonism	Lewy bodies
PARK3	Unknown	2p13	Late-onset parkinsonism	Lewy bodies
PARK5	*UCHL1*	4p14	Late-onset parkinsonism	Unknown
PARK6	*PINK1*	1p35–p36	Early-onset, slowly progressing parkinsonism	One case exhibiting Lewy bodies
PARK7	*DJ1*	1p36	Early-onset parkinsonism	Unknown
PARK8	*LRRK2*	12q12	Late-onset parkinsonism	Lewy bodies (usually)
PARK9	*ATP13A2*	1p36	Early-onset parkinsonism with Kufor–Rakeb syndrome	Unknown
PARK10	Unknown	1p32	Unclear	Unknown
PARK11	*GIGYF2*	2q36–q37	Late-onset parkinsonism	Unknown
PARK12	Unknown	Xq	Unclear	Unknown
PARK13	*OMI/HTRA2*	2p13	Unclear	Unknown
PARK14	*PLA2G6*	22q13.1	Parkinsonism with additional features	Lewy bodies
PARK15	*FBX07*	22q12–q13	Early-onset parkinsonism	Unknown
PARK16	Unknown	1q32	Late-onset parkinsonism	Unknown
FTDP-17	*MAPT*	17q21.1	Dementia, sometimes parkinsonism	Neurofibrillary tangles
SCA2	*ATXN2*	12q24.1	Usually ataxia, sometimes parkinsonism	Unknown
SCA3	*ATXN3*	14q21	Usually ataxia, sometimes parkinsonism	Unknown
Gaucher locus	*GBA*	1q21	Late-onset parkinsonism	Lewy bodies

Adapted from Martin et al. (2011)

synucleinopathy associated with cognitive decline, hallucinations, and parkinsonism and neuropathology showing SNCA aggregates in classical LB and diffuse cortical LB

- Multiple system atrophy is a sporadic, adult onset neurodegenerative disease with unknown cause, clinically characterized by variable parkinsonism cerebellar and pyramidal signs, and autonomic failure; histologically characterized by SNCA-positive glial cytoplasmic inclusions

- Parkin (PARK2)
  - PARK2 functions as an E3 ubiquitin protein ligase import for ubiquitination of proteins targeted for proteosomal degradation (Fig. 16.15)
  - PARK2 may also function as a neuroprotectant molecule by interacting with mitochondria and preventing apoptosis
  - Mutations cause a loss of function of this ubiquitin–proteosomal system and accumulation of neurotoxic proteins and/or loss of the neuroprotectant function
  - PARK2- and PTEN-induced kinase-1 (PINK1) have been shown to act in a common genetic pathway
- Ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1)
  - UCH-L1 is a highly abundant, neuron-specific protein that belongs to a family of deubiquitinating enzymes that are responsible for hydrolyzing polymeric ubiquitin chains to free ubiquitin monomers

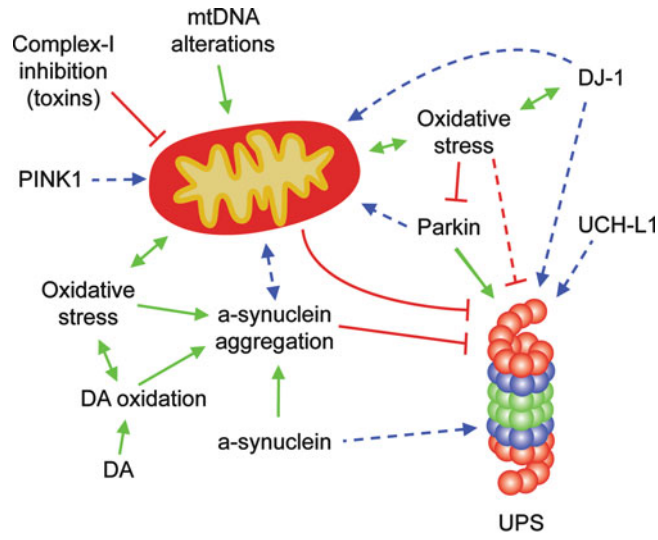

Fig. 16.15 Common pathways underlying PD pathogenesis. Mutations in five genes encoding *SNCA*, *PARK2*, *UCH-L1*, *PINK1*, and *DJ1* are associated with familial forms of PD through pathogenic pathways that may commonly lead to deficits in mitochondrial and ubiquitin–proteosomal system function. Mitochondrial and ubiquitin–proteosomal system dysfunction, oxidative stress, and SNCA aggregation ultimately contribute to the demise of DA neurons in PD. *Red lines* indicate inhibitory effects, *green arrows* depict defined relationships between components or systems, and *blue-dashed arrows* indicate proposed or putative relationships (adapted from Moore DJ et al., from Annual Reviews)

- It may also function as an ubiquitin protein ligase
- UCH-L1 can be found in LB in sporadic PD
- How mutated UCH-L1 contributes directly to PD is not known

◦ PINK1
- PINK1 physiologic function is not currently known
- PINK1 has a mitochondrial targeting sequence and a conserved domain similar to calcium-calmodulin kinase family
- Mutations are thought to cause a loss of function in the putative kinase activity leading to mitochondrial dysfunction and PD

◦ DJ1
- Ubiquitously expressed protein in neurons and glia belonging to the DJ1/ThiJ/PfpI superfamily
- DJ1 does not colocalize in LB but is found associated within a number of neurodegenerative tauopathies and with SNCA-positive glial inclusions in multiple system atrophy
- Insoluble forms are increased in brains of sporadic PD patients
- Physiologic function of DJ1 is unclear but it may function as an antioxidant protein or as a sensor of oxidative stress
- DJ1 may be a component of the ubiquitin–proteosomal system and may confer protection by functioning as a molecular chaperone or protease to refold or promote degradation of misfolded proteins

• Diagnosis
– Clinical diagnosis relies on history, observation of clinical manifestations, and initial responsiveness to dopaminergic agonist therapy
– Neuropathological examination at autopsy is required to confirm diagnosis

Amyotrophic Lateral Sclerosis/Motor Neuron Disease (MND)

- Clinical
  - Amyotrophic lateral sclerosis (ALS) is a disease with patients presenting both upper and lower motor neuron signs, which leads to paralysis and death usually within 2–5 years
  - Generally affects older individuals (50–70 years) with an annual incidence of 1–2/100,100 and overall lifetime risk of 1/800
  - Slight male to female preponderance (1.3:1–1.6:1)
  - There is patient-to-patient variability in terms of muscle areas affected initially and the pattern of progressive spread to eventually most muscle groups
  - Some patients present with prominent bulbar symptoms secondary to early and more extensive loss of cranial nerve motor neurons
  - Upper motor neuron signs include: clonus and hyperreflexia
  - Lower motor neuron signs include: muscle atrophy, weakness, and fasciculations
  - The cause is unknown with 90% of cases being sporadic and 10% familial
  - Some epidemiological studies suggest the incidence is increasing
- Gross and histological neuropathology
  - Gross changes are not usually noted in the brain, though in long-term surviving patient's, atrophy of the precentral gyrus can be seen
  - Spinal cord anterior motor nerve roots are notably thinned compared with posterior sensory nerve roots
  - Depletion of upper (corticospinal, Betz cells) and lower motor neurons as well as cranial nerve motor neurons are the histological hallmarks of ALS
  - The lateral and anterior medial corticospinal tracts are depleted (lateral sclerosis)
  - Skeletal muscle deprived of innervation shows grouped atrophy, small acutely angulated fibers, and fiber-type grouping
  - Skein-like inclusions and Bunina bodies are cytoplasmic inclusions that are often found in motor neurons of ALS patients
  - Reactive astrocytes and microglia are found in the anterior horns of the spinal cord and motor cortex of the cerebrum
- Genes associated with and possible molecular mechanisms of pathogenesis of ALS
  - Familial ALS represents about 10% of cases (Table 16.5), and within this group there is significant phenotypic and genotypic heterogeneity
  - While genetic loci have been identified for many of the familial cases of ALS, the function of these genes and their relationship to ALS pathogenesis is largely unknown
  - The search is on for modifier genes and polymorphisms in sporadic ALS
  - Small studies of sporadic ALS patients have identified possible altered genes/polymorphisms that need to be confirmed in larger studies: *VEGF*, *EAAT2*, *GRIA2*, *CNTF*, *SMN1*, *SMN2*, *APOE*, and *NEFH*
  - Epigenetic and genetic causes have been postulated to bring about a number of cytotoxic phenotypes, though direct links are not well established (Fig. 16.16)
    - *SOD1*
      - Copper-zinc superoxide dismutase-1 was the first gene identified for familial ALS, and mutations account for 20% of familial cases
      - Ubiquitously expressed cytoplasmic protein that detoxifies the reactive molecule superoxide to oxygen and H_2O_2, which can be then be cleared by catalase and glutathione peroxidase
      - >100 mutations have been identified, but the phenotypic variability even in families with the same mutation suggests that other genes and/or environmental factors are important
      - Rodent transgenic models with human *SOD1* mutations display similar clinical and pathological features and have been important research tools

Table 16.5 Genes associated with amyotrophic lateral sclerosis (ALS)

Reported FALS/MND loci	Gene	Chromosomal location
Adult onset dominant typical ALS		
ALS1[a]	*SOD1*	21q22.1
ALS3		18q21
ALS6	*FUS*	16q11.2
ALS7		20p13
ALS9	*ANG*	14q11.2
ALS10	*TARDBP*	1p36.22
ALS11	*FIG4*	6p21
ALS13	*ATXN2*	12q24.12
Adult onset dominant atypical ALS		
ALS with/without frontotemporal dementia	*MAPT* *C9ORF72* *UBQLN2*	9q21–q22 9 Chr X
ALS14	*VCP*	9q13.3
ALS with dementia/parkinsonism	*DCTN1*	17q21.1
Progressive lower motor neuron disease		
ALS8	*VAPB*	20q13.3
ALS12	*OPTN*	10p13
Juvenile onset dominant ALS		
ALS4	*SETX*	9q34.13
Juvenile onset recessive ALS		
ALS2	*ALS2*	2q33.1
ALS5		15q15.1–q21.1

FALS familial form of ALS; *MND* motor neuron disease
[a]Note that both dominant and recessive linked-*SOD1* mutations have been reported (modified from Gros-Louise et al. 2006; Lill et al. 2011)

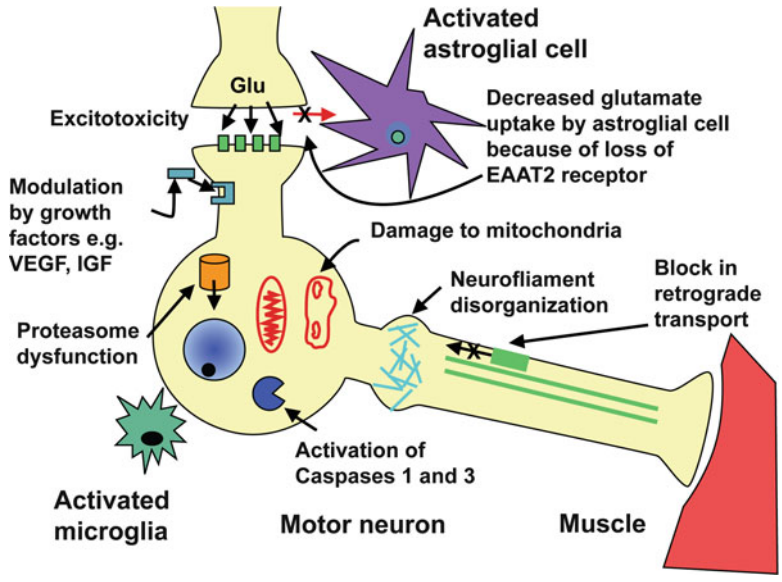

Fig. 16.16 Molecular and cellular processes possibly implicated in pathogenesis of ALS (adapted from Bruijn et al. 2004; from Annual Reviews)

- The exact mechanism by which mutated *SOD1* causes ALS is not known despite its discovery >13 years ago
- A dominant negative toxic gain of function mechanism is thought to be involved in *SOD1* pathogenesis
- Recent animal studies have suggested that mutant *SOD1* expression must be in both glial as well as neuronal cells for the ALS-like disease to manifest
- Hypothesized mechanisms for *SOD1* toxicity include: excitotoxicity, oxidative stress, mitochondrial dysfunction, inflammation, axonal transport defect, and/or toxic aggregation with likely some combination of the above

° TDP-43
- Transactivating region (TAR) DNA-binding protein 43, a recently identified protein that aggregates with ubiquitin and other proteins in frontotemporal dementias and many cases of ALS both sporadic and some familial
- Abnormal aggregation occurs both in the presence and absence of mutation in the gene
- Interestingly, reports show that abnormal cell localization to the cytoplasm of affected neuronal and glial cells is found with all sporadic ALS cases and some familial cases but not with mutant *SOD1* familial cases, suggesting possible different modes of degeneration in familial and sporadic ALS
- Normal physiological function and role in the pathophysiology of ALS is not fully understood and an active area of investigation

- Diagnosis
  - Patients are diagnosed with ALS based on the E1 Escorial criteria that categorizes patients as clinically definite, probable, or possible for ALS based on degree of upper and lower motor neuron findings
  - Less commonly muscle biopsies are performed (often to rule out other diseases)
  - *SOD1* genetic testing is available and should be considered in the setting of familial ALS with appropriate genetic counseling
  - Autopsy findings confirm the diagnosis

Tauopathies

- General comments
  - Tau is a microtubule-associated protein that binds microtubules and promotes microtubule assembly, essential components of the cytoskeleton
  - Tau is abundantly expressed in the CNS and exists in six isoforms created by alternative mRNA splicing of a single gene
  - The different isoforms have either three or four microtubule-binding repeat sequences with similar ratios of either three or four repeat isoforms normally expressed; varying this ratio appears to confer susceptibility to some neurodegenerative disease later in life
  - Tau is also a major component of atypical protein aggregates found in paired helical filaments in AD and in a number of other neurodegenerative diseases collectively thought of as tauopathies
  - Mutations in the tau gene and/or altered phosphorylation states have been identified in many of these diseases

FTDP-17T

- Frontotemporal dementia and parkinsonism linked to chromosome 17 associated with tau gene mutations
- Adult onset, slowly progressive neurodegenerative disease
- Clinically characterized by variable cognitive, behavioral, and motor dysfunction
- Diffuse deposition of tau aggregates in neurons and glia can be identified with silver stains and tau immunohistochemistry
- Mutations have been identified in multiple sites (exonic and intronic) of the tau gene
- Autosomal dominant transmission

Progressive Supranuclear Palsy

- Multisystem degeneration characterized by symptoms of parkinsonism and supranuclear ophthalmoplegia
- Slowly progressive disease affecting middle- and late-aged individuals
- No established genetic or epigenetic etiologies and most (if not all) cases are sporadic
- Polymorphisms in the tau gene have been identified, and sporadic cases are associated with a homozygous H1 haplotype and overexpression of the 4-repeat tau isoform
- Neurofibrillary tangles, neuropil threads, and glial fibrillary tangles can be identified in multiple areas but tend to concentrate in substantia nigra, basal ganglia, subthalamic nucleus, and brainstem
- Kinase/phosphatase dysregulation may be involved in the pathogenesis of progressive supranuclear palsy

Corticobasal Degeneration

- Adult onset, neurodegenerative disease with focal pathology and corresponding clinical phenotype of primary aphasia, dementia, visual inattention, or rapidly progressive mutism
- Neuropathology consists of focal cortical or deep gray matter degeneration with tau-positive neurons and glia
- Most cases are sporadic; several familial cases have been reported, though they share overlapping pathology and genetics with tau mutations similar to *FTDP-17*
- Sporadic cases are associated with a homozygous H1 haplotype and overexpression of the 4-repeat tau isoform
- The histological hallmark of corticobasal degeneration is swollen or "balloon" neurons in the affected area
- Balloon neurons, neuropil threads, as well as occasional other neurons and glia contain tau-positive aggregates within their cytoplasm

Pick Disease

- Frontotemporal degeneration with three clinical patterns: behavioral syndrome referred to as frontotemporal dementia, progressive nonfluent aphasia, and semantic dementia
- Majority of cases are sporadic
- Familial cases with defined tau mutations have been reported and called "atypical Pick disease" but may be better recognized as some other tauopathy distinct from Pick
- The sporadic form is not related to H1 or H2 haplotypes and appears to be a 3-repeat tau disorder
- Gross neuropathology shows variable marked atrophy ("knife-edge" atrophy) of the frontal, temporal, and parietal lobes with consistently preserved precentral gyrus and posterior two-thirds of the superior temporal gyrus (Fig. 16.17)
- Pick bodies, round cytoplasmic fibrillar inclusions, are consistently found in the fascia dentata of the hippocampus and less common in other nuclei and cortical neurons
- Pick bodies are highlighted with silver stains or tau or ubiquitin immunohistochemistry
- Extensive neuronal loss, gliosis, and occasionally ballooned neurons are seen in affected areas

Trinucleotide Repeat Diseases

- General comments
  - These disorders are caused by expanding triplet repeat of nucleotides and affect primarily the CNS/PNS (Table 16.6)

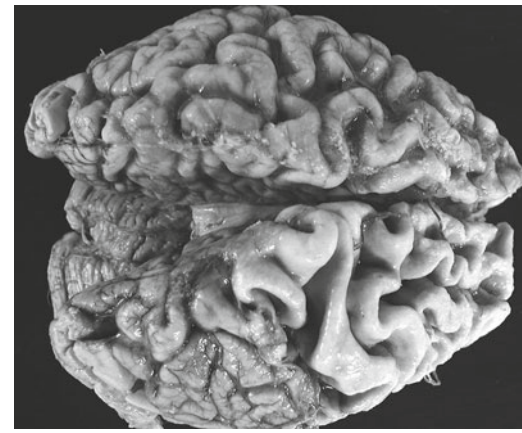

Fig. 16.17 Gross image of superior surface of a brain from a patient with Pick type frontotemporal dementia. Note the widely spaced, so-called "knife-edge" gyri in the frontal lobes

Table 16.6 Triplet repeat disorders affecting the nervous system

Disease	Symptoms	Gene	Locus	Protein
Noncoding repeats				
Friedreich ataxia	Ataxia, weakness, sensory loss	*FXN*	9q13–q21.1	Frataxin
Fragile X syndrome A	Mental retardation	*FMR1*	Xq27.3	Fragile X mental retardation 1 protein
Fragile X syndrome E	Mental retardation	*FMR2*	Xq28	Fragile X mental retardation 2 protein
Dystrophia myotonica 1	Weakness, myotonia	*DMPK*	19q13	Dystrophia myotonica protein kinase
Spinocerebellar ataxia 8	Ataxia	Antisense to *KLHL1*	13q21	Undetermined
Spinocerebellar ataxia 12	Ataxia	*PPP2R2B*	5q31–q33	Regulatory subunit of the protein phosphatase PP2A
Huntington disease-like 2	Chorea, dementia	*JPH3*	16q24.3	Junctophilin 3
Polyglutamine disorders				
Spinal and bulbar muscular atrophy	Weakness	*AR*	Xq13–q21	Androgen receptor
Huntington disease	Chorea, dementia	*IT15*	*4p16.3*	Huntingtin
Dentatorubral-pallidoluysian atrophy	Ataxia, myoclonic epilepsy, dementia	*DRPLA*	12p13.31	Atrophin 1
Spinocerebellar ataxia 1	Ataxia	*SCA1*	6p23	Ataxin 1
Spinocerebellar ataxia 2	Ataxia	*SCA2*	12q24.1	Ataxin 2
Spinocerebellar ataxia 3 (Machado–Joseph disease)	Ataxia	*SCA3/MJD*	14q32.1	Ataxin 3
Spinocerebellar ataxia 6	Ataxia	*CACNA1A*	19p13	α-la Voltage-dependent calcium channel subunit
Spinocerebellar ataxia 7	Ataxia	*SCA7*	3p12–p13	Ataxin 7
Spinocerebellar ataxia 17	Ataxia	*TBP*	6q27	TATA box binding protein
Polyalanine disorders				
Oculopharyngeal dystrophy	Weakness	*PABPN1*	14q11.2–q13	Poly(A)-binding protein 21
Congenital central hypoventilation syndrome	Respiratory difficulties	*PHOX2B*	4p12	Paired-like homeobox 2B
Infantile spasms	Mental retardation, epilepsy	*ARX*	Xp22.13	Aristaless-related homeobox, X-linked
Synpolydactyly	Limb malformation	*HOXD13*	2q31–q32	Homeobox D13

Adapted from Di Prospero and Fischbeck (2005)

  - Generally the larger the number of repeats the more severe the disease
  - "Anticipation," a key genetic feature in these diseases, is the earlier age of onset in successive generations within a family
  - A variety of modes of inheritance exist including: AR, AD, X-linked
  - Pathogenesis is not well understood, but likely involves loss of function and/or toxic gains of function of affected proteins

Huntington Disease (HD)

- Clinical
  - Autosomal dominant transmitted disease with near complete penetrance characterized by chorea and progressive cognitive and behavioral disorders
  - Mean age of onset: 40 years
  - Prevalence of 5–10/100,000
- Gross and histologic neuropathology
  - Classic gross appearance of the brain on coronal section is widened lateral ventricles and atrophied, flattened caudate and putamen nuclei
  - Advanced cases show global brain atrophy
  - Neuronal loss and reactive astrocytosis in affected areas
  - Polyglutamine expanded repeats cause an accumulation of the abnormal protein and formation of nuclear inclusions within cells of the striatum and cortex
  - Atrophy and neuronal loss correlate with severity of clinical disease
- Genes associated with HD
  - Autosomal dominant form of disease (90% cases; 10% occur de novo) caused by expanded CAG repeats (>36) coding for polyglutamines in the *HTT* gene (*IT15*) product huntingtin
- Possible molecular mechanisms of pathogenesis of HD
  - Normal functions of HD are thought to include roles in transport, transcription, and neurogenesis
  - The abnormal mutated form of huntingtin with increased glutamines in the amino terminus can form protein aggregates with other proteins including ubiquitin
  - How the inclusions lead to cellular dysfunction and degeneration is not known
  - Energy depletion, increased apoptosis, impairment of the proteasome–ubiquitin system, oxidative stress, and excitotoxicity of susceptible neurons have all been hypothesized to be involved in the pathogenesis of HD
- Diagnosis
  - Based on a thorough clinical history, physical and cognitive examination
  - CT scanning to look for striatal atrophy can be helpful
  - Genetic testing for the mutations of the *HTT* gene can be done in affected individuals and family members
  - Genetic counseling is essential
  - Fetal genetic testing and in vitro fertilization with preimplantation screening can be performed at some medical centers

Friedrich Ataxia (FA)

- Clinical
  - Most common inherited ataxia with worldwide distribution
  - European prevalence of 1/29,000 and carrier status of 1:85
  - Autosomal recessive
  - 85% have onset before age 20; late adult onset is also seen less commonly
  - Slow progress with loss of ambulation in about 5–15 years
  - Patients have loss of deep sensation and deep tendon reflexes and cerebellar signs including an ataxic speech disorder
  - Cardiomyopathy is frequent and can lead to cardiac failure
  - Adult onset diabetes in 10–32%
- Gross and histological neuropathology
  - Spinal cord and dorsal roots are atrophic
  - Loss of large myelinated axons and dorsal root ganglion cells
  - Degeneration of dorsal column tracts and spinocerebellar tracts
  - Cerebellum shows white matter gliosis and dentate nucleus degeneration

  - Polyglutamine expanded repeats cause an accumulation of the abnormal protein and formation of nuclear inclusions within cells of the striatum and cortex
- Genes associated with FA
  - Homozygous GAA-repeat expansion within the first intron of the frataxin (*FXN*) gene
  - Repeats in FA can range from 67 to 1,700 (normal 6–34)
- Possible molecular mechanisms of pathogenesis of FA
  - Frataxin is a mitochondrial protein involved in iron metabolism
  - Increased repeats lead to decreased frataxin levels and iron accumulation
  - Increased iron is thought to contribute to increased oxidative stress, decreased oxidative phosphorylation, and reduced activity of mitochondrial enzyme complexes containing iron-sulfur clusters
- Diagnosis
  - Based on satisfying major and minor clinical criteria with emphasis on early onset, progressive ataxia, and sensory dysfunction
  - Genetic testing is widely available to support clinical diagnosis; especially helpful in atypical presentations

Suggested Readings

CNS Tumors

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Molecular Pathology of Endocrine Cancer

17

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Endocrine Tumors

Overview

- Morphological and immunohistochemical analyses are the most definitive approaches for the diagnosis of endocrine tumors
  - Molecular techniques and cytogenetic analyses are being used more frequently for diagnostic and prognostic purposes
- Specific genetic mutations can influence prognosis as well as therapeutic approaches
- The most commonly used techniques for molecular analyses include:
  - Fluorescence in situ hybridization (FISH)
  - Polymerase chain reaction (PCR)
  - Reverse transcription (RT)-PCR
  - DNA sequencing
  - Loss of heterozygosity (LOH)
  - Comparative genomic hybridization (CGH)
- Gene expression profiling of endocrine tumors by cDNA microarrays provide genetic fingerprinting which may be used for diagnostic and therapeutic purposes

L. Cheng and J.N. Eble (eds.), *Molecular Surgical Pathology*,
DOI 10.1007/978-1-4614-4900-3_17,

Follicular-Patterned Thyroid Tumors

- General molecular concepts in follicular-patterned thyroid carcinomas
- Definition
  - A wide spectrum of follicular-derived cancers of varying aggressiveness from papillary thyroid carcinoma to anaplastic thyroid carcinoma
- Several inherited tumor syndromes are associated with follicular-patterned thyroid carcinomas and include:
  - Familial adenomatosis coli
  - PTEN–hamartoma tumor syndrome
  - Carney complex
  - Multiple endocrine neoplasia type 1 (MEN1)
- Genetic alterations implicated in the pathogenesis of follicular-patterned thyroid carcinoma
- BRAF mutation
  - BRAF maps to chromosome 7q34
  - BRAF is a member of the RAF family
  - The V600E mutations activate the MAPK pathway
  - BRAF mutation is probably an early event in the pathogenesis of PTC
    - The frequency of BRAF mutations vary with the subtype of PTC
    - It is high in tall cell carcinomas, less high in conventional PTC and columnar cell carcinoma and relatively uncommon in follicular variant of papillary thyroid carcinoma
    - Anaplastic carcinomas also have BRAF mutation
    - The most common mutation is V600E which is present around 95% of the time
    - Other mutations rarely found in thyroid carcinoma include K601E point mutation, small in-frame insertions or deletions surrounding codon 600, and AKAP9–BRAF rearrangement
- RET/PTC rearrangement is common in papillary thyroid carcinoma
  - RET protooncogene on chromosome 10q11.2
  - Encodes a cell membrane receptor tyrosine kinase
    - It consists of an extracellular domain, a transmembrane domain, and an intracellular tyrosine kinase domain
  - Wild type RET is expressed at high levels in the calcitonin-producing C-cells but not in thyroid follicular cells
  - Chromosomal rearrangement leads to activation of RET/PTC
  - The most common rearrangements include: RET/PTC1 formed by fusion with the H4 gene
    - RET/PTC3 is formed by the fusion with the NCOA4 (ELE1) gene
  - RET/PTC1 and RET/PTC3 are intrachromosomal paracentric inversions
    - In RET/PTC2, since the genes are on chromosome 10 and 17 this results in RET fusion with the regulatory R1 alpha of the cAMP-dependent protein kinase 4
  - RET/PTC shows a wide variation in different geographic regions
    - In the USA, the frequency ranges from 11 to 43%; while in Canada it is 40% and in Italy it is 29–35%
    - It is around 3% in Saudi Arabia and 85% in Australia
    - RET/PTC1 accounts for 60–70% of positive cases, RET/PTC 3 for 20–30% of cases, and RET/PTC 2 for less than 10% of cases
- TRK rearrangements
  - NTRK1 gene located on chromosome 1q22
  - TRK rearrangement involves another tyrosine kinase gene NTRK1 in papillary thyroid carcinoma
  - It encodes one of the nerve growth factor receptor genes expressed in neurons
    - It is involved in cell growth, differentiation, and survival
  - In the thyroid, the gene is activated through chromosomal rearrangement with juxtaposition of the intracellular tyrosine kinase domain of NTRK1 to the 5′ terminal sequence of different genes
    - Two of the fusion partner genes include tropomyosin (TPM) and the translo-

cated promoter region gene, both on the q arm of chromosome 1
    - A third fusion partner, the TF6 gene, is on chromosome 3, so there is a t(1;3) translocation
    - TRK rearrangement promotes neoplastic transformation of thyroid cells
    - TRK rearrangements are present in 10–15% of papillary thyroid carcinoma
- RAS mutations
  - RAS genes and their chromosomal locations include NRAS (1p13), KRAS (12p12), and HRAS (11p15)
  - RAS genes all encode distinct 21 kD proteins
  - Somatic mutations in codons 12/13 and 61 of the three RAS genes are found in 40–50% of follicular carcinomas
  - RAS point mutations are present in 10–20% of papillary thyroid carcinomas
  - RAS mutation in papillary thyroid carcinoma is usually associated with the follicular variant pattern
- Phosphate and tensin (PTEN) mutations
  - Maps to chromosome 10q23.3
  - PTEN gene products possess protein tyrosine phosphatase and three phosphoinositol phosphatase activities
    - These are important in regulating cell migration, invasion, and cell proliferation
  - PTEN mutations are present in 5–15% of anaplastic carcinomas and most mutations are in the kinase domain in exon 20 and the helical domain in exons 4 or 9
- TP53 mutations
  - Maps to chromosome 17q13.1
  - TP53 is a transcriptional transactivator
  - Regulating functions associated with the cell cycle, cell differentiation, angiogenesis, apoptosis, and DNA repair
  - TP53 is found in 17–38% of poorly differentiated carcinomas and 67–83% of anaplastic thyroid carcinomas
  - p53 induces overexpression of p21
  - The immunohistochemical expression of p53 is not necessarily associated with p53 mutations
- PAX8–PPAR gamma
    - PAX8 is located on chromosome 2q13
    - PAX8 encodes a paired domain transcription factor which has a critical role in thyroid development and differentiation of follicular cells
    - The protein binds to the promoters of thyroglobulin, thyroperoxidase, and sodium iodide symporter genes and regulates their thyroid-specific expression
    - There are several PAX8 splice variants
    - The peroxidase proliferative-activated receptor (PPAR) is part of the rearrangement
    - PPARs are nuclear hormone receptors and control some of the genes involved in lipid metabolism
  - The PAX8/PPAR gamma fusion protein contains the partial homeobox domains of PAX8 fused with the DNA binding ligand of PPAR gamma
  - PAX8/PPAR gamma is present in 30–35% of follicular carcinomas with a lower prevalence in Hurtle cell carcinoma
    - The rearrangement is also present in ~2–10% of follicular adenomas
  - A higher prevalence of PAX8/PPAR gamma is associated with follicular carcinomas in irridiated patients
- Beta-catenin (CTNNB1)
  - Beta-catenin is located on chromosome 3p22–3p21.3
  - Beta-catenin has a role in E-cadherin-mediated cell–cell adhesion
  - Point mutations in the phosphorylation sites of beta catenin in exon 3 stabilizes the protein and leads to nuclear accumulation of beta-catenin
  - Exon 3 mutation of CTNNB1 is present in about 25% of poorly differentiated carcinomas and about 50–60% of anaplastic carcinomas
- PI3K/AKT point mutations
  - Mutations in the PIK3CA and PTEN involving the PI3K/AKT signaling portions are common in anaplastic carcinomas
  - In anaplastic thyroid carcinomas 10–20% of tumors have PIK3CA mutations and 5–15% PTEN mutations

  - In the PIK3CA gene most mutations are in exon 20 which codes for the kinase domain and exon 9, which codes for the helical domain resulting in the AKT pathway activation
  - There is also an increase in the PIK3CA gene copy numbers in about 30–40% of anaplastic carcinomas
- RET point mutations
  - Located on chromosome 10q11.2
  - Mutations occur in sporadic tumors and in tumors associated with multiple endocrine neoplasia (MEN) type 2A or 2B or in familial medullary thyroid carcinoma (FMTC)
  - Germline mutations in certain functional regions are associated with MEN2A, MEN2B, and FMTC
  - In MEN2A and FMTC the mutations are present in exons 10 and 11
    - In MEN2A around 90% of the mutations are in codon 634
    - In MEN2B more than 90% of the mutations involve codon 918
  - In sporadic MTC somatic RET mutations are present in 25–70% of cases
  - Most mutations affect codon 918

Papillary Thyroid Carcinoma

- Definition
  - A well-differentiated group of carcinomas derived from the follicular cell and characterized by distinct cytologic features including irregular nuclear shapes, prominent nuclear grooves, nuclear clearing, and cytoplasmic invagination into the nuclei
  - The conventional subtype has prominent papillae with fibrovascular cores
- Clinical features
  - Children and young adults up to fourth and fifth decades are more commonly affected
  - Previous exposure to radiation increases incidence
  - Radiographically usually appears as a cold nodule
- Pathologic features (Fig. 17.1)
  - Variable prominence of papillary architecture

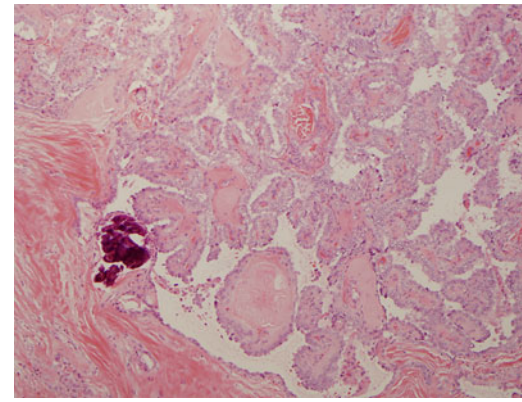

Fig. 17.1 Papillary thyroid carcinoma showing prominent papillae and one focus of calcification

  - Elongated nuclei with nuclear clearing and nuclear overlap
  - Cytoplasmic invagination into nuclei
  - Prominent nuclear grooves
- Genetic findings
  - BRAF mutations (30–60% of cases) (Table 17.1) (Fig. 17.2)
  - RET/PTC rearrangements in 10–43% of cases in the USA (Table 17.2) (Fig. 17.3)
  - TRK rearrangements in 10–15% of cases
  - RAS point mutations in 10–20% of cases

Follicular Thyroid Carcinoma

- Definition
  - A malignant tumor derived from the thyroid follicular cells that shows a follicular architecture and does not show the nuclear feature of papillary thyroid carcinoma
  - Oncocytic or Hurtle cell carcinoma is a subtype characterized by abundant cytoplasmic mitochondria
- Clinical features
  - Rare in children
  - Most common in fifth decade
  - Radiologically often presents as a cold nodule
- Pathologic features (Fig. 17.4)
  - Follicular cells with a follicular architecture
  - Lacks nuclear features of papillary carcinoma
  - May show capsular invasion only, vascular invasion (angioinvasive), or may be widely invasive

Table 17.1 BRAF mutations in papillary thyroid carcinomas (PTC)

Mutation	Distribution	Histologic variants of PTC
BRAF V600E	30–60%	Conventional PTC, tall cell variant
BRAF K601E	~3%	Follicular variant of PTC
AKAP9/BRAF rearrangement	Rare	Conventional PTC

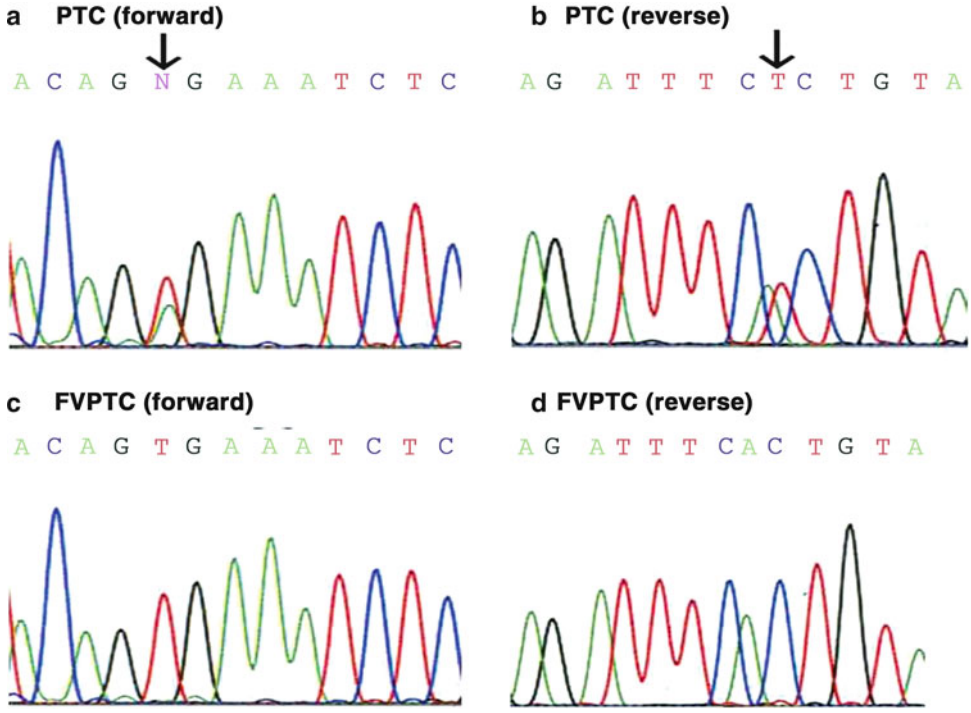

Fig. 17.2 BRAF mutation in papillary thyroid carcinomas. Bidirectional DNA sequencing shows a T1799A (V600E) mutation in exon 15 of the BRAF gene (**a**, **b**), and wild type BRAF sequence in a follicular variant of papillary thyroid carcinoma (FVPTC) (**c**, **d**)

Table 17.2 RET/PTC rearrangements in papillary thyroid carcinoma

RET/PTC variant	Fused genes	Rearrangement	Prevalence (%)
RET/PTC 1	H4	inv(10)(q11.2;q21)	10–43
RET/PTC 2	R1alpha	t(10;17)(q11.2;q23)	5–10
RET/PTC 3 and RET/PTC 4	NCOA4 (RF6,ELE1)	inv(10)(q11.2)	20–30

- Genetic findings
  - PAX8–PPAR gamma rearrangements in 30–35% of cases
  - RAS mutations in 40–50% of cases of conventional follicular carcinomas (also in 20–40% of follicular adenomas)
  - RAS mutations in 15–25% of Hurtle cell carcinomas (also in 0–4% of Hurtle cell adenomas)

Poorly Differentiated Carcinoma

- Definition
  - A heterogeneous group of carcinomas which by the Turin proposal may include necrosis, convoluted nuclei, and greater than three mitoses per ten high power fields
  - Insular carcinoma is one distinct subtype of poorly differentiated carcinoma

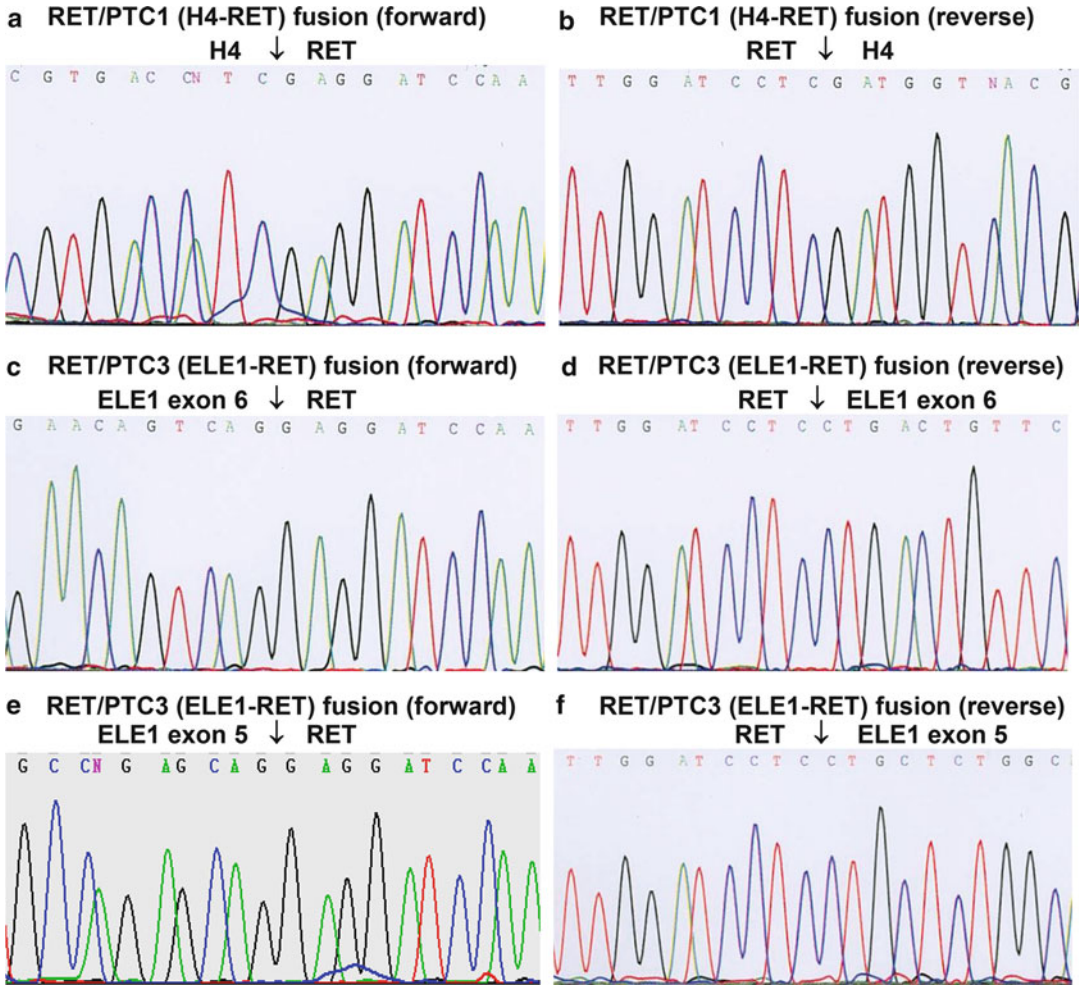

Fig. 17.3 RET/PTC rearrangement in papillary thyroid carcinomas. Bidirectional DNA sequencing of RT-PCR products shows the RET/PTC1 (H4-RET) fusion sequences (**a**, **b**), and RET/PTC3 (ELE1-RET) fusion sequences (**c**–**f**) in two conventional papillary thyroid carcinomas

- Clinical features
  - Rare in children
  - Most common in adults in fifth and sixth decades
  - Often associated with a multinodular goiter
- Pathologic features (Fig. 17.5)
  - Tumor usually large and arises in a background of a multinodular goiter
  - Necrosis, convoluted nuclei, and three or more mitoses per ten high power fields
  - Vascular invasion common
- Genetic findings
  - RAS mutations in 18–27% of cases
  - TP53 mutations in 17–38% of cases
  - BRAF mutations in less than 2% of cases
  - Beta-catenin mutations in 25% of cases

Anaplastic Thyroid Carcinoma

- Definition
  - A highly aggressive undifferentiated thyroid carcinoma usually developing in older patients
  - Many undifferentiated carcinomas are derived from dedifferentiation of papillary or follicular carcinomas
- Clinical features
  - Rapidly growing carcinoma with compression of adjacent neck structures
  - Patient may present with stridor, hoarseness, or dysphagia
  - Most common in seventh and eighth decades of life

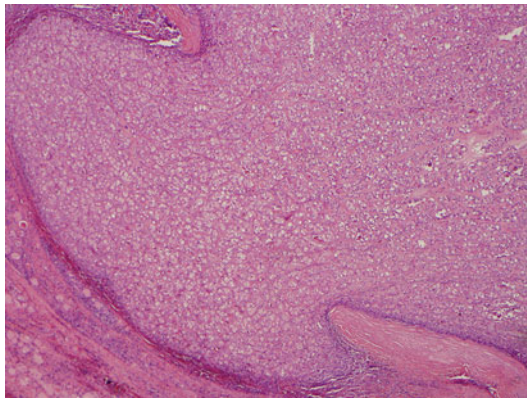

Fig. 17.4 Follicular thyroid carcinoma showing invasion through the capsule

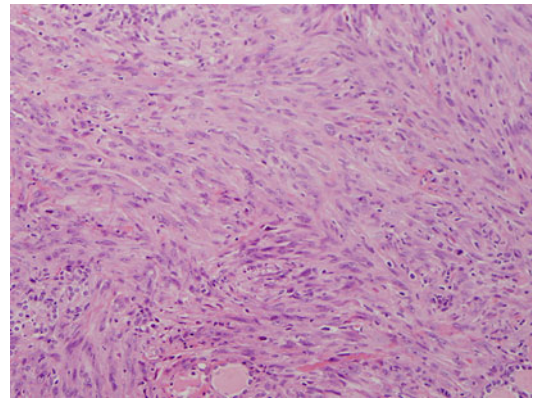

Fig. 17.6 Anaplastic thyroid carcinoma showing mostly spindled cells and a few entrapped normal follicles

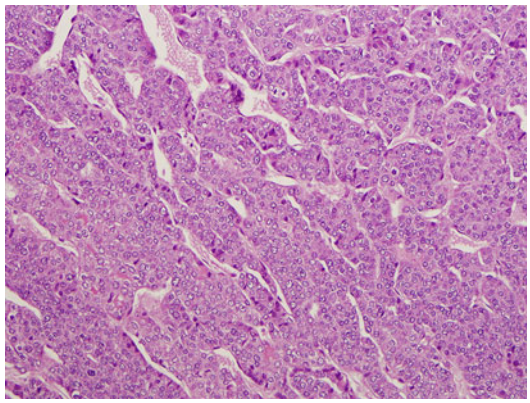

Fig. 17.5 Poorly differentiated thyroid carcinoma showing cells with convoluted nuclei, increase mitotic activity and focal insular pattern of growth

- Pathologic features (Fig. 17.6)
  - Large tumors, usually greater than 4.0 cm
  - May replace the entire thyroid lobe and infiltrate the soft tissues of the neck
  - Various patterns including spindle cell, giant cell, squamoid, and paucicellular
- Genetic features
  - BRAF mutations in about 30–50% of cases
  - RAS mutations in 55% of uses
  - TP53 mutations in 67–83% of cases
  - Beta-catenin mutations in 50–60% of cases
  - PIK3CA mutations in 30–40% of cases

Medullary Thyroid Carcinoma

- Definition
  - Carcinoma derived from the thyroid calcitonin-producing cells
  - Tumors secrete calcitonin and other peptides and may develop sporadically or in a familial pattern
- Clinical features
  - Occurs as a sporadic tumor as well as with several familial syndromes including MEN 2A and 2B (both associated with pheochromocytomas) and FMTC (not associated with pheochromocytomas)
  - Patients with familial disease may develop carcinomas at a much younger age
  - Patients with sporadic medullary thyroid carcinoma present with a solid mass
  - Familial tumors are usually bilateral and multifocal and are preceded by C-cell hyperplasia
  - Increased serum levels of calcitonin and carcinoembryonic antigen are often present
- Pathologic features (Fig. 17.7)
  - May range from micromedullary thyroid carcinomas (less than 1 cm) to much larger tumors
    - Most tumors occur in the upper and middle third of the thyroid gland

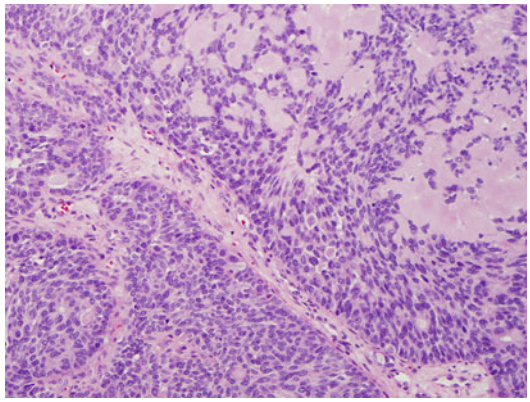

Fig. 17.7 Medullary thyroid carcinoma composed of spindled and epithelioid cells. Amyloid shown as pale eosinophilic amorphous material is present

  - The histologic features are variable ranging from spindle and epithelioid cells to less common patterns such as solid, oncocytic, and papillary
    - Amyloid is present in the stroma in about 75% of cases
  - Immunohistochemical staining is positive for chromogranin, synaptophysin, calcitonin, CEA, keratin, and sometimes somatostatin
  - Ultrastructural features include dense core secretory granules 100–600 μm in diameter
- Genetic findings
  - RET protooncogene mutations
  - Sporadic MTC RET mutation in 25–70% of cases, usually in codon 918
  - MEN2A: Most RET mutations (90%) in codon 634
  - MEN2B: Most RET mutations (90%) involve codon 918

Parathyroid Adenomas and Carcinomas

General Molecular Concepts in Parathyroid Tumors

- Definition: Increase in size of one or more parathyroid glands which may be hyperplastic, associated with benign tumors (adenomas) or malignant tumors (carcinomas)
- Several inherited conditions are associated with parathyroid neoplasia (Table 17.3)
  - MEN1 on chromosome 11q13
  - Cyclin D1 on chromosome 11q13
  - RET on chromosome 10q11.2
  - Rb on chromosome 13q14
  - TP53 on chromosome 17p13.1
  - Hyperparathyroidism 2 protein (HRPT2) on chromosome 1q21
- Genetic alterations implicated in the pathogenesis of parathyroid neoplasia
- Tumor suppressors
  - MEN1 is located on chromosome 11q13
    - The active protein is menin
  - Loss of expression is associated with hyperplasia or adenomas
  - Rb is located on chromosome 13q14
  - The Rb protein shows decreased expression in parathyroid carcinomas
  - HRPT2 is located on chromosome 1q2–q32
  - HRPT2 is lost in familial isolated hyperparathyroidism, hyperparathyroidism jaw tumor syndrome, and in parathyroid carcinomas
  - TP53 located on chromosome 17p13.1; mutations may occur in adenomas and carcinomas
- Oncogenes
  - RET is a member of the tyrosine kinase family
  - Mutations are found in hyperplasia and in adenomas
  - Cyclin D1 is a cell cycle regulator which may be rearranged or amplified in parathyroid adenomas and carcinomas

Parathyroid Adenoma

- Definition: Common benign neoplasm usually involving only one parathyroid gland and causing hypercalcemia and hyperparathyroidism
- Clinical features
  - Hyperparathyroidism and hypercalcemia
  - Most common in fifth and sixth decades
  - Most common in female patients
- Pathologic features (Fig. 17.8)
  - Enlarged glands may range from 80 mg to more than a few grams

Table 17.3 Genes involved with parathyroid adenomas and carcinomas

Histological type	Syndromes	Genes	Chromosome
Adenoma/hyperplasia	MEN1 MEN2	MEN1 RET	11q13 10q11.2
Carcinoma	Hyperparathyroidism–jaw tumor syndrome	HRPT2	1q21

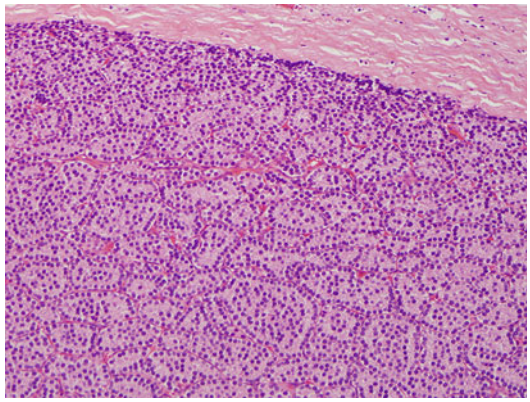

Fig. 17.8 Parathyroid adenoma showing chief cells with uniform cells and no infiltration of the adjacent capsule

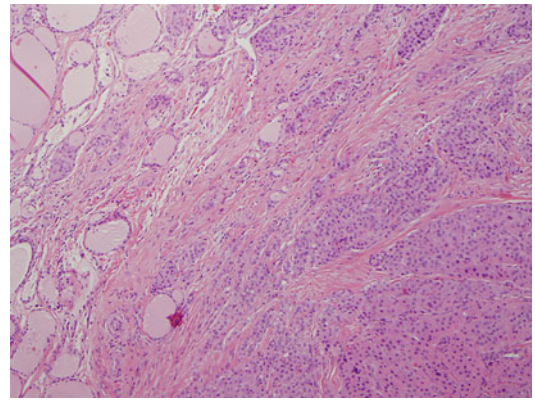

Fig. 17.9 Parathyroid carcinoma showing tumor cells infiltrating into the adjacent thyroid tissue

  - Chief cells are most common type seen
  - A rim of normal parathyroid may be present adjacent to the adenoma
  - Cystic changes may be present
    - More commonly in hyperparathyroidism jaw–tumor syndrome
  - Occasionally mitotic figures may be present
- Molecular features
  - MEN1 gene mutation
  - Cyclin D1 rearrangement
  - RET mutations
  - HPRT2 mutations

Parathyroid Carcinoma

- Definition: Malignant neoplasm of parathyroids usually involving only one parathyroid gland and associated with severe hypercalcemia and hyperparathyroidism
- Clinical features
  - Severe hyperparathyroidism
  - Peak age usually in the fifth and sixth decades
  - Equal sex distribution
  - May have a palpable neck mass
  - Metastases to regional lymph nodes, lungs, and liver
- Pathologic features (Fig. 17.9)
  - Usually weighs between 2 and 10 g
  - Composed mainly of chief cells
  - Mitotic rate variable, but often high
  - May see necrosis, vascular invasion, extension to adjacent soft tissues, or thyroid invasion
- Genetic features
  - Overexpression of cyclin D1
  - Rb protein is decreased or absent
  - HRPT2 shows LOH and may show mutations
  - Loss of HRPT2 expression in many tumors
  - TP53 mutations

Adrenal Cortical Tumors

General Molecular Concepts in Adrenal Cortical Tumors

- Definitions: Steroid producing neoplasms which may produce glucocorticoids or mineralocorticoids

Table 17.4 Hereditary tumor syndromes associated with adrenal cortical tumors

Hereditary tumor syndrome	Gene (chromosome location)	Prevalence of adrenal cortical tumors syndrome
Li–Fraumeni syndrome	TP53 (17p13) hCHK2 (22q121)	ACC 3–4%
Beckwidth–Wiedemann syndrome	IGF2 (11p15) H19 CDKN1C KCNQ1	ACC 4–5%
Carney complex	PRKAR1A (17q23–q2), 2pl6	PPNAD 90–100%
Multiple endocrine neoplasia I	MEN1 (11q13)	ACA 55% ACC rare
Congenital adrenal hyperplasia/tumors	CYP21B (6p21.3)	Adrenal tumors 82% Hyperplasia 100%

ACA adrenal cortical adenoma; *ACC* adrenal cortical carcinoma; *PPNAD* primary pigmented nodular adrenocortical disease

- Several inherited tumor syndromes are associated with adrenal cortical tumors (Table 17.4)
  - Beckwith–Wiedemann syndrome
  - Li–Fraumeni syndrome
  - MEN1
  - Carney complex
- Genetic alterations implicated in the pathogenesis of adrenal cortical tumors
  - TP53: Located on chromosome 17
  - Mutations in exons 5–8 are found in 20–27% of sporadic adrenal cortical carcinomas and 0–6% of adrenal cortical adenomas
    - Most mutations are in the DNA-binding domain
- IGF system
  - Located on chromosome 11p15.5 locus
  - Consists of IGF1 and IGF2
    - More commonly overexpressed in adrenal cortical carcinomas than in adenomas
    - LOH in 34% of adenomas and 83% of carcinomas
    - Overexpression of IGF2 is associated with a higher risk of recurrence in adrenal cortical carcinomas
- $P57^{Kip2}$ gene
  - Located within the 11p15 region and is paternally imprinted
  - Encodes a CDK inhibitor which binds to cyclin-CDK complexes and inactivates their catalytic domain
    - Functions as a negative regulator of cell cycle progression
- MEN1 gene
  - Located in chromosome 11q13
  - LOH of the MEN1 gene occurs in about 20% of sporadic adrenal cortical tumors
- PRKAR1A gene
  - Located on chromosome 17q23–q24
  - Main mediator of cAMP signaling
  - LOH of 17q23–24 in 23% of adenomas and 53% of carcinomas
    - Direct sequencing of PRKAR1A found mutations in 10% of adenomas
- Guanine nucleotide-binding protein
  - Located on chromosome 20q13.2
  - Associated with McCune–Albright syndrome
  - GNAs mutated in sporadic adrenal cortical adenomas
  - CYP21B located on chromosome 6p21.3
  - Associated with adrenal hyperplasia in 100% of cases and in adrenal neoplasia in 100% of cases

Adrenal Cortical Adenoma

- Definition
  - Benign neoplasm derived from the adrenal cortex

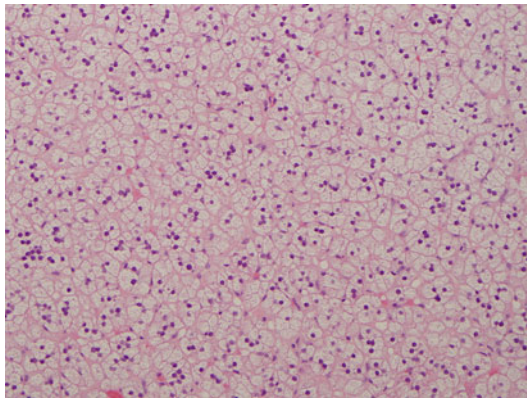

Fig. 17.10 Adrenal cortical adenoma showing cells with abundant clear cytoplasm and small nuclei

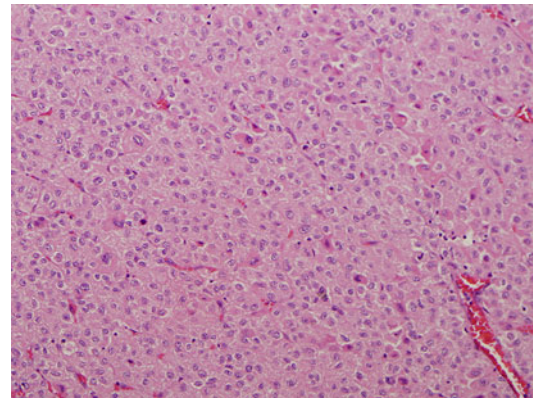

Fig. 17.11 Adrenal cortical carcinoma showing a diffuse growth pattern of tumor cells with enlarged nuclei and several mitotic figures

  - May be composed of adrenal cortical cells which can range from cells of the zona glomerulosa, fasciculata to reticularis
- Clinical features
  - Adenomas may be associated with excess production of mineralocorticoids, glucocorticoids, or sex steroids
  - Occurs in children, but more common in adults
- Pathologic features (Fig. 17.10)
  - Usually composed of zona fasciculata cells, but may be zona glomerulosa or zona reticularis cells
  - Cells have abundant lipid laden cytoplasm and small nuclei
  - Ultrastructural features include distinct mitochondrial cristae
- Genetic findings
  - TP53 mutations in 0–6%
  - IGF1 and IGF2
  - LOH in 34% of adenomas
  - MEN1 gene mutations
  - PRKAR1A mutations in 10% of adenomas

Adrenal Cortical Carcinomas

- Definition: Malignant neoplasm of adrenal cortical origin
- Clinical features
  - May occur in pediatric patients as well as in adults
  - Various familial conditions associated with carcinomas
- Pathologic features (Fig. 17.11)
  - Large tumors
  - Associated with increased mitotic activity including atypical mitoses
  - Necrosis and vascular invasion often present
- Genetic features
  - TP53 mutation in 20–27% of carcinomas
  - IGFI and IGFII overexpression in up to 83% of carcinomas
  - PRKAR1A mutations in 53% of carcinomas
  - $P57^{kip2}$ alterations in some carcinomas
  - MEN1 gene LOH

Adrenal Medullary Tumors and Paragangliomas

General Molecular Concepts in Adrenal Medullary Tumors and Paragangliomas

- Definition: Spectrum of benign and malignant tumors associated with adrenal medulla and paraganglionic tissues including pheochromocytomas and paragangliomas
- Several inherited tumor syndromes associated with adrenal medullary tumors and paragangliomas (Table 17.5)
  - MEN2A and MEN2B
  - Von Hippel–Lindau disease

Table 17.5 Syndromes associated with the pathogenesis of pheochromocytomas and paragangliomas

Syndrome	Gene	Chromosome	Adrenal pheochromocytoma	Paraganglioma
Von Hippel–Lindau	VHL	3p25–26	++	Rare
MEN2	RET	10q11.2	++	Rare
NF1	NF1	17q11	+	Rare
PGL1	SDHD	11q23	+	++
PGL2	SD5 (SDHAF2)	11q13.1	Rare	++
PGL3	SDHC	1q21–23	Rare	++
PGL4	SDHB	1q23–25	+	++

  - Neurofibromatosis type I
  - Familial paraganglioma/pheochromocytoma syndrome
- Genetic alterations implicated in the pathogenesis of pheochromocytomas and paragangliomas
- MEN2A—located on chromosome 10q11.2
  - Autosomal dominant with genetic mutation of RET gene
  - About 50% of patients with MEN2A develop pheochromocytomas
    - Missense mutations in RET in exons 10 and 11 are common
- MEN2B—located on chromosome 10q11.2
  - Mutations affect the catalytic site of the kinase
  - Mutations of exon 16 and less commonly exon 15 are seen in MEN2B
- Von Hippel–Lindau
  - Located on chromosome 3p25
    - Disease inherited in an autosomal-dominant fashion
  - Type 1 VHL disease is associated with a low risk for pheochromocytomas
  - Type 2 VHL separated into three categories
    - Type 2A
    - Type 2B
    - Type 2C
- Neurofibromatosis type 1 located on chromosome 17q11.2
  - Autosomal-dominant inherited disease
  - About 0.1–5.7% of cases may develop pheochromocytomas
    - There is 100% disease prevalence in families
  - NF1 gene encodes for the neurofibromin protein
- Familial paragangliomas/pheochromocytoma syndrome
  - Associated with genes encoding several of the subunits of the succinate dehydrogenase (SDH) mitochondrial complex 2, a tumor suppressor
  - PGL type 1 on chromosome 11q23 associated with SDHD mutations
  - PGL type 2 on chromosome 11q13.1 associated with SDH5 (SDHAF2) mutations
  - PGL type 3 on chromosome 1q21–23 associated with SDHC mutations
  - PGL type 4 on chromosome 1q23–25 associated with SDHB mutations
  - Tumors with SDHB mutations are more likely to be malignant

Pheochromocytoma

- Definition: A benign tumor of chromaffin cells in the adrenal medulla
- Clinical features
  - May present with paroxysmal or sustained hypertension
  - Palpitation, tachycardia, tremors, and headaches may be present
  - Elevated serum and urine catecholamines and metabolites of catecholamines such as vanilmandelic acid
- Pathologic features (Fig. 17.12)
  - Tumors are of neural crest origin
  - Composed of large polygonal cells with basophilic cytoplasm forming cell nests (Zellballen)

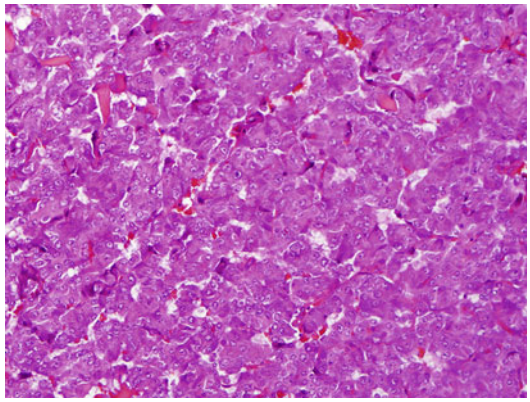

Fig. 17.12 Pheochromocytoma showing cells with abundant basophilic granular cytoplasm and small round nuclei

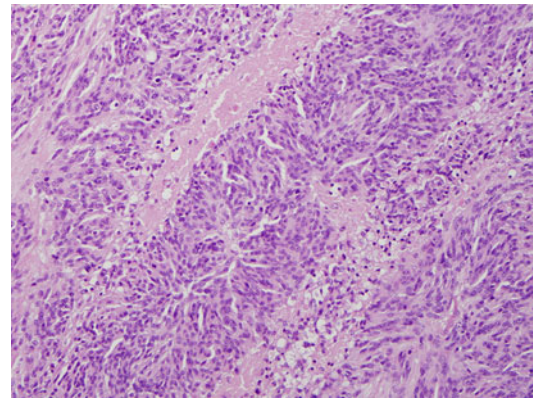

Fig. 17.13 Malignant pheochromocytoma with proven metastases to the liver showing spindled cells with necrosis and increased mitotic figures

  - Immunohistochemistry is positive for chromogranin A and for synaptophysin and negative for cytokeratin
  - Electron microscopy shows cytoplasmic dense core secretory granules
  - S100 protein-positive sustentacular cells
- Genetic findings
  - MEN2A and MEN2B mutations common in familial pheochromocytomas
  - VHL mutations in 4% of pheochromocytomas
  - NF1 mutations present in about 4% of pheochromocytomas
  - PGL1 or SDHD rare in pheochromocytomas (approximately 4%)
  - PGL3 or SDHC mutations rare in pheochromocytomas
  - PGL4 or SDHB mutations in some pheochromocytomas (approximately 3%)

Malignant Pheochromocytomas

- Definition: A pheochromocytoma with proven metastatic disease to sites where paraganglionic tissues are usually not found
- Clinical features
  - Rate of malignancy varies with locations and specific genetic mutations
  - Age of patients with malignant pheochromocytoma is quite variable
- Pathologic features (Fig. 17.13)
  - Malignancy defined by metastatic disease
  - Tumors with increased weight, size, mitotic activity, necrosis, spindle cell morphology are more likely malignant
  - Metastatic disease present with malignant tumors
- Genetic features
  - MEN2A and MEN2B mutations are more common in familial tumors
  - VHL mutations
  - NF1 mutations are uncommon in malignant pheochromocytomas
  - PGL1 or SDHD mutations present in a small percentage of malignant pheochromocytomas
  - PGL3 (SDHC) and PGL4 (SDHB) mutations are rare in malignant pheochromocytomas

Paragangliomas

- Definition: Neuroendocrine tumors arising from the extraadrenal sympathetic and parasympathetic paraganglia
- Clinical features
  - Tumors are distributed from the head and neck to the urinary bladder and organs of Zuckerkandl
  - Multifocal tumors in familial cases

  - Tumors may produce norepinephrine and less commonly epinephrine
  - Tumors of the cauda equina are unusual in that they frequently express cytokeratin
- Pathologic features (Fig. 17.14)
  - Tumors are of neural crest origin
  - Growth in cell nests (Zellballen)
  - Immunoreactivity for chromogranin A and synaptophysin
  - S100 protein stains sustentacular cells
  - Dense core secretory granules present on electron microscopy
  - Most are benign; malignant tumors with proven metastases are uncommon
- Genetic features
  - PGL1 or SDHD mutations associated with head and neck paragangliomas
  - PGL2 or SDH5 (SDAF2) mutations in head and neck paragangliomas
  - PGL3 or SDHC mutations in head and neck paragangliomas
  - PGL4 or SDHB mutations are commonly associated with malignancy in retroperitoneal paragangliomas (Fig. 17.15)

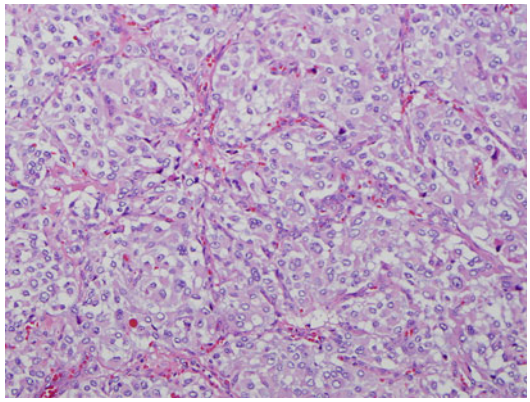

Fig. 17.14 Paraganglioma showing cells with abundant eosinophilic cytoplasm and round nuclei. Some cells form cell nests (zellballen)

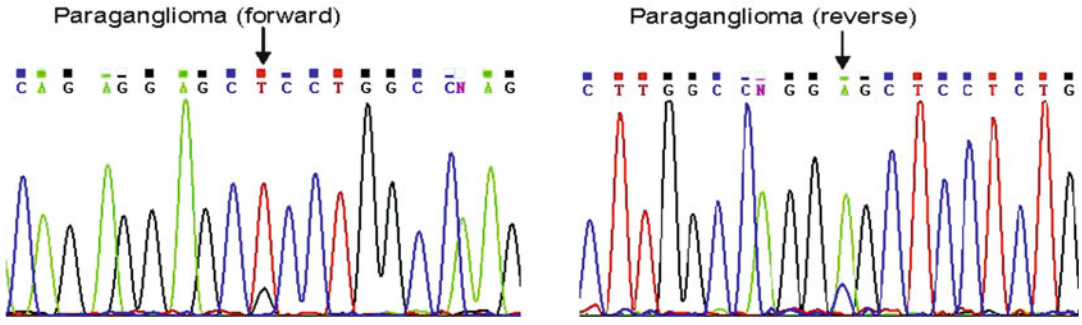

Fig. 17.15 Paraganglioma showing a mutation in the succinate dehydrogenase gene. Bidirectional DNA sequencing shows a CGC > CTC (R230L) mutation in exon 7 of the SDHB gene

Suggested Readings

Thyroid Tumors

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L. Cheng and J.N. Eble (eds.), *Molecular Surgical Pathology*,
DOI 10.1007/978-1-4614-4900-3, © Springer Science+Business Media New York 2013