Cancer Pathology

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3.1 Introduction

This text is intended as an overview of cancer pathology with particular reference to classification, tumorigenesis, microscopic features, sampling, and diagnostic techniques for ocular cancer.

3.2 Classification of Neoplasia

Tumors may be either benign or malignant, where benign tumors may be precursors to malignant tumors. The most significant difference between benign and malignant tumors is that the malignant tumors have metastatic potential, whereas benign tumors do not metastasize. However, any benign or malignant tumor may cause death if inappropriately located. Characteristic differences between benign and malignant tumors are summarized (Table 3.1). Below, different benign and malignant tumors are exemplified.

3.2.1 Benign Tumors

Benign tumors are usually labeled by the suffix -oma. Some exceptions tend to cause confusion; lymphoma and melanoma are by definition malignant, irrespective of the suffix -oma. To emphasize this, terms like malignant lymphoma and malignant melanoma are sometimes used:

- Adenoma is composed of cells originating from glandular epithelium.
- Hamartoma is composed of physiologic cells originating at the affected site.

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Table 3.1 Characteristic differences between benign and malignant tumors

Feature	Benign	Malignant
Cellular pleomorphism	None or mild	Mild to severe
Cellular dedifferentiation	None or mild	Mild to severe
Necrosis	Rare	Occasionally
Basement membrane invasion	Never	Frequent
Metastatic spread	Never	Occasionally

- Choristoma is composed of cells not normally occurring at the affected site, but otherwise histologically physiologic.
- Teratoma is composed of pluripotent cells forming different types of tissue originating from one or more of the three germ cell layers. A teratoma may be either benign or malignant.

3.2.2 Malignant Tumors

- Carcinoma is a neoplasm of epithelial origin. For example, an adenocarcinoma of the lacrimal gland is a cancer derived from the glandular epithelium of the lacrimal gland.
- Sarcoma is derived from mesenchymal tissue.
- Blastoma is a malignant tumor of embryonic origin. For example, retinoblastoma is derived from retinoblasts in the developing retina.
- Leukemia is a malignancy of blood cells that arises from the bone marrow precursor cells and is present in the peripheral blood.
- Lymphoma is a malignancy derived from lymph nodes, but may occasionally be present in other organs such as the lacrimal gland or in peripheral blood.
- Melanoma is a malignant tumor that originates from melanocytes, i.e., cells containing intracytoplasmic pigment lodged in specific organelles, melanosomes. These cells appear in the skin, uvea, conjunctiva, and a variety of other tissues.

3.3 Tumorigenesis

Tumorigenesis is a multistep process in which normal cells progressively evolve to a neoplastic state. Advances in techniques for studying cancer genetics have revolutionized our knowledge of tumorigenesis [1]. The rapidly accumulating information on the genetic and epigenetic constitution of malignancies has made it possible to tailor novel therapeutic agents, several of which are in clinical use. Hanahan et al. structured a succession of "hallmarks" of tumor cells which mark the steps of tumorigenesis [1]. These hallmarks include *genomic instability and mutations*, *evading immune destruction*, *proliferative signaling and reprogramming energy metabolism*, *resisting growth suppressors*, *escaping cell death*, *replicative immortality*, *angiogenesis*, *invasion and metastasis*, and *the tumor microenvironment*. The hallmarks are briefly outlined below; the genes involved in tumorigenesis are discussed in greater detail elsewhere (Chap. 6).

3.3.1 Genomic Instability and Mutations

Believed to underlie many of the hallmark capabilities in neoplastic cells, genomic instability generates random mutations including chromosomal rearrangements (Fig. 3.1). Tumors progress by stepwise accumulation of enabling genetic mutations. Random mutations occur in genomic regions that drive malignant progression. Non-tumorigenic regions may also harbor "passenger" mutations that are not pathogenic but may serve as biomarkers [2]. Recurrent genetic aberrations and mutations across several different tumor types indicate that some genetic regions are important drivers of tumorigenesis [3]. The recent application of deep sequencing of entire cancer cell genomes will increase our knowledge of these apparently random mutations [4]. Studies have shown that mutations of telomerase and telomeres are required to achieve endless replication and survival [5]. However, recent evidence suggests that cells that survive the telomere erosion enter breakage-fusion-bridge cycles with resulting gross genetic aberrations. Tumor cells with these complex genetic alterations will have an accelerated acquisition of mutations and rapidly progress to more malignant states [6].

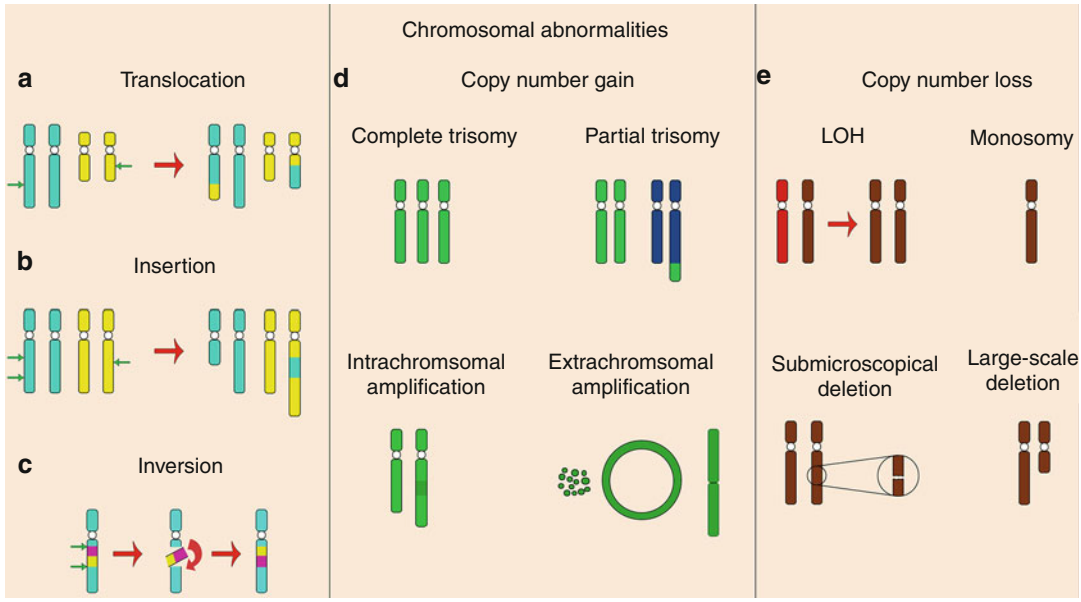

Fig. 3.1 Chromosomal abnormalities in human cancer. A multitude of different chromosomal aberrations underlie tumorigenesis. These may lead to the formation of fusion gene or over- or underexpression of structurally normal gene. The most common are included in this figure. (**a**) Translocation: one chromosomal segment is translocated from one chromosome to another and vice versa. (**b**) Insertion: a chromosomal segment is inserted into another chromosome. (**c**) Inversion: a 180° rotation of a segment. (**d**) Copy number gain can occur through, e.g., complete or partial trisomy, intrachromosomal, and extrachromosomal amplifications. (**e**) Copy number loss includes copy number neutral loss of heterozygosity (LOH), submicroscopic, or large-scale deletions

3.3.2 Evading Immune Destruction

The immune system plays an important role in eliminating both micrometastases and late stage tumors [1]. Studies on mouse models have revealed that deficiencies of NK and T cells result in a higher risk of developing cancer, which suggests that both the adaptive and innate immune systems serve as barriers to tumor progression [7, 8]. Tumor cells must therefore be able to evade immunological killing in order to thrive. This can be achieved, e.g., by altering immunological reactions by secreting immunosuppressive factors such as TGF-β [9].

3.3.3 Proliferative Signaling and Reprogramming Energy Metabolism

Proliferative signaling has been recognized as one of the earliest and most important steps in tumorigenesis [1]. It is mediated in large by growth factor ligands that bind to cell-surface receptors (frequently tyrosine kinase domains), which mediate proliferative signals, such as progression through the cell cycle, growth, survival, and energy metabolism [1]. By altering the proliferative signaling network, neoplastic cells become independent of their environment and are placed in a continuous state of "on." The genes involved in growth signaling are referred to as oncogenes. Recently, new light has been shed on the adjustments of energy metabolism to suit the rapid growth of cancer cells [1]. The energy metabolism of cancer cells has been demonstrated to be different compared to normal cells; by using aerobic glycolysis tumor cells can increase their uptake and utilization of glucose, which has been documented in many tumor types and is utilized to visualize tumor dissemination in PET-scan examination [10].

3.3.4 Resisting Growth Suppressors

Apart from activating proliferation, tumor cells must also be able to avoid signaling pathways that negatively regulate cell proliferation. The

genes controlling progression in the cell cycle and inhibitory signaling are commonly referred to as tumor suppressor genes. One of the most common suppressor genes is the *RB1* gene. This gene is mutated in a wide range of tumors [11]. Individuals with germline mutations of *RB1* suffer a great risk of developing bilateral retinoblastoma at young age, as well as several other tumor types. In normal cells, cell-to-cell contact mediates suppression of growth [1]. If normal cells lose the cell-to-cell contact, they undergo programmed cell death. This inhibitory signal is often lost in tumor cells. A set of cell-surface adhesion molecules, most notably the cadherins (E-cadherin and N-cadherin), are commonly altered in tumor cells enabling the tumor cell to let go and then reattach, thus serving as an important step in metastatic spread [12, 13].

3.3.5 Escaping Cell Death

Apoptosis is triggered by, e.g., environmental stress, elevated levels of oncogene signaling, and loss of attachment to other cells and functions as a guardian against malignant genetic change [14]. By escaping programmed cell death, tumor cells manage to survive even though they harbor multiple genetic changes.

3.3.6 Replicative Immortality

Normal cells are only able to replicate a limited number of times before they enter either senescence or cell death. Telomeres and telomerase protect the ends of chromosomes and are intimately involved in replicative immortality [1]. While telomerase is barely expressed in normal cells, its overexpression in malignancies allows the cells to escape destruction [4, 15].

3.3.7 Angiogenesis

Tumors must acquire new vasculature by angiogenesis to be able to grow beyond a size of approximately 2 mm^2 (Chap. 4).

3.3.8 Invasion and Metastasis

Tumor metastases account for approximately 90 % of tumor-related death [16]. By pinpointing the processes underlying metastasis, greater understanding and treatment options will emerge [17]. The metastatic process is thought to start with individual tumor cells dislodging from the primary tumor and spreading by blood or lymphatic vessels. The timing of tumor dissemination remains a subject of debate. There are two major models: the linear progression model where a cell acquires a set of characteristics through stepwise alteration before dissemination and the parallel progression model where cells that are not yet fully neoplastic are circulating, suggesting that they are disseminated from an early malignant lesion [18, 19]. The invasion-metastasis cascade is a complex, multistep process where tumor cells must be able to invade locally through the extracellular matrix and stromal cell layers. The precisely organized architecture of surrounding normal epithelium serves as an effective barrier, which is overcome by invading tumor cells when the metastatic process starts [17]. The matrix metalloproteinases secreted by macrophages at the tumor periphery contribute to the loss of the basement membrane by proteolysis, which facilitates invasion of the stromal compartment [17, 20]. Invading cells must also become motile to escape the primary tumor; this is achieved by alterations in cell-surface proteins that promote migration. Alteration of the cytoskeleton allows for movement along the extracellular matrix and surface of other cells. Further, the Ras family of GTPases alters actin and myosin activity, which promotes movement [1].

After leaving the primary tumor and invading the stroma, the tumor cells must intravasate into blood or lymph vessels. Intravasation into blood vessels is enabled because malignant blood vessels have insufficient pericyte coverage and the interaction is weak between adjacent endothelial cells [17]. Acquiring an intrinsic vasculature is important for primary tumors in order to metastasize and for metastases to grow beyond a certain size. Indirect evidence for this has been obtained

in uveal melanoma (and several other tumors) as tumor vessel counts have been associated with a poor prognosis [21]. Following intravasation, the tumor cells must survive the detachment from the supporting tumor matrix, the hemodynamic forces in the circulation, as well as the hostile immune system. The process of extravasation begins when the cell attaches to the vessel wall in the target tissue. This is achieved either by a tumor forming in the vessel wall, which eventually ruptures the vessel wall, or by penetrating the endothelial cells and pericyte layers and thereby establishing micrometastasis (Box 3.1) [22]. Tumors originating within the eye undergo hematogenous dissemination, because there is no lymphatic drainage. Tumors originating in the orbit or eyelids may undergo either hematogenous or lymphatic spread, depending on the tumor type.

Box 3.1: Steps in Metastatic Process

- Tumor invasion of the vasculature or lymph vessels
- Tumor cell survival in the circulation
- Cellular extravasation
- Establish a metastasis at a distant site
- Acquiring an intrinsic vasculature

Little is known about the predilection for particular metastatic targets, but two major theories have emerged: the mechanistic theory where tumor cells arrest within capillary beds due to size restrictions of the capillary vessels. The other theory describes receptor-ligand binding between tumor cells and capillaries, also known as the "seed and soil" theory: the provision of a fertile environment in which compatible tumor cells can grow [23]. Most likely the combination of both theories is true for the majority of metastatic malignancies. Some microenvironments seem to be more hospitable than others, exemplified by preferential dissemination by a wide range of different tumor types to the liver, bone marrow, and lung tissue. Extravasation of tumor cells is more challenging than intravasation since intravasation occurs at the primary tumor site where the vasculature is already quite leaky and therefore easy to traverse [17]. After intravasation the cancer cells must be able to adapt to a very different environment from that of the primary tumor. The process of metastasis is very inefficient. Indeed, it has been suggested that only <0.01 % of tumor cells that enter the circulation go through the metastatic process to establish clinical metastasis [24]. Even if the tumor cells initially survive in the new microenvironment, it is not certain that they will grow rapidly. On the contrary, most micrometastasis seems to go through a state of dormancy. This may be because of incompatibilities with the foreign environment; it may also be that the cells proliferate, though a net increase does not occur because of the counteracting effects of a high apoptotic rate [17]. An explanation of the high cell turnover and dormancy may be the failure to form new vessels [24].

3.3.9 The Tumor Microenvironment

Tumors are rarely isolated masses of homogenous proliferating cells, but rather a makeup of different tumor subpopulations as well as tumor-associated stroma, normal tissue, and infiltrating lymphocytes. Recent studies have shed light on the importance of recruitment of normal cells, which play a distinct role in the development of neoplasms, and also have been implicated in processes such as drug resistance [25]. The stroma surrounding the tumor is frequently reactive and shares similar upregulated pathways, e.g., chronic inflammation or wound healing tissue [26]. The tumor-associated stroma is induced by the neoplasm, and in turn, the stromal cells may increase the aggressive behavior of the tumor by releasing growth stimulating factors [17, 27]. Other incidents in the tumor microenvironment may increase the reactivity of the tumor surrounding stroma further. As an example, necrotic cell death releases pro-inflammatory signals into the surrounding microenvironment, which recruits inflammatory cells [26]. These cells may promote angiogenesis, cancer cell proliferation, and invasiveness [28]. Necrosis may

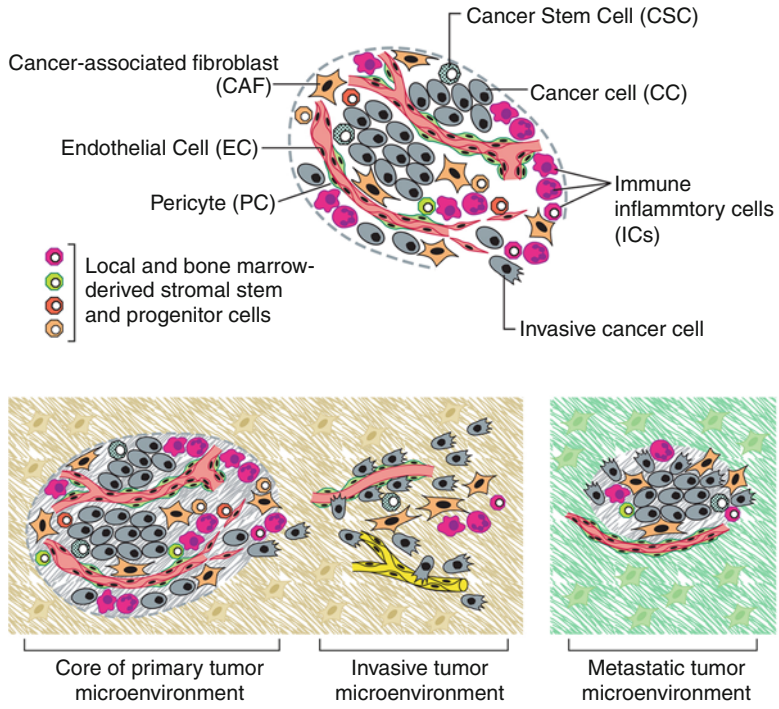

Fig. 3.2 The cells of the tumor microenvironment. *Upper:* An assemblage of distinct cell types constitutes most solid tumors. Both the parenchyma and stroma of tumors contain distinct cell types and subtypes that collectively enable tumor growth and progression. Notably, the immune inflammatory cells present in tumors can include both tumor-promoting as well as tumor-killing subclasses. *Lower*: The distinctive microenvironments of tumors. The multiple stromal cell types create a succession of tumor microenvironments that change as tumors invade normal tissue and thereafter seed and colonize distant tissues. The abundance, histologic organization, and phenotypic characteristics of the stromal cell types, as well as of the extracellular matrix (*hatched background*), evolve during progression, thereby enabling primary, invasive, and then metastatic growth. The surrounding normal cells of the primary and metastatic sites, shown only schematically, likely also affect the character of the various neoplastic microenvironments (Reprinted from Hallmarks of Cancer: The Next Generation, 144, Douglas Hanahan, Robert A. Weinberg, Page No 662, Copyright (2012), with permission from Elsevier [1])

therefore act tumor promoting; indeed tumors with necrosis are associated with worse clinical prognosis.

Solid tumors constitute a mixture of different tumor clones at different stages of development, which lead to genetic heterogeneity [29]. The existence of cancer stem cells as an important composition of the proliferating neoplasm has been a source of ongoing discussion (Fig. 3.2). Cancer stem cells are defined by their ability to seed new tumors when inoculated in host mice [30]. They have been suggested as the culprits of acquired chemotherapy resistance as well as disease recurrence after successful debulking of primary disease where no residual disease can be detected [31].

3.4 Microscopic Features of Neoplasia

Certain histopathologic features differentiate benign and malignant tumors from surrounding normal tissues. In general, the differences from the normal tissue are more marked in malignant tumors than in benign tumors.

3.4.1 Cellular Proliferation

Many malignant tumors feature a large number of dividing cells with abnormal mitotic patterns (Fig. 3.3a). Cell proliferation markers like

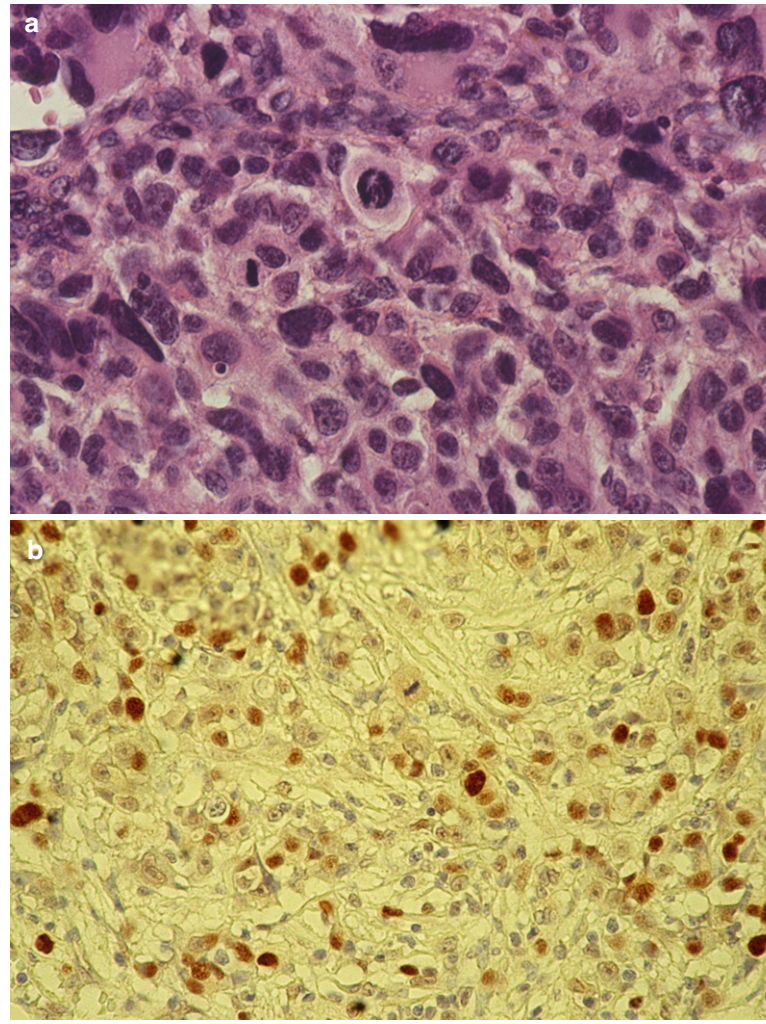

Fig. 3.3 Many malignant tumors feature a large number of dividing cells with abnormal. mitotic figures (**a**). Cell proliferation markers like proliferating cell nucleolar antigen and Ki-67 often reveal a larger proportion of proliferating cancer cells than detected by mitotic counts alone (**b**)

proliferating cell nucleolar antigen (PCNA) and Ki-67 often reveal a larger proportion of proliferating cancer cells than detected by mitotic counts alone (Fig. 3.3b). A high mitotic index (mitotic count per unit of microscopic area) is often found in rapidly growing tumors although this is usually balanced by the presence of many apoptotic cells. Not all cancers show high cell proliferation rates; typically uveal melanoma features comparatively low counts, but nonetheless may metastasize. However, in many cancers, including uveal melanoma, a high cell proliferation rate is associated with poor prognosis [32, 33].

3.4.2 Cellular Pleomorphism

When tissue and cellular architecture is distorted, the tissue is classified as dysplastic. Epithelial dysplasia may be divided into mild, moderate, and severe dysplasia. Severe dysplasia is characterized by full thickness dysplasia with prominent cellular atypia and is synonymous with carcinoma in situ. It is debatable whether a slight or even moderate degree of dysplasia is precancerous, a condition inevitably leading to cancer. When tissue architecture is considerably distorted with individual cells showing a significant degree of variation in shape and size including abnormal nuclei sometimes

featuring binucleate or multinucleate forms, this is referred to as pleomorphism at the cellular level. Cellular pleomorphism, including the extreme state when the tissue of origin is no longer recognizable (anaplasia), is a hallmark of cancer.

3.4.3 Cellular Differentiation

Typically, cancers recapitulate the architecture of the tissue of their origin to some extent. This recapitulation may closely resemble the original structure and such cancers are highly differentiated, whereas others are poorly differentiated or even anaplastic. Usually, poorly differentiated cancers carry a worse prognosis than cancers more closely mimicking the original tissue appearance. In retinoblastoma, the rosettes appearing in moderately and highly differentiated retinoblastoma are believed to be an attempt to recapitulate the original retinal structure (Fig. 3.4a). In uveal melanoma, the tumor spindle cell morphology resembles the original melanocytes, and the presence of less differentiated epithelioid cells is associated with an adverse outcome (Fig. 3.4b, c) [34].

3.4.4 Nuclear Cytoplasmic Ratio

Most neoplastic cells have a relatively large nucleus in relation to the amount of cytoplasm. However, this varies significantly with the type of neoplasia. Clear cell carcinoma of the kidney features large cells with abundant cytoplasm, whereas the retinoblastoma typically is composed of cells with relatively large nuclei and small amounts of cytoplasm.

3.4.5 Invasion of Surrounding Tissues

Tumor cells invade surrounding tissue by direct infiltration or by dissemination along blood or lymphatic vessels. In carcinoma, breakdown of the basement membrane and stromal invasion signify the progression of an in situ carcinoma to invasive carcinoma. Cancers with minimally invasive features are sometimes referred to as microinvasive.

Some malignant tumors show a particular affinity for spread along peripheral nerves extending a considerable distance away from the primary site (e.g., perineural growth in adenocystic carcinoma of the lacrimal gland). In some of these tumors, severe pain due to invasion or compression of sensory nerve fibers may be the first clinical presentation.

3.4.6 Tumor Infiltration by Normal Cells

In some malignant tumors, infiltration by macrophages or lymphocytes is a characteristic feature reflecting recruitment of the immune system as well as physiologic cells by neoplastic tissue. Macrophage infiltration in uveal melanoma is associated with a poor prognosis [35].

3.5 Tissue Sampling and Processing

3.5.1 Cytological Sampling

Sampling typically includes a number of individual cells disrupted from their original tissue. Diagnosis is therefore usually made on the morphological appearance of individual cells because the relationship to surrounding cells is lost. Cytological samples may be used for immunocytochemistry, which is conducted to characterize protein expression, and for auxiliary techniques such as flow cytometry. Two basic techniques are used for sampling.

3.5.1.1 Exfoliative Cytology

Exfoliative cytology involves sampling of cells that are dispersed in fluids (e.g., cerebrospinal fluid sampled by lumbar puncture) or forcibly removed by a spatula, brush, or some other instrument or filter paper. Cells may also be dislodged from a surface by a touch preparation, for example, imprint cytology for conjunctival tumors. The exfoliative sample is then spread on a glass slide and stained. Vitrectomy samples may be filtered though a membrane (e.g., Millipore filter), centrifugated in a pellet (cytospin preparation) or paraffin embedded as a cell block.

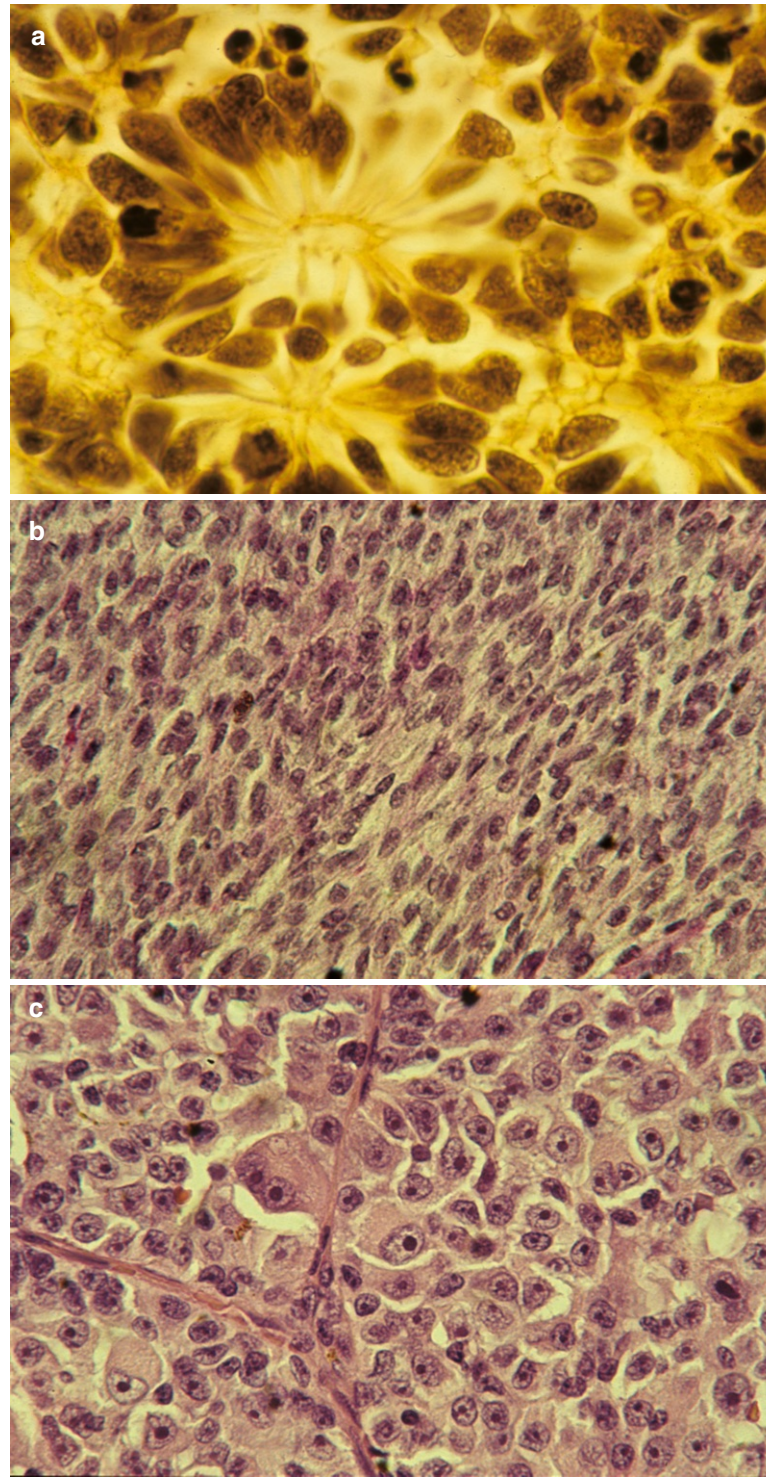

Fig. 3.4 Highly differentiated retinoblastoma with tumor cells arranged in a radiating, flowerlike pattern referred to as Flexner-Wintersteiner rosettes (**a**). Uveal melanoma specimens featuring spindle-type tumor cells (**b**) and cells with epithelioid appearance (**c**)

3.5.1.2 Aspiration Cytology

Aspiration cytology may be applied to palpable lesions, guided by ultrasound or computerized tomography imaging. Intraocular fine-needle aspiration biopsy (FNAB) is performed with needles between 21 and 25 gauges [36]. A pars plana approach guided by indirect ophthalmoscopy may be used, but clear cornea and transscleral routes have also been advocated [37–39]. The various techniques of intraocular biopsy are discussed in detail under uveal tumors. Local tumor spread using FNAB in loosely cohesive tumors is a concern. For this reason, FNAB should only be used with extreme caution and almost never in suspected cases of retinoblastoma. The requirements for tissue handling vary between laboratories; therefore, it is advisable to contact the local cytopathologist before sampling.

3.5.2 Histopathologic Sampling and Processing

Tissue is obtained by incisional or excisional biopsy of the lesion. Caution not to coagulate or otherwise maltreat the tissue sample is recommended. Incisional biopsies or core needle biopsies should be avoided in tumors prone to local recurrence, e.g., the pleomorphic adenoma of the lacrimal gland. In such tumors, primary complete excision or possibly diagnostic FNAB followed by complete excision is recommended. The optimal fixative for the surgical biopsy varies depending on the local setting and the technique used for histopathologic examination, but for most cases, formaldehyde is sufficient.

When biopsying tissues like the conjunctiva or iris, care should be taken to orientate the specimen and to make sure the tissue is maintained flat and does not curl. This can be achieved by attaching the tissue to a piece of filter paper and annotating the specimen mount before immersion in the fixative. Assessment of the surgical margins is pivotal in any excisional tumor biopsy. To facilitate this, the surgical margins may be marked by the pathologist before gross sectioning and paraffin embedding. Also, special techniques like Mohs technique using cryosectioning or modifications using vertical paraffin-embedded sections have been advocated for eyelid and skin tumors [40, 41]. Cryosectioning allows for a rapid assessment (within 30 min) of margins or malignancy and can be used as a preoperative procedure; however, paraffin sections allow a more reliable microscopic assessment. Specific guidelines for histopathology reports on cancer specimens from the eye and adnexa have been discussed elsewhere [42].

3.6 Diagnostic Techniques

Traditionally, cancer diagnosis was made using light microscopic examination, but recent advances in molecular diagnostics have created a completely new set of tools with which tumors may be more accurately diagnosed and better characterized. Some of these techniques are outlined below.

3.6.1 Light Microscopy

When processed for routine light microscopy, samples are usually fixed in formaldehyde and then embedded in paraffin. This allows for the cutting of 3–4 μm sections. Paraffin sections are usually routinely stained with hematoxylin and eosin, although this may differ depending on the local routine (Fig. 3.5a). Other stains like the periodic acid-Schiff dye preferentially stain mucopolysaccharides and glycogen and are appropriate for study of basement membrane material like the lens capsule.

3.6.2 Immunohistochemistry

This technique has rapidly evolved from a research tool to a diagnostic technique and has revolutionized diagnostic pathology. Several commercially available antibodies may be used in combination with routine fixatives like formaldehyde and staining may be enhanced by antigen retrieval techniques (Fig. 3.5b). Fixation over

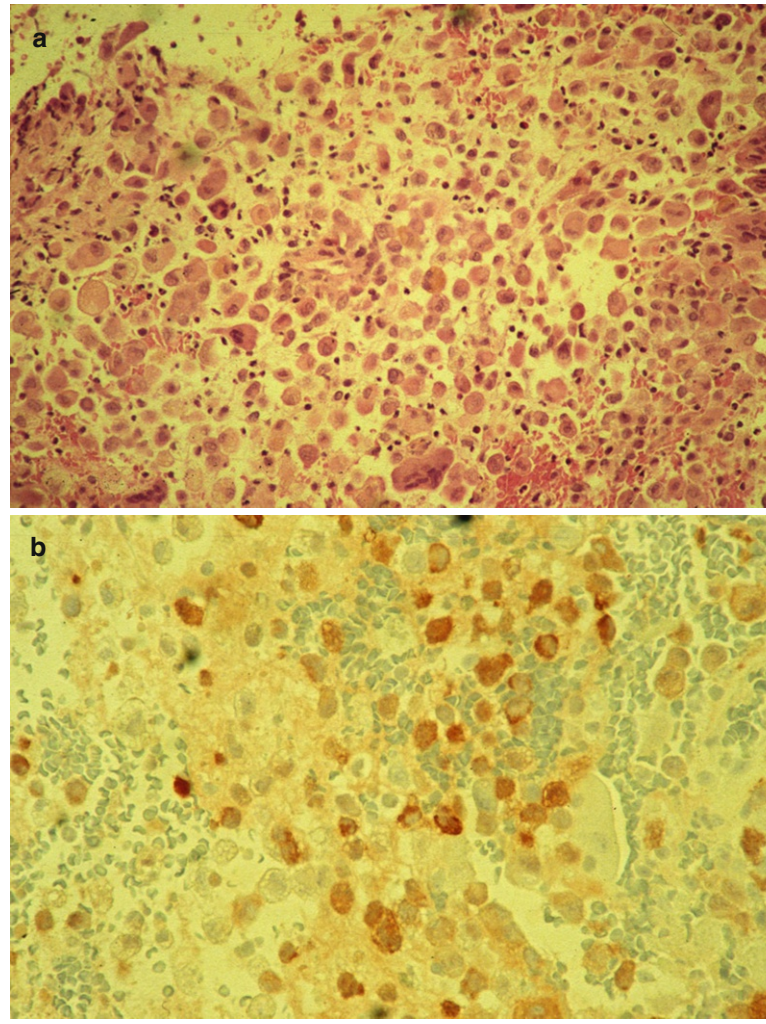

Fig. 3.5 Langerhans' cell histiocytosis. Light microscopy (**a**) and immunohistochemical stain recognizing S-100 protein (**b**)

prolonged periods of time may cause reduced staining and the wary pathologist uses negative and positive controls.

3.6.3 Additional Techniques

Recent advances in molecular pathology have generated numerous techniques for the genetic and epigenetic study of ocular tumors. One of the most important laboratory techniques is the polymerase chain reaction (PCR). Several different types of PCR have been developed, including competitive or real-time PCR, which allows for sensitive assays of gene expression at the RNA level. Further, several experimental laboratory techniques have been translated into clinical use, e.g., flow cytometry in lymphoma and tissue imprints for gene rearrangements in rhabdomyosarcoma. In several cases, tissue needs to be submitted fresh, which requires close collaboration with the examining laboratory for optimal results. Microarrays have revolutionized our understanding of the tumor genome and transcriptome by making it possible to study thousands of genes in one experiment at a single gene resolution. Distinct cancer biomarkers and gene expression profiles have been identified through microarray studies. After vigorous evaluation, several assays have been developed and are now in clinical use. Two of the earliest assays developed include the MammaPrint® and Oncotype DX® assays

[43, 44]. These assays predict prognosis and assess the likely benefit from treatment by analyzing signatures of multiple genes. In uveal melanoma, genome-wide expression profiling has identified two subsets of tumors: nonmetastasizing low-grade tumors and metastasizing high-grade tumors [45]. From these results, a 15-gene assay has been developed, which successfully separates the tumors into two distinct subgroups [12, 46]. Through fine-needle biopsies, the assay can be used to determine metastatic potential and patients can be selected for interventions like adjuvant therapies [47, 48]. Functional analysis of the genes involved in the high-grade profile has allowed identification of upregulated genes and pathways in uveal melanomas. This constitutes an important basis for novel strategies for early tumor identification and application of targeted therapies. Several antibodies have been investigated in ocular tumors, including aflibercept (VEGF-Trap) and ipilimumab (anti-CTLA4) [49, 50]. Ipilimumab is applied in routine clinical treatment, and several drugs are in various stages of clinical investigation (www.clinicaltrials.gov). In line with the findings in microarray studies, proteomics hold a similar promise and generate vast amount of data on protein expression.

Next generation sequencing of the whole genome has become a groundbreaking tool in the study of cancer cells. It is used for the characterization and identification of, e.g., DNA, RNA, exome, and transcriptome sequences of tumor cells. It enables identification of, e.g., copy number variants, sequence variants, mutations, and structural changes such as chromosomal translocations, and fusion genes. Various open source projects aim at collecting sequence variants and mutations critical in the development of human cancers. Public sharing of data has enabled inclusion in larger data sets for reanalysis and construction of databases of cancer profiles.

Described here are only a handful of the techniques developed for research and clinical study of tumors. In the near future many of the techniques used for experimental purposes will delineate central pathways in cancer cells with prognostic, diagnostic, and treatment implications in human cancer.

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