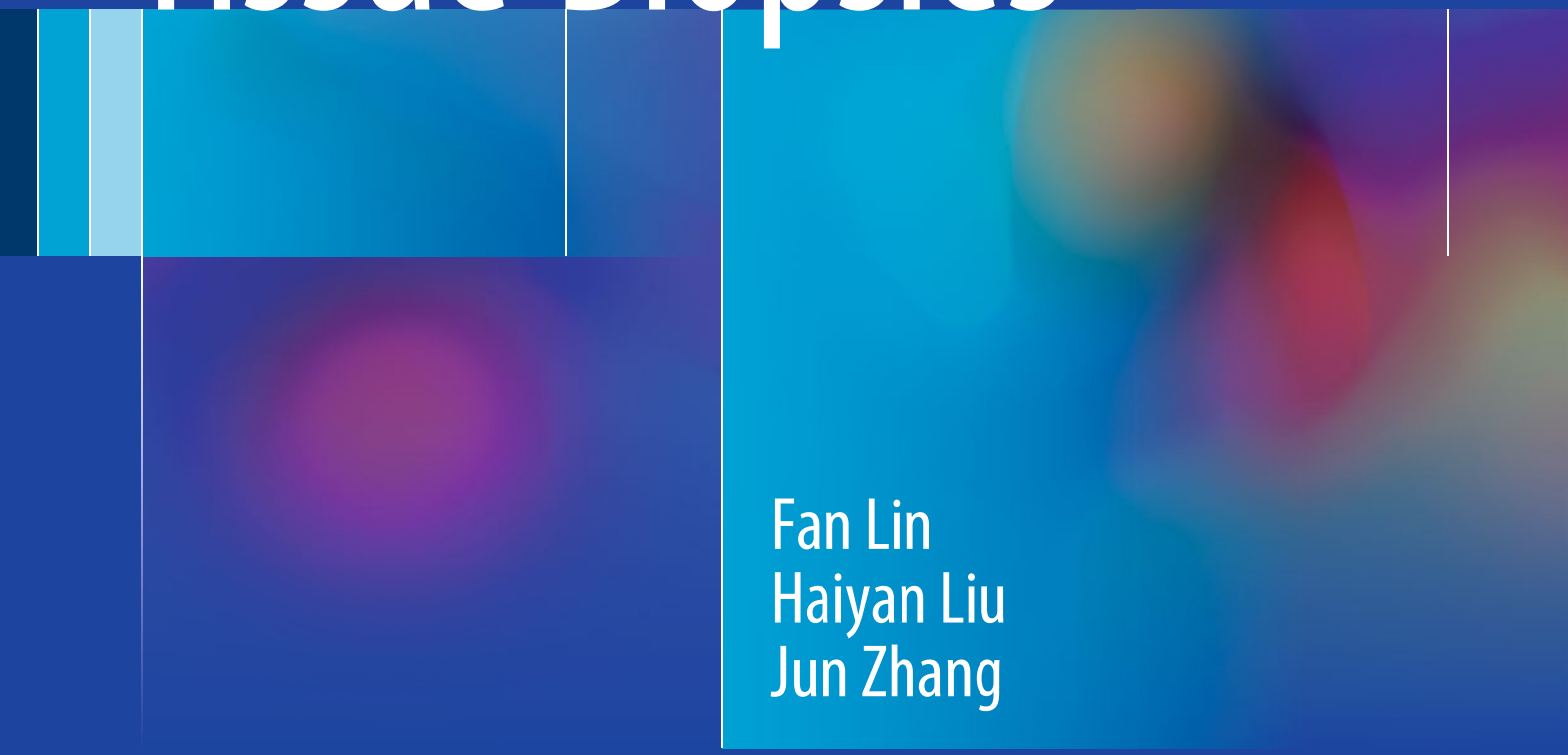
Handbook of Practical Fine Needle Aspiration and Small Tissue Biopsies



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Springer

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To my loving and supportive wife Danquing,
and my sons Dustin and Benjamin

–Fan Lin

To my dear husband Shu for his support,
and my two beautiful daughters, Jenni and Vivian

–Haiyan Liu

To my wife, Peiwen for her unlimited love and support,
and my son, David for his inspiration

–Jun Zhang

Preface

Fine needle aspiration (FNA) biopsies with cellblock preparation or small tissue biopsies have become a primary modality to achieve a definitive diagnosis of a mass-like lesion of the superficial organs (such as thyroid, salivary glands, lymph nodes, breast, and soft tissue) and deep-seated organs (such as lung, pancreas, liver, kidney, adrenal, and retroperitoneum). The primary goal of this book is to address how to obtain maximal, accurate diagnostic information from the minimal available material. This book is intended to be practical, accurate, concise, and yet up-to-date and user-friendly. Some important features are summarized as follows. This book:

- Lists diagnostic pearls and pitfalls at the beginning of each chapter; these were based on the available literature and our personal practicing experience over last 20 years.
- Applies the most current WHO Classifications of Tumors for each organ.
- Delineates cytologic and histologic features with bullet points for each neoplastic and non-neoplastic lesion, including many recently introduced entities.
- Emphasizes the importance of triple tests (clinical, radiologic, and histologic/cytologic).
- Describes the utilities and pitfalls of diagnostic IHC, including over 50 IHC panels, in a tabular format and numerous recently introduced and useful diagnostic markers.
- Highlights the application of important molecular tests.
- Provides over 1400 color microphotographs.

One of the unique features of this book is the highly practical presentations of typical case scenarios seen in an anatomic pathology laboratory at the end of each chapter to re-emphasize the crucial points illustrated throughout each chapter. These are in the form of case presentations with step-by-step critical and logical analysis. Sample cases include common but challenging situations, such as evaluation of well-differentiated malignant tumors vs benign/reactive lesions, distinction of two benign entities, subclassification of a malignant tumor, identification of newly described tumor and non-tumor entities, workup of a tumor of unknown origin, and implementation of best practice in immunohistochemistry and molecular testing in a difficult case.

In short, this book will provide a unique and valuable resource in the field of cytopathology and surgical pathology, both for those currently in training and for those already in clinical practice at various skill levels. It does not seek to duplicate or completely replace other large textbooks; rather, it is a new, comprehensive yet concise and practical resource on these timely and critical topics.

Danville, PA, USA — Fan Lin, MD, PhD
Danville, PA, USA — Haiyan Liu, MD
Scottsdale, AZ, USA — Jun Zhang, MD, MS

Acknowledgments

It was a very challenging task to produce this handbook, and we wish to acknowledge the assistance and tremendous support we received. Dr. Conrad Schuerch, Chair of Geisinger Medical Laboratory, supported and encouraged this project from conception through completion. Sandy Mullay, Operations Director of Anatomic Pathology, always ensured we had the secretarial and clerical support needed throughout the project. Melissa Erb served as our project coordinator, scheduling many meetings, pulling out many interesting cases, and keeping us organized. Kathy Fenstermacher was invaluable in editing, formatting, and polishing book chapters. Without Kathy's talent and tireless effort, it would not have been possible to meet the submission deadline. Finally, we are in debt to our families and close friends for their understanding while we buried ourselves in this project for such a long period of time. We are very fortunate to have your love and incredible support.

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Introduction and Application of Fine Needle Aspiration Biopsy

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Fan Lin, Jun Zhang, and Haiyan Liu

Indications for Fine Needle Aspiration (FNA) Biopsy

- Mass lesion with a clinical suspicion of malignant tumor – palpable or deep-seated
- Infections – virus, fungus
- Granulomatous inflammation
- Infiltration – amyloidosis

Complications of FNA

- Pain
- Bleeding
- Faintness
- Hematoma
- Pneumothorax
- Seeding of tumor cells

Advantages of FNA

- FNA is *SAFE*.
  - *S*imple
  - *A*ccurate
  - *F*ast
  - *E*conomic

Primary Applications of FNA

- Primary diagnosis of a malignant tumor.
- Confirm a reactive/benign condition.
- Metastatic tumor of unknown primary.
- Deep-seated organ/tumor.
- Confirm a recurrent tumor.

Target Organs of FNA

Superficial Organs

- Thyroid
- Lymph node
- Salivary gland
- Soft tissue
- Breast

Deep-Seated Organs

- Liver
- Pancreas
- Lung/mediastinum
- Kidney/adrenal
- Retroperitoneum

Sensitivity and Specificity of FNA (Table 1.1)

How to Perform an FNA

Supplies

- 23- or 25-gauge, 1.0-inch or 1.5-inch needle
- Syringes – 10 mL
- Syringe holder (Fig. 1.1)

Procedure

- Stabilize the target lesion.
- Pass needle through the skin and advance into the lesion.

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Table 1.1 Summary of sensitivity and specificity of FNA

Organ	Sensitivity (%)	Specificity (%)
Thyroid	83	92
Breast	92.5	99.8
Salivary gland	90	95
Lymph node	90	98
Lung	89	96
Liver	85	100
Pancreas	90	100
Kidney	85	98
Adrenal gland	85	100
Soft tissue	96	96

Note: When a sample is adequate for evaluation

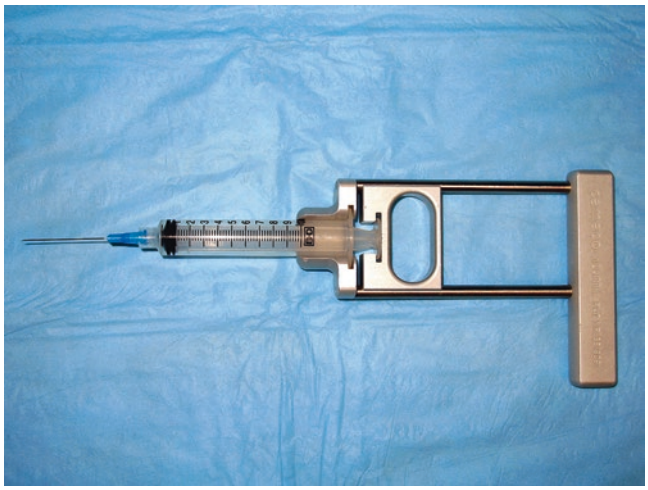

Fig. 1.1 Showing an aspiration gun (Cameco AB, Tägy, Sweden)

- Apply suction.
- Move the needle back and forth for 10 s.
- Release suction.
- Remove the needle from patient.
- Detach the needle from the syringe.
- Fill the syringe with air and replace needle on syringe.
- Express the specimen onto microscopic slides.
- Prepare air-dried and fixed smears.

How to Interpret an FNA

Overall Cellularity

- High – lymphoma, melanoma, neuroendocrine tumor (NET) (Fig. 1.2)
- Low – lobular carcinoma, schwannoma (Fig. 1.3)

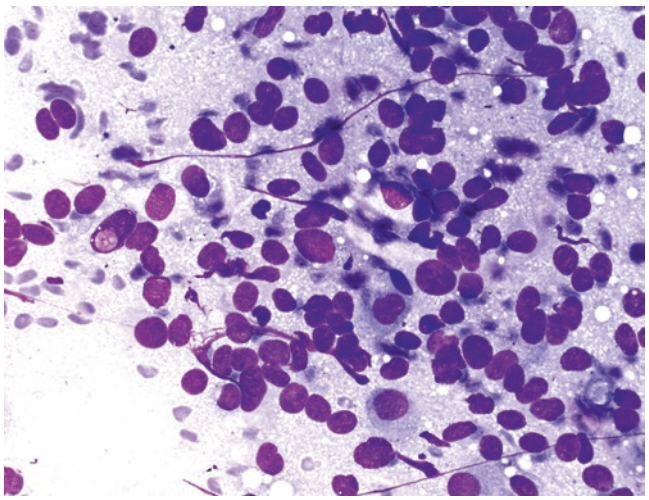

Fig. 1.2 Hypercellularity in melanoma

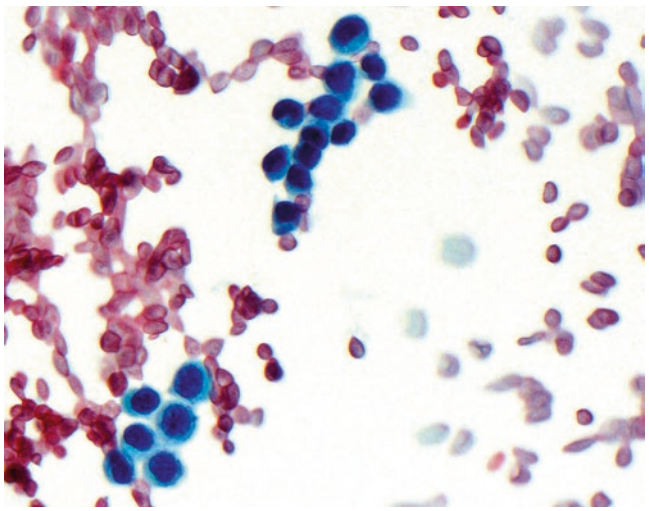

Fig. 1.3 Low cellularity in breast lobular carcinoma

Cellular Architectures

- Papillary (Fig. 1.4)
- Tightly cohesive groups (Fig. 1.5)
- Loosely cohesive groups (Fig. 1.6)
- Acinar (Fig. 1.7)
- Glandular (Fig. 1.8)

Cell Shapes

- Epithelial (Fig. 1.9)
- Epithelioid (Fig. 1.10)
- Spindle (Fig. 1.11)
- Bizarre
- Small round cell (Fig. 1.12)
- Giant cell (Fig. 1.13)

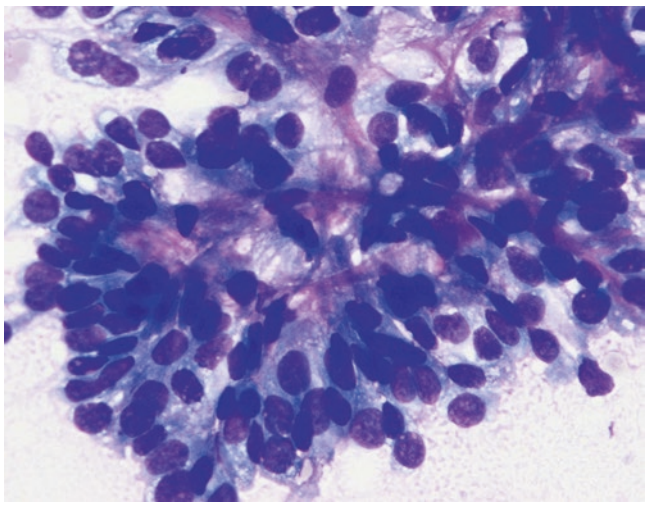

Fig. 1.4 Papillary structure in papillary RCC

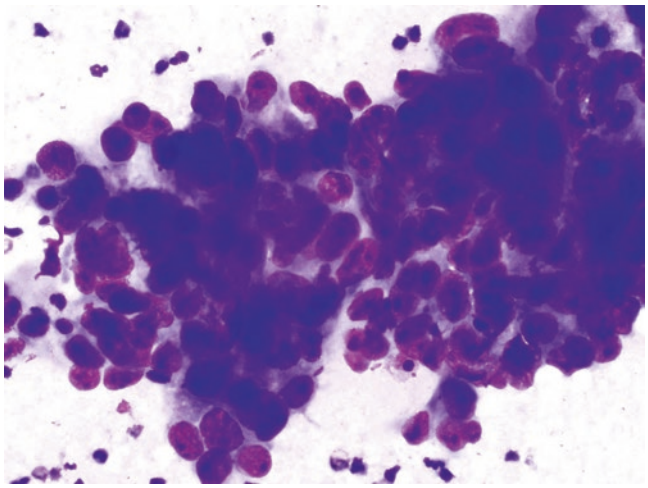

Fig. 1.5 Cohesive cellular group in medullary carcinoma of the breast

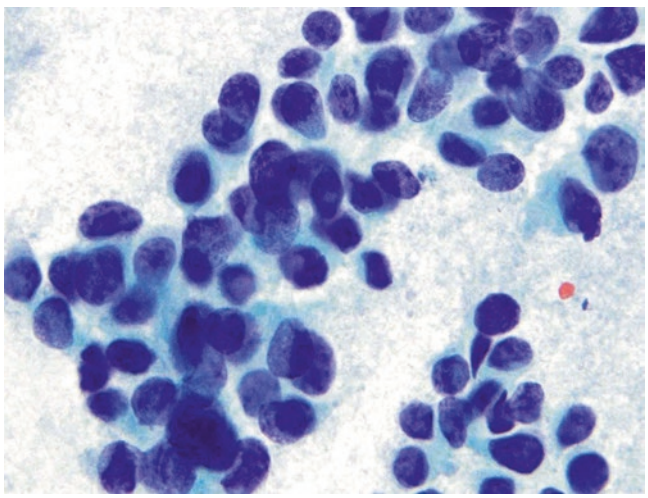

Fig. 1.6 Loosely cohesive group in breast carcinoma

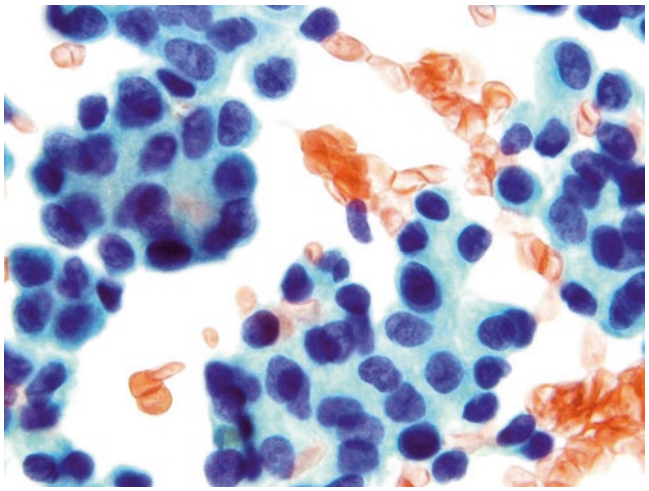

Fig. 1.7 Acinar formation in acinar cell carcinoma of the pancreas

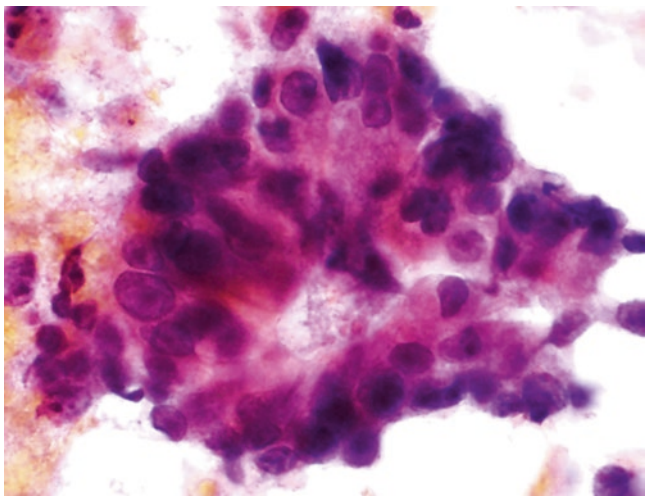

Fig. 1.8 Glandular formation in colonic ADC

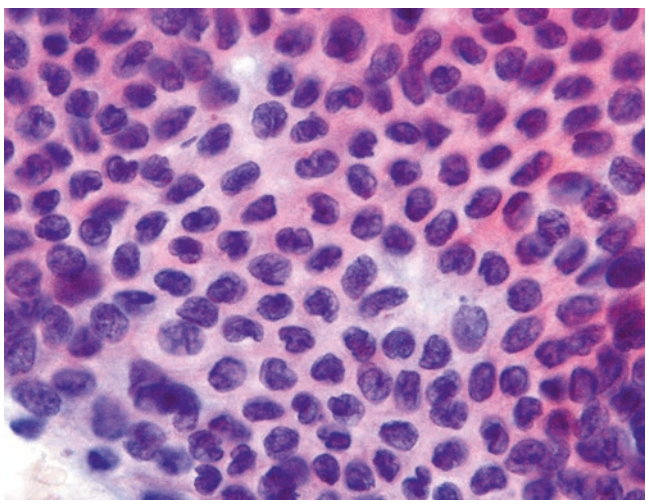

Fig. 1.9 Epithelial cells in well-differentiated ADC of the pancreas

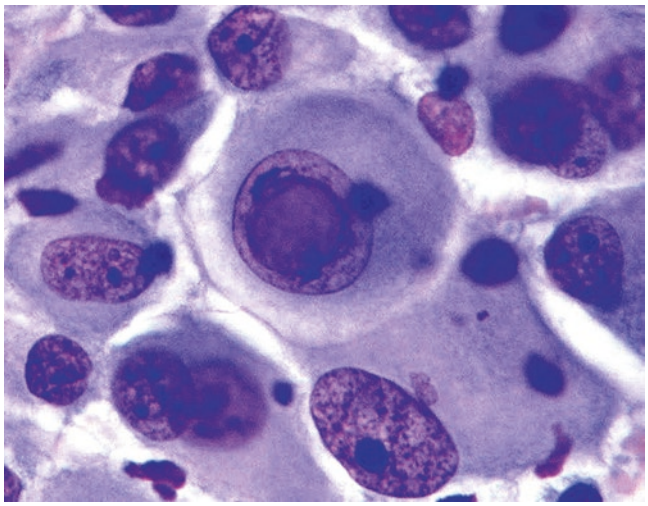

Fig. 1.10 Epithelioid cells in melanoma

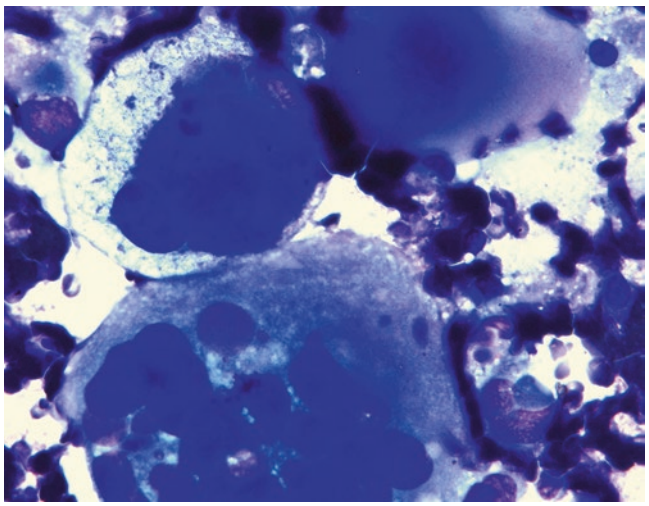

Fig. 1.13 Giant tumor cells in rhabdomyosarcoma on Diff-Quik (DQ)

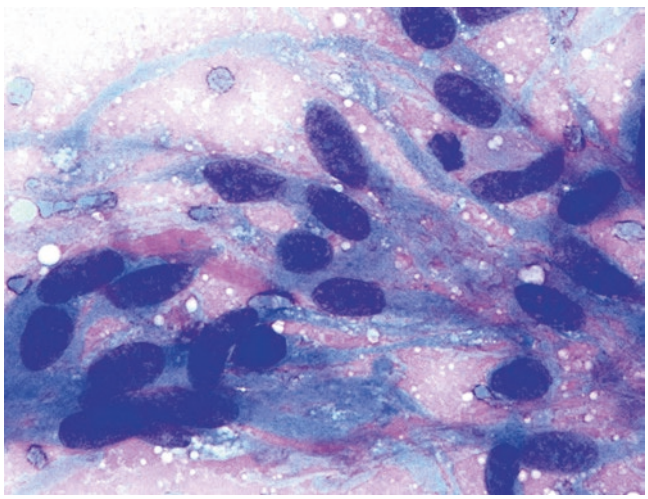

Fig. 1.11 Spindle cell in gastrointestinal stromal tumor

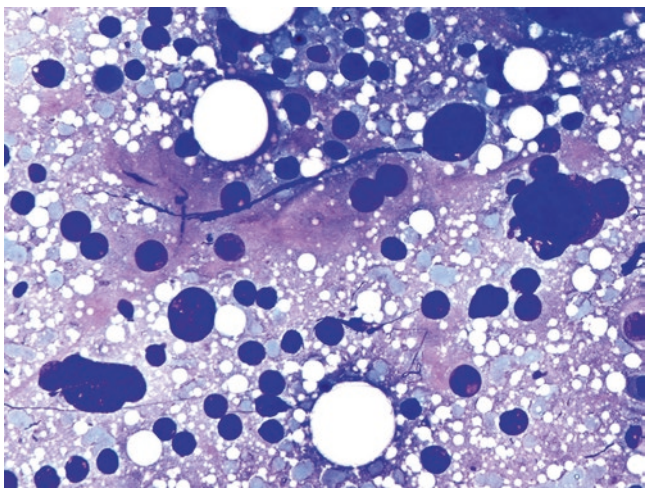

Fig. 1.14 Naked nuclei in HCC

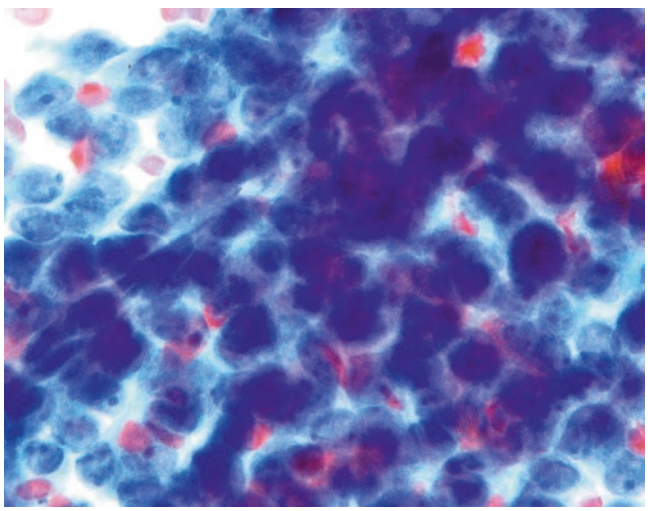

Fig. 1.12 Small blue cell in Ewing's sarcoma

Naked Nuclei

- Hepatocellular carcinoma (HCC) (Fig. 1.14)
- Acinar cell carcinoma
- Granular cell tumor
- Lactating adenoma
- Fibroadenoma

Nuclear Details

- Intranuclear inclusion – melanoma (Fig. 1.15), renal cell carcinoma (RCC), papillary thyroid carcinoma, HCC, and paraganglioma
- Nuclear grooves – papillary thyroid carcinoma, adult granulosa cell tumor, histiocytosis X (Fig. 1.16), solid-pseudopapillary tumor of pancreas

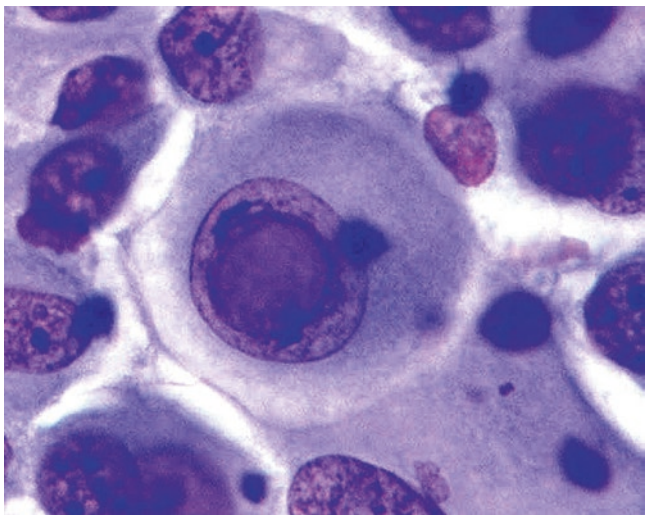

Fig. 1.15 Intranuclear inclusion in a melanoma

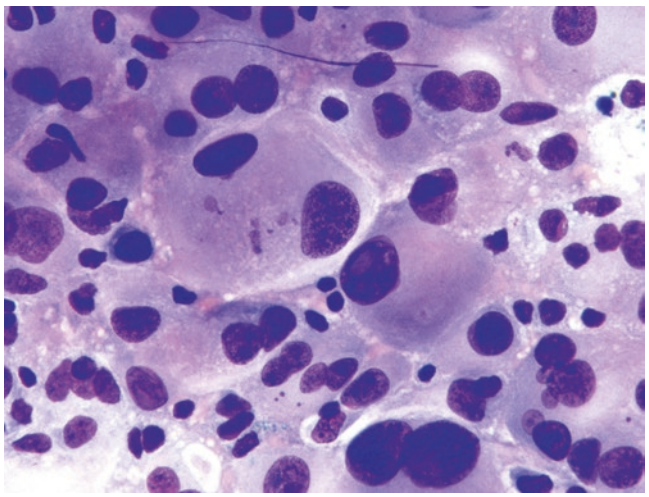

Fig. 1.17 Nuclear pleomorphism in a melanoma

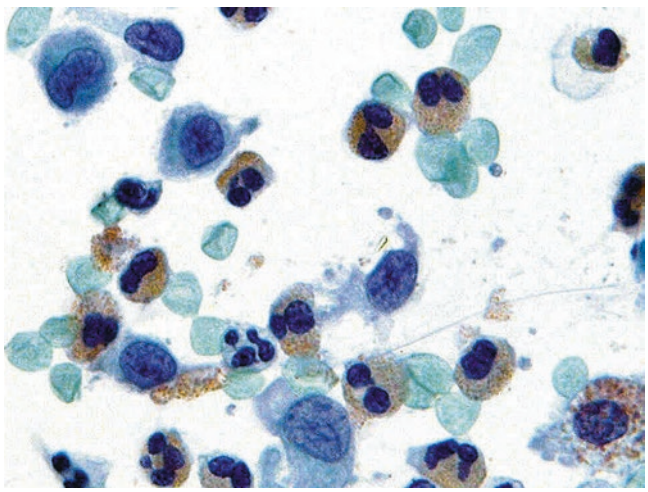

Fig. 1.16 Nuclear grooves in Langerhans cell histiocytosis

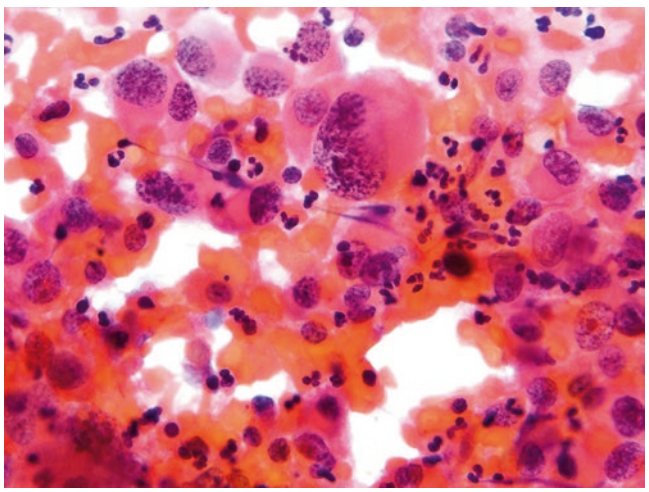

Fig. 1.18 Anaplastic carcinoma of the thyroid with marked nuclear pleomorphism

- Anisonucleosis – high-grade neoplasms (Figs. 1.17 and 1.18)
- Nuclear chromatin clearing – pancreatic carcinoma
- Prominent nucleoli – melanoma, high-grade lymphoma, HCC, high-grade RCC, adenocarcinoma (ADC), and sarcoma

Cytoplasm

- Clear – clear cell RCC (Fig. 1.19), clear cell carcinoma of the ovary and the uterus, melanoma, ADC with clear cell changes (such as pancreas), and squamous cell carcinoma (SCC) with clear cell changes
- Granular – oncocytoma (Fig. 1.20), HCC, granular cell tumor (Fig. 1.21), high-grade RCC, medullary carcinoma of the thyroid, and other NETs/carcinomas

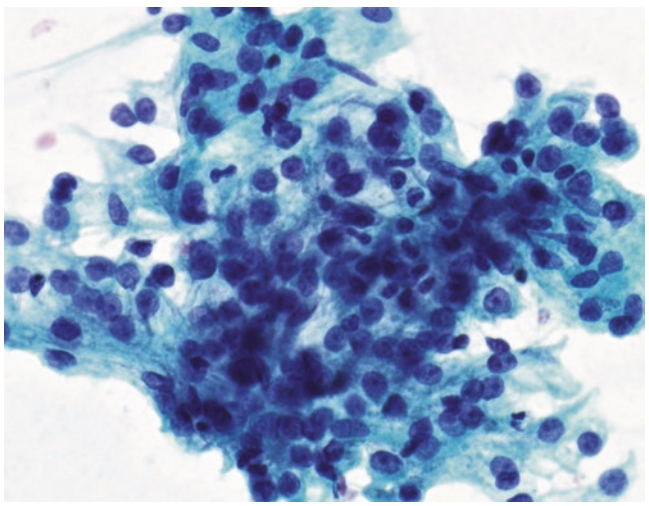

Fig. 1.19 Clear cytoplasm in a clear cell RCC

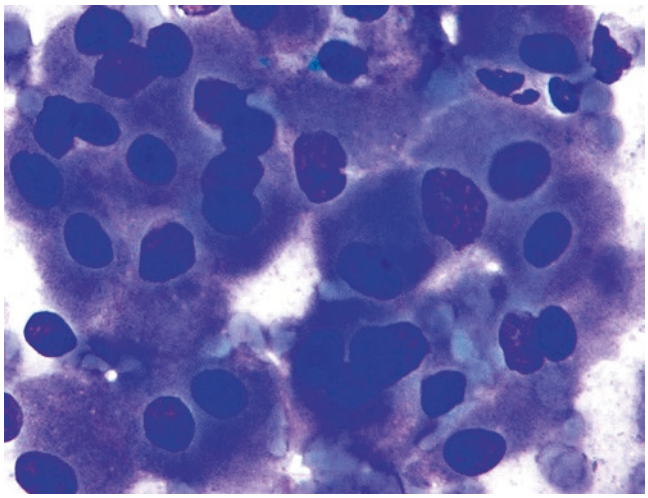

Fig. 1.20 Oncocytic cytoplasm in an oncocytoma

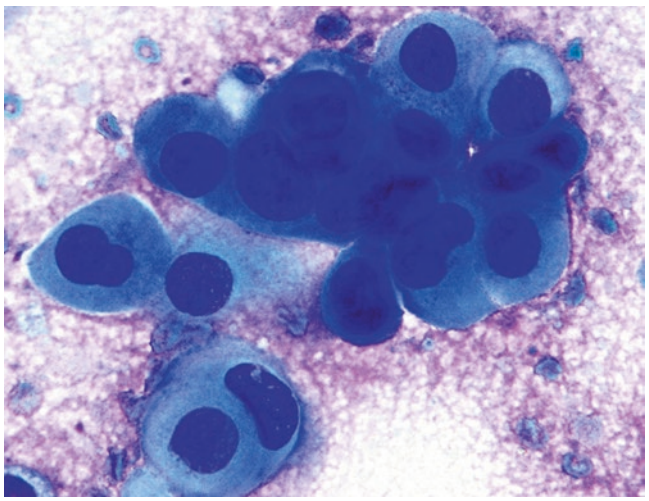

Fig. 1.22 Squamous tumor cell in a SCC on DQ

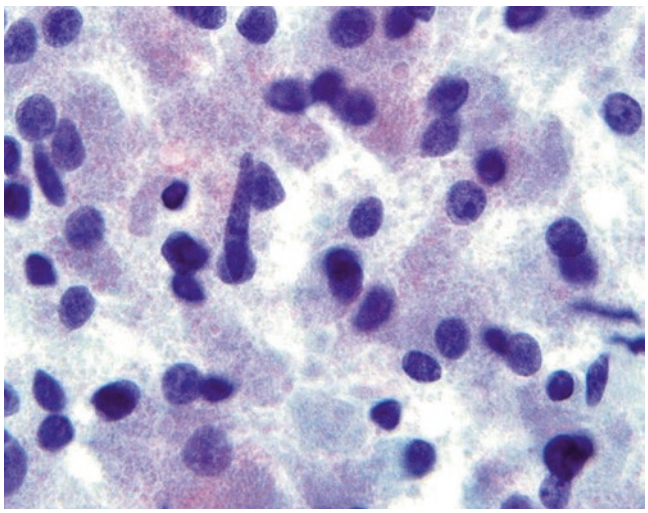

Fig. 1.21 Coarse, granular cytoplasm in a granular cell tumor

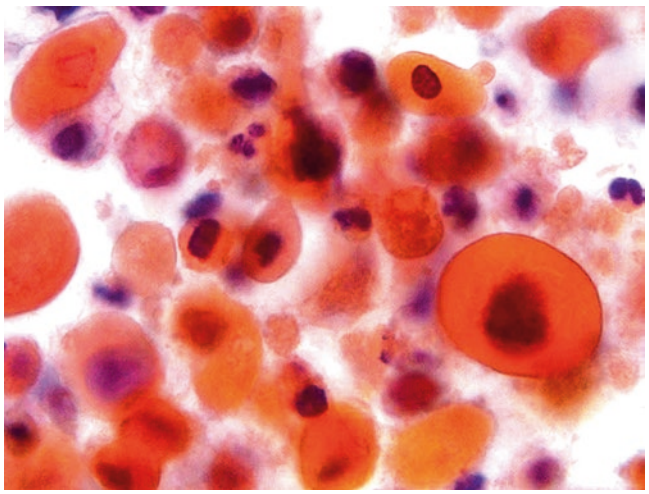

Fig. 1.23 Keratinizing squamous tumor cell in an SCC on Papanicolaou (Pap) stain

- Foamy – RCC, carcinoma of the breast, lung, and pancreas, melanoma
- Squamoid/dense – SCC (Figs. 1.22 and 1.23), papillary thyroid carcinoma, carcinoma of the lung and pancreas, and high-grade mucoepidermoid carcinoma
- Intracytoplasmic lumen – lobular carcinoma and low-grade ductal carcinoma of the breast (Fig. 1.24), signet-ring cell carcinoma, and melanoma

Background Material

- Abundant mucin – colloid carcinoma of breast and pancreas (Figs. 1.25 and 1.26) and mucoepidermoid carcinoma
- Abundant colloid – thyroid nodular goiter

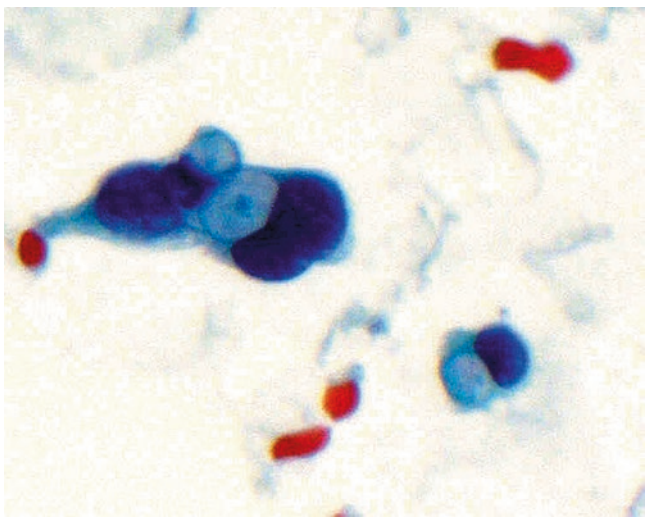

Fig. 1.24 Intracytoplasmic lumen in a breast lobular carcinoma

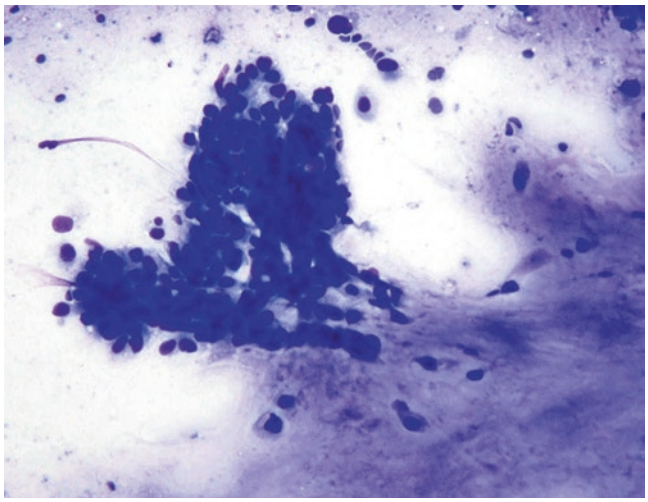

Fig. 1.25 Mucinous background in a colloid carcinoma of the breast on DQ

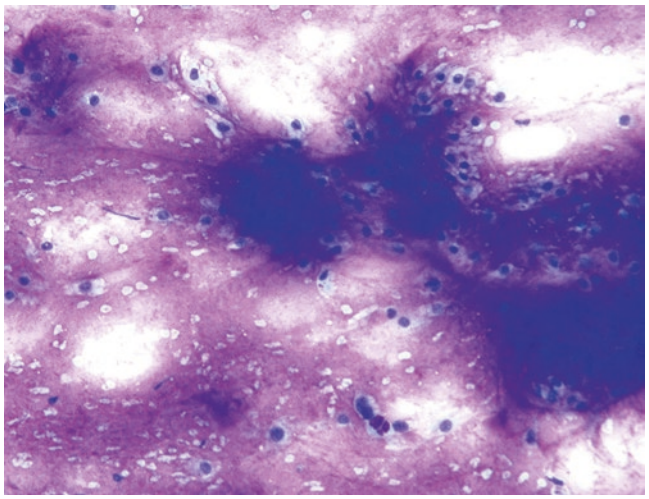

Fig. 1.27 Chondroid background in a chodorma on DQ

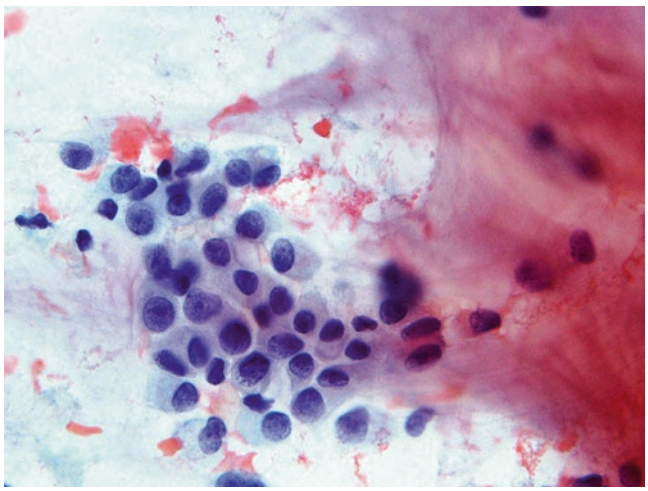

Fig. 1.26 Mucinous background in a colloid carcinoma of the breast on Pap stain

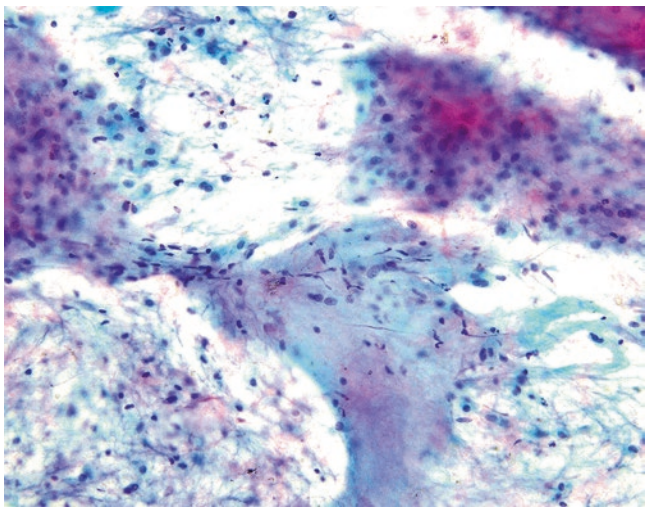

Fig. 1.28 Chondroid background in a chodorma on Pap stain

- Myxoid/chondroid – benign mixed tumor, chondrosarcoma, and myxoid and chondroid neoplasms (Figs. 1.27 and 1.28)
- Amyloid – medullary carcinoma of the thyroid, NET, and endocrine tumor of the pancreas
- Necrosis – colorectal ADC, small-cell undifferentiated carcinoma, lymphoma, and high-grade carcinoma or sarcoma
- Crushed artifact – small blue cell tumor, lymphoma, and lymphoid tissue
- Acute inflammation – infection, inflammatory process, anaplastic carcinoma of the thyroid, anaplastic large-cell lymphoma, and SCC with cystic degeneration

Single Cell Population

- NET (Figs. 1.29)
- Lymphoma/plasmacytoma/myeloid sarcoma (Fig. 1.30)
- Melanoma
- Sarcoma

Two Populations of Cells

- Seminoma (Figs. 1.31 and 1.32)
- Thymoma
- Hodgkin's lymphoma (Fig. 1.32)

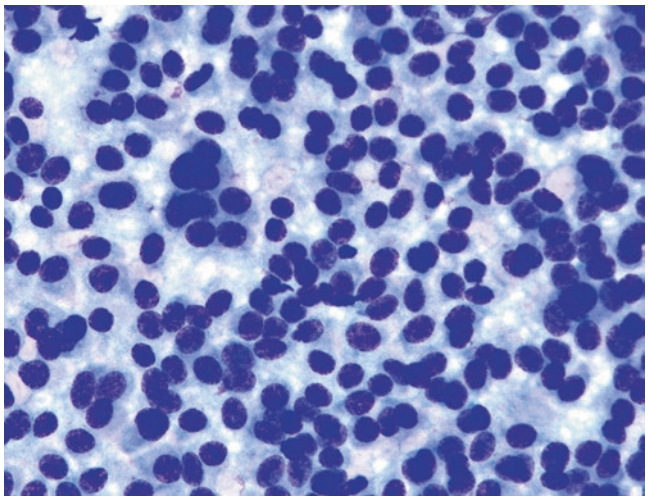

Fig. 1.29 Single cell population in a carcinoid tumor of lung on DQ

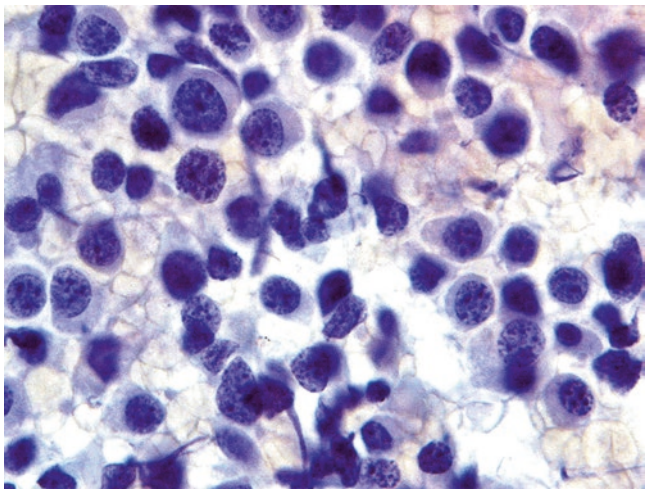

Fig. 1.30 Single cell population in a plasmacytoma on Pap stain

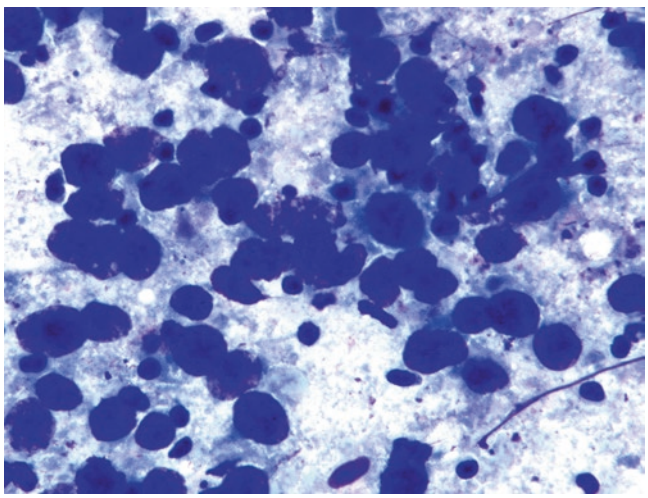

Fig. 1.31 Two populations of cells in a seminoma on DQ

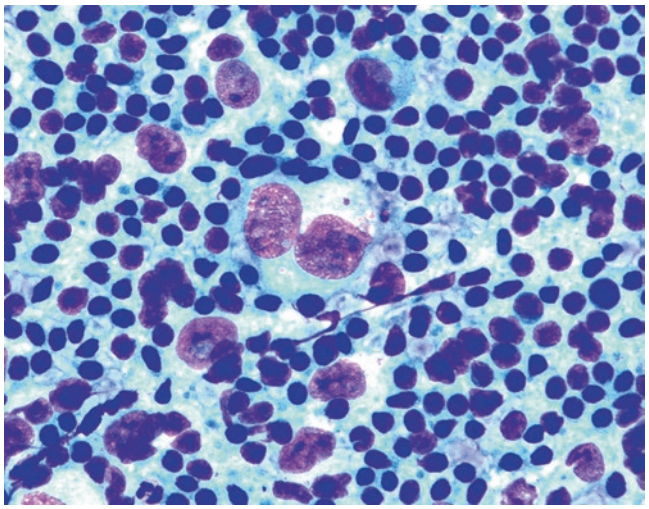

Fig. 1.32 Two populations of cells in a Hodgkin's lymphoma on DQ

- Lymphoepithelial carcinoma
- Medullary carcinoma of the colon
- Metastasis

How to Report an FNA

Category

1. Positive for malignant cells/malignant
2. Suspicious for malignant cells
3. Atypical cytology/atypical cells of undetermined significance
4. Negative for malignant cells/benign
5. Indeterminate

Specimen Adequacy

1. Adequate/satisfactory
2. Inadequate/unsatisfactory
3. Suboptimal/limited

An Example of a Formal Report of an FNA of the Thyroid

Thyroid, right, FNA
Positive for malignant cells
Papillary thyroid carcinoma
Adequately cellular specimen

Comment: Tall cell variant of papillary carcinoma of the thyroid is suspected.

Cytological Criteria of Common Neoplasms

1. *Papillary Carcinoma of Thyroid*
   Major Criteria (Fig. 1.33)
   - Nuclear enlargement
   - Nuclear overlapping
   - Nuclear clearing
   - Nuclear grooving
   - Intranuclear inclusion

   Minor Criteria
   - Squamoid cytoplasm
   - Cytoplasmic vacuoles
   - Psammoma body
   - Thick colloid
   - Multinucleated giant cells

2. *Medullary Carcinoma of the Thyroid*
   Major Criteria (Fig. 1.34)
   - Two populations of cells, epithelioid and spindle cells
   - Salt-pepper chromatin
   - Small to inconspicuous nucleoli
   - Plasmacytoid features

   Minor Criteria
   - Intranuclear inclusion
   - Granular and dense cytoplasm
   - Amyloid
   - Hyaline globules

3. *Adenoid Cystic Carcinoma of Salivary Gland*
   Major Criteria (Fig. 1.35)
   - Small uniform, basaloid cells with high nuclear-to-cytoplasmic ratio, bland nuclei, but hyperchromatic chromatin
   - Hyaline globules

   Minor Criteria
   - Usually absence of myoepithelial cells
   - Few stromal fragments
   - Exclude other entities

4. *Benign Mixed Tumor of the Salivary Gland*
   Major Criteria (Fig. 1.36)
   - Epithelial cells
   - Stromal cells
   - Metachromatic stroma
   - Spindle stromal cells
   - Plasmacytoid myoepithelial cells

   Minor Criteria
   - Epithelial cell dominant
   - Stromal cell dominant
   - Stromal acellular component dominant

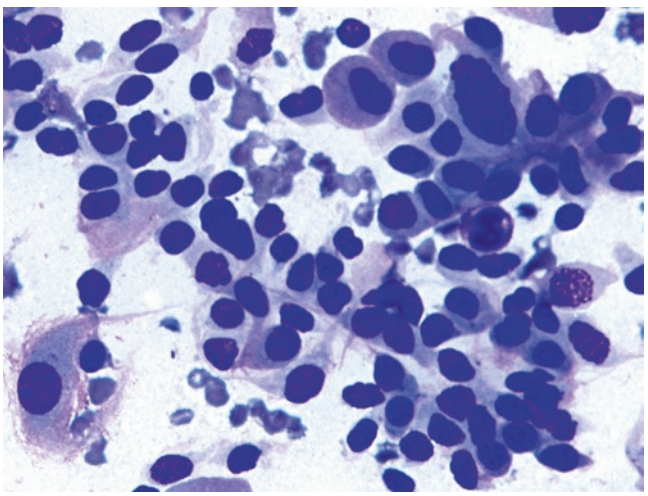

Fig. 1.34 Classic cytological features for a medullary carcinoma of the thyroid on DQ

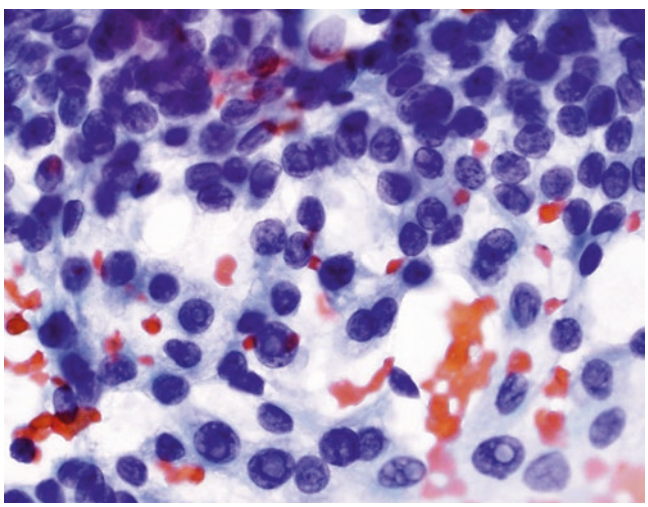

Fig. 1.33 Classic nuclear changes in a papillary carcinoma of the thyroid on Pap stain

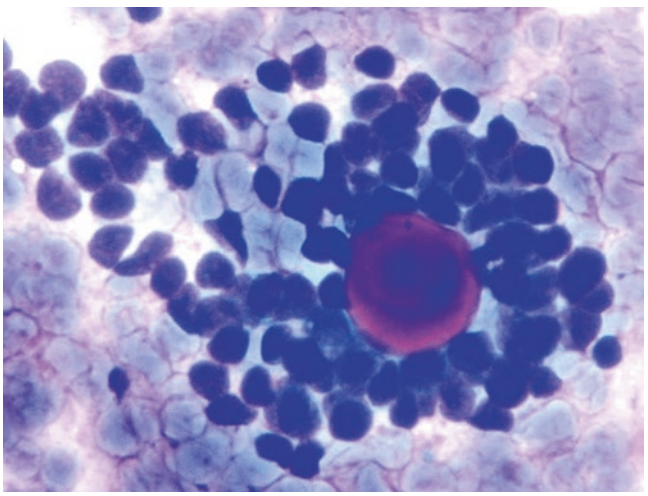

Fig. 1.35 Diagnostic hyalinizing globules in an adenoid cystic carcinoma on DQ

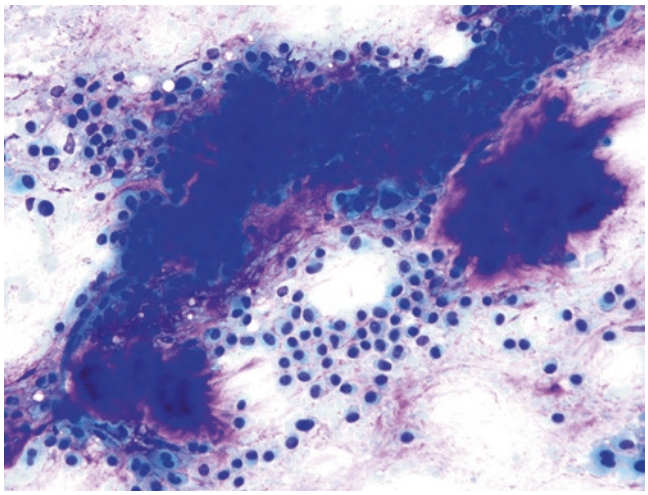

Fig. 1.36 Showing an example of benign mixed tumor on DQ

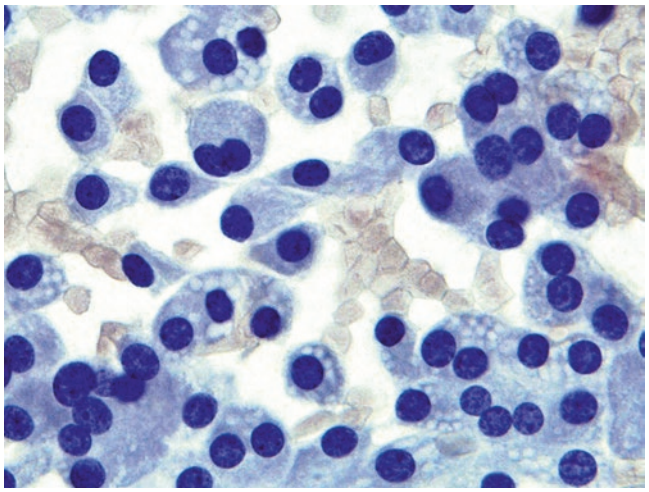

Fig. 1.38 Showing an example of pancreatic NET

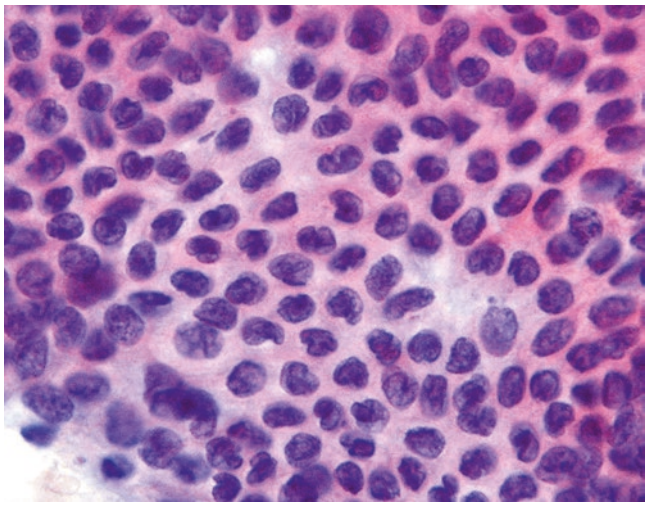

Fig. 1.37 Showing an example of well-diff ADC of the pancreas on Pap stain

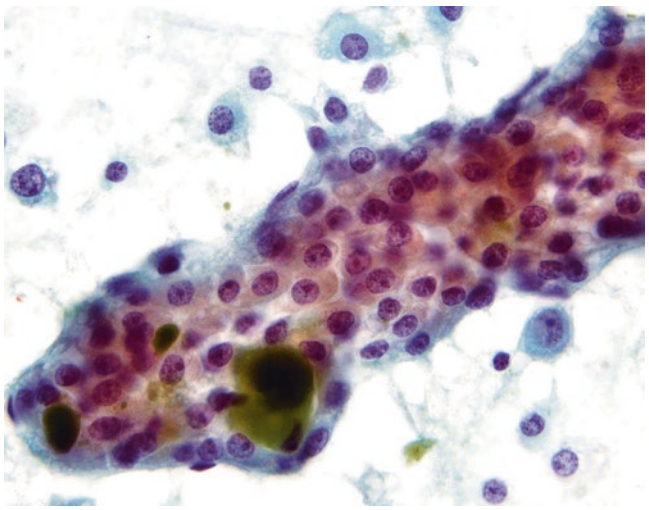

Fig. 1.39 Showing an example of HCC producing bile on Pap stain

- Myoepithelial cell dominant
- Extensive squamous metaplasia

5. *ADC of the Pancreas*
   Major Criteria (Fig. 1.37)
   - Variation in nuclear size in the same group (1:4)
   - Nuclear enlargement (>2 red blood cells [RBCs])
   - Nuclear overlapping/three dimensionality
   - Nuclear membranous irregularity

   Minor Criteria
   - Single atypical cells
   - Tumor necrosis
   - Prominent nucleoli
   - Mitosis
   - Chromatin clearing
   - Giant tumor cells
   - Hyperchromatic nuclei

6. *NET of the Pancreas*
   Major Criteria (Fig. 1.38)
   - A mixture of small cohesive groups and single cells
   - Round nuclei with salt-pepper nuclear chromatin
   - Small nucleoli
   - Plasmacytoid features
   - Binucleation

   Minor Criteria
   - Occasional large atypical cells
   - Crushed artifact
   - Focal necrosis
   - Multinucleated giant cells
   - Granular cytoplasm
   - Striped nuclei

7. *HCC*
   Major Criteria (Fig. 1.39)

- Trabecular fragment >3 cells thick and wrapped by endothelial cells or pseudoglandular formation with production of bile
- Special stain for reticulin and an immunostain for cluster of differentiation (CD)34 performed on the cell block section or core biopsy are useful

Minor Criteria

- Hypercellularity
- Many single cells
- Naked nuclei
- High nuclear-to-cytoplasmic ratio
- Few ductal cells

8. *Ductal Carcinoma of the Breast*

Major Criteria (Fig. 1.40)

- Hypercellularity
- Nuclear enlargement (>2.5 RBCs)
- Disordered, loosely cohesive epithelial group
- Single atypical cells
- Nuclear chromatin changes

Minor Criteria

- Marked nuclear atypia
- Tumor necrosis
- Mitosis
- Prominent nucleoli
- Intracytoplasmic lumens
- Foamy cytoplasm

9. *Small-Cell Carcinoma of the Lung*

Major Criteria (Figs. 1.41 and 1.42)

- Pleomorphic nuclei with salt-and-pepper nuclear chromatin
- Very high nuclear-to-cytoplasmic ratio
- Single cell necrosis and mitosis
- Inconspicuous nucleoli

Minor Criteria

- Crushed artifact
- Hypercellularity
- Many single cells
- Blue body
- Nuclear molding
- Extensive necrosis
- Marked atypical cells

10. *ADC of the Lung*

Major Criteria

- Three dimensional groups
- Glandular, tubular, acinar, or papillary formation
- Nuclear enlargement
- Chromatin clearing and clumping
- Irregular nuclear membrane
- Prominent nucleoli
- Mucinous material in the background

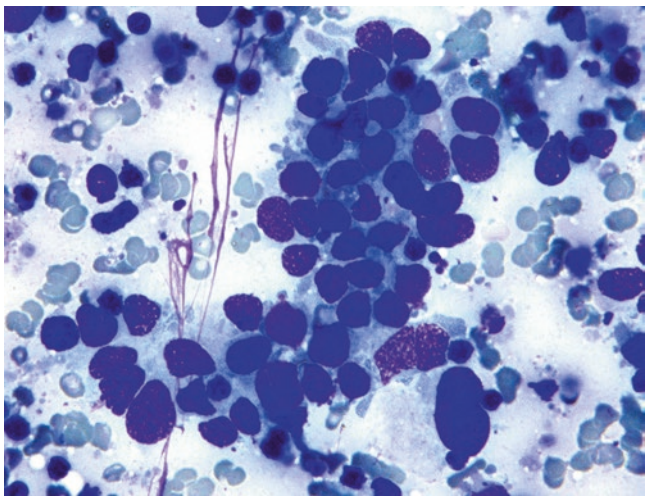

Fig. 1.41 Showing an example of small-cell carcinoma of the lung on DQ

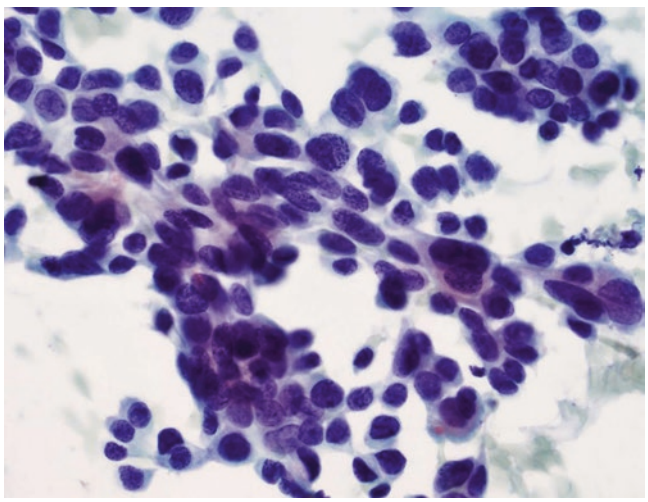

Fig. 1.40 Showing an example of breast ductal carcinoma on Pap stain

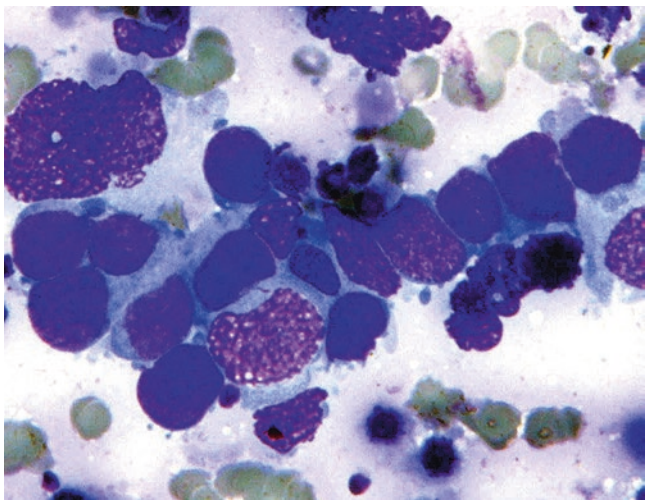

Fig. 1.42 Showing nuclear molding in a small-cell carcinoma on DQ

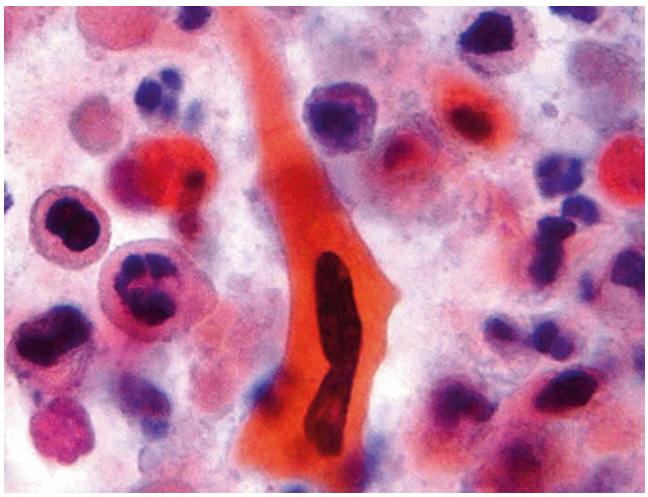

Fig. 1.43 Showing an example of well-differentiated SCC of the lung on Pap stain

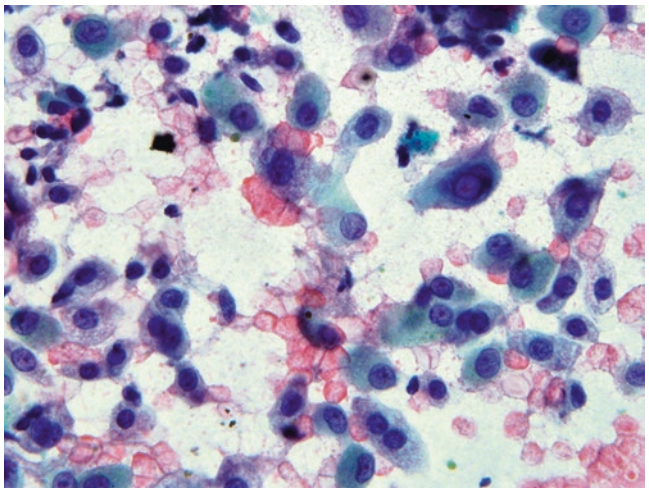

Fig. 1.44 Showing an example of melanoma with clear cytoplasm on Pap stain

Minor Criteria
- Single atypical cells with plasmacytoid features
- Mitosis
- Vacuoles in cytoplasm

11. *SCC of the Lung*
Major Criteria (Fig. 1.43)
- Two or three dimensional groups
- Keratinization
- Single atypical cells with dense cytoplasm
- Nuclear enlargement
- Small nucleoli
- Irregular nuclear membrane
- No glandular, tubular, acinar, or papillary formation
- Mucinous material in the background

Minor Criteria
- Tumor necrosis
- Mitosis
- Marked pleomorphic cells
- Bizarre cell sharps

12. *Melanoma*
Major Criteria (Fig. 1.44)
- Large epithelioid cells
- Abundant cytoplasm
- Large nuclei
- Prominent nucleoli
- Binucleation
- Intranuclear inclusion

Minor Criteria
- Two populations of cells – epithelioid and spindle
- Plasmacytoid appearance
- Marked pleomorphic cells
- Pigments

13. *Clear Cell RCC*
Major Criteria (Fig. 1.45)
- Clusters of tumor cells with vascular-rich network
- Low nuclear-to-cytoplasmic ratio

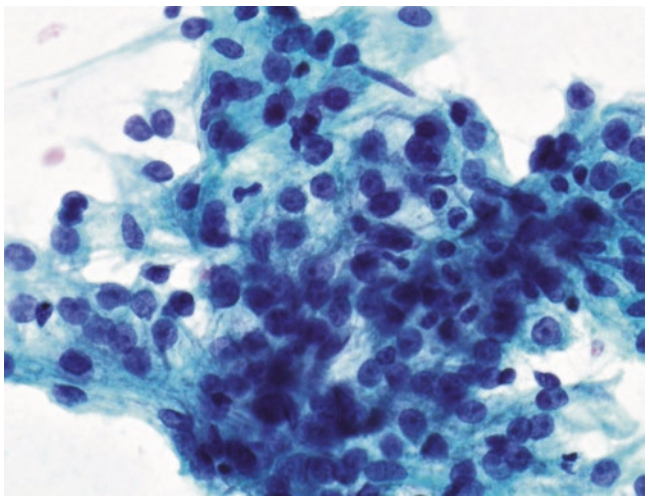

Fig. 1.45 Showing an example of clear cell RCC on Pap stain

- Clear or granular cytoplasm
- Small to prominent nucleoli
- Intranuclear inclusion

Minor Criteria
- Naked nuclei
- Mixed neutrophils, RBCs, and pigment-laden histiocytes with tumor cells

14. *Non-Hodgkin's Lymphomas*
Major Criteria (Fig. 1.46)
- Uniform population of lymphoid cells
- Classified into small, medium, and large cell size using histiocytes as a reference
- Lymphoglandular body

Minor Criteria
- Cleaved or noncleaved nuclei
- Fine granular chromatin
- Many mitoses
- Single cell or extensive necrosis

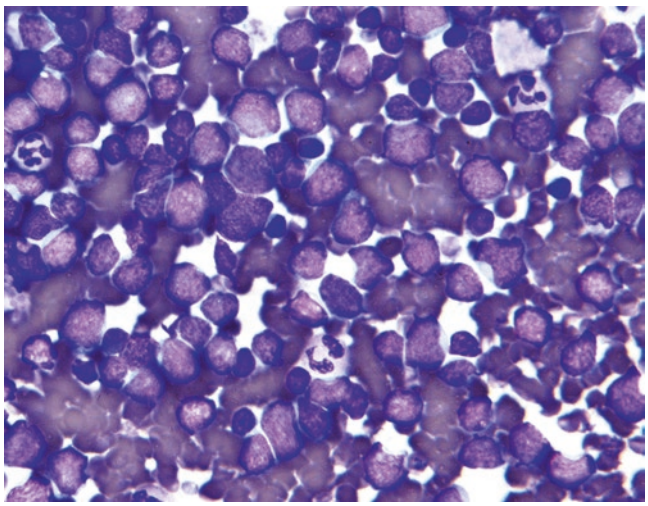

Fig. 1.46 Showing an example of lymphoblastic lymphoma on DQ

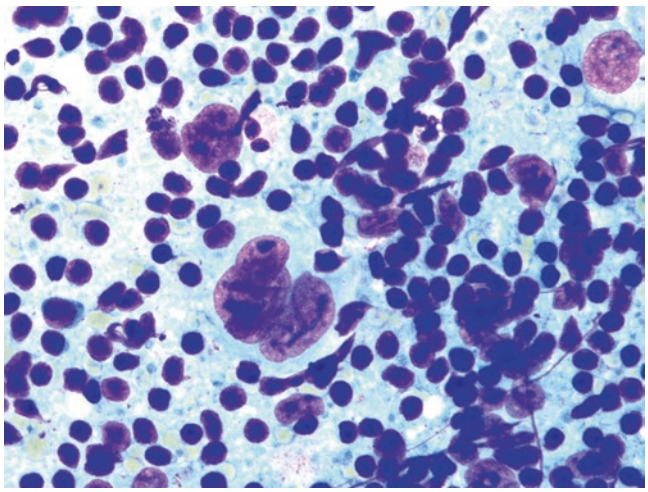

Fig. 1.47 Showing an example of Hodgkin lymphoma with Reed-Sternberg cells on DQ

- Prominent nucleoli
- Cytoplasmic vacuoles

15. *Hodgkin's Lymphoma*

Major Criteria (Fig. 1.47)

- Reed-Sternberg cells
- Hodgkin's cells
- Mixed population of small lymphoid cells, histiocytes, plasma cells, and eosinophils in the background

Minor Criteria

- Granulomas
- Fibrosis
- Necrosis

Diagnosis of lymphomas should not solely rely on cytological features; instead, it should include (1) cytomorphology, (2) immunohistochemistry (IHC), (3) flow cytometry, and (4) FISH/molecular diagnosis.

Ancillary Studies

IHC

In this section, the focus will be on the application of IHC to undifferentiated neoplasms if a cell block or a small tissue biopsy sample is available, especially carcinoma of unknown origin. The utilities of IHC on other specific entities on each organ will be delineated in each organ-based chapter.

How to Approach Undifferentiated Neoplasms/Tumors of Uncertain Origin

- *Review hematoxylin- and eosin (H&E)-stained slides.*
  Morphologic features are fundamental. The very first step is to determine if the lesion is malignant. If a benign/reactive condition is included in the differential diagnosis, caution should be taken when applying any immunostains, since IHC may or may not contribute to this process or may lead one to come to the wrong conclusion. If the lesion is malignant, it is important to review the slides and generate a broad differential diagnosis based on the morphologic features alone. One can be misled by incomplete or inaccurate clinical information.
- *Consider the basic clinical information such as age, sex, tumor location, and prior malignancy.*
  After formulating the initial differential diagnostic categories, it is time to consider the patient's age, sex, tumor location, and any prior malignancy. One should follow the statistics and focus on the common entities in that particular age group of patients and tumor location. Jumping to a conclusion of an uncommon entity in the initial diagnostic workup is not a wise choice.
- *Re-evaluate morphologic features of the tumor and predict the most likely category, such as carcinoma, melanoma, sarcoma, lymphoma, or germ cell tumor.*
  Based on the patient's age, sex, tumor location, prior malignancy, and morphologic features, one should narrow down the initial differential diagnosis to one to three options, if possible. For example: Is this a carcinoma? Is this an ADC? If it is an ADC, what is the likely primary site? Based on the tumor morphology, patient's age, and tumor location, the literature demonstrated that pathologists were able to correctly identify the tumor origin as their first choice in 50–55% of cases or as their first, second, or third choice in 67–74% of cases.

- *Determine the first diagnostic IHC panel to order.*

There are two likely scenarios. In the first, there is a clear lineage differentiation, such as an ADC/carcinoma. The next question will be: What is the likely primary site? A broad-spectrum cytokeratin cocktail (AE1/3 and CAM5.2), cytokeratin (CK)7, CK20, plus relatively organ-specific markers are recommended.

Determination of a Broad Category of Neoplasm

A cocktail of AE1/AE3 and CAM 5.2 is an effective panel of markers for identifying an epithelial lineage. AE1/AE3 by itself is insufficient to exclude an epithelial lineage. Other broad-spectrum cytokeratins containing keratin 8 and keratin 18, such as clones KL1, OSCAR, MAK6, and 5D3/LP3, are also excellent choices as a screening cytokeratin.

Leukocyte common antigen (LCA) itself is insufficient to exclude a potential diagnosis of hematopoietic neoplasm. Some diffuse large B-cell lymphomas, plasmablastic lymphomas, and anaplastic lymphomas can be negative for LCA. A combination of LCA and CD43 will cover a broad spectrum of lymphomas/myeloid sarcomas.

Vimentin is a non-specific marker; however, a vimentin-negative tumor is unlikely to be a sarcoma (with the exception of alveolar soft part sarcoma), lymphoma, or melanoma. Some carcinomas frequently co-express vimentin. A combination of S100 and sex-determining region Y-box (SOX)10 will detect nearly 100% of melanomas and greater than 80% of spindle cell/desmoplastic melanomas.

Sal-like protein 4 (SALL4) and lin-28 homolog A (LIN28) are highly sensitive and specific markers for identifying a tumor of germ cell origin. The markers for determination of a broad category of neoplasms are summarized in Table 1.2.

Tissue-Specific Markers

No single antibody is absolutely sensitive and specific for a particular tumor; however, some are especially useful when used in a small panel. Frequently used tissue-specific biomarkers are summarized in Table 1.3.

Co-expression of Cytokeratin and Vimentin

Follicular, papillary, and medullary thyroid carcinomas are nearly 100% positive for vimentin. Metaplastic breast carcinoma usually expresses both cytokeratin and vimentin in addition to high molecular weight cytokeratins and myoepithelial markers. Alveolar soft part sarcoma is a rare sarcoma which has no immunoreactivity for vimentin. Tumors that express both cytokeratin and vimentin are described in Table 1.4.

Expression of Epithelial Markers in Non-epithelial Neoplasms

Expression of cytokeratin is not restricted to epithelial neoplasms. Keratin is commonly expressed in some tumors with evidence of epithelial differentiation, such as synovial sarcomas, epithelioid sarcomas, desmoplastic small round cell tumors, chordomas, adamantinoma, and myoepithelial carcinomas. Other mesenchymal tumors can also express cytokeratin, although with a low frequency, including angiosarcomas, epithelioid hemangioendotheliomas, epithelioid leiomyosarcomas, and meningiomas. Aberrant expression of cytokeratin, which tends to be focal, has been reported in other tumors, including undifferentiated pleomorphic sarcomas, rhabdomyosarcomas, malignant rhabdoid tumors, and peripheral nerve sheath tumors, clear cell sarcomas, plasmacytomas, diffuse large B-cell lymphomas, anaplastic large-cell lymphomas, and melanomas.

Expression of Hematopoietic Markers in Non-hematopoietic Neoplasms

CD5 has been reported in thymic carcinoma, breast carcinoma, colonic ADC, pancreatic ADC, and lung ADC. CD138 is also frequently positive in SCC and can be positive in breast carcinoma, ovarian carcinoma, adrenal cortical carcinoma, and RCC.

CD56 is the most sensitive but not an entirely specific marker for neuroendocrine neoplasms, including some small-cell carcinomas which may lose expression of cytokeratins and other neuroendocrine markers but still show expression of CD56. A significant percentage of thyroid carcinomas are immunoreactive for CD56 as well, as reported in the literature. Hematopoietic markers expressed in non-hematopoietic neoplasms are listed in Table 1.5.

Table 1.2 Markers for determination of a broad category of neoplasms

Marker/Tumor	Carcinoma	Sarcoma	Melanoma	Lymphoma	GCT	Mesothelioma
CK	+	−	−	−	+/−	+
Vimentin	−/+	+	+	+	−	+/−
S100/SOX10	−/+	−	+	−	−	−
LCA/CD43	−	−	−	+	−	−
SALL4/LIN28	−	−	−	−	+	−

Note: *GCT* giant cell tumor, *CK* a broad spectrum cytokeratin, *SOX10* sex-determining region Y-box 10, *LCA* leukocyte common antigen, *CD43* cluster of differentiation 43, *SALL4* sal-like protein 4, *LIN28* lin-28 homolog A, "+" >75% of cases are positive, "−" <5% of cases are positive, "+/−" 50–75% of cases are positive, "−/+" <50% of cases are positive

Table 1.3 Useful markers for identifying tumor origin

Primary site	Markers
Lung ADC	TTF1, napsin A
Breast carcinoma	GATA3, ER, GCDFP-15, TFF1, MGB
Urothelial carcinoma	GATA3, UPII/UPIII, S100P, CK5/6, CK903, p63, CK20
Squamous cell carcinoma	p40, CK5/6, p63, SOX2, desmocollin-3
RCC, clear cell type	PAX8/PAX2, RCCma, pVHL, CD10, KIM-1
Papillary RCC	P504S, RCCma, pVHL, CD10, PAX8, KIM-1
Translocational RCC	TFE3
HCC	Arg-1, glypican-3, HepPar-1, AFP
Adrenal cortical neoplasm	Mart-1, inhibin-alpha, calretinin, SF-1
Melanoma	S100, Mart-1, HMB45, MiTF, SOX10, PNL2
Merkel cell carcinoma	CK20 (perinuclear dot staining), MCPyV
Mesothelial origin	Calretinin, WT1, D2-40, CK5/6, mesothelin
Neuroendocrine origin	Chromogranin, synaptophysin, CD56,
Upper GI tract	CDH17, CDX2, CK20
Lower GI tract	CDH17, SATB2, CDX2, CK20
Intrahepatic cholangiocarcinoma	pVHL, CAIX, albumin by RNA in situ hybridization
Pancreas, acinar cell carcinoma	Glypican-3, Bcl-10, antitrypsin
Pancreas, ductal ADC	MUC5AC, CK17, maspin, S100P, IMP3
Pancreas, NET	PR, PAX8, PDX1, islet-1
Pancreas, solid pseudopapillary tumor	Nuclear beta-catenin, loss of E-cadherin, PR, CD10, vimentin
Prostate, ADC	NKX3.1, PSA, PSAP, ERG
Ovarian serous carcinoma	PAX8, ER, WT1
Ovarian clear cell carcinoma	pVHL, HNF-1B, KIM-1, PAX8
Endometrial stromal sarcoma	CD10, ER
Endometrial ADC	PAX8/PAX2, ER, vimentin
Endocervical ADC	PAX8, p16, CEA, HPV in situ hybridization, loss of PAX2
Thyroid follicular cell origin	TTF1, PAX8, thyroglobulin
Thyroid medullary carcinoma	Calcitonin, TTF1, CEA, chromogranin
Parathyroid neoplasm	PTH, GATA3, chromogranin
Hyalinizing trabecular adenoma of the thyroid	MIB-1 (unique membranous staining pattern)
Salivary duct carcinoma	AR, GCDFP-15, HER2, GATA3
Mammary analogue secretory carcinoma of the salivary gland	S100, GATA3, MGB, GCDFP15
Thymic origin	PAX8, p63, CD5
Seminoma	SALL4, OCT4, CD117, D2-40
Yolk sac tumor	SALL4, glypican-3, AFP, GATA3
Embryonal carcinoma	SALL4, LIN28, OCT4, NANOG, CD30, SOX2
Choriocarcinoma	Beta-HCG, CD10, GATA3
Sex cord-stromal tumors	SF-1, inhibin-alpha, calretinin, FOXL2
Vascular tumor	ERG, CD31, CD34, Fli-1
Synovial sarcoma	TLE1, cytokeratin
Chordoma	Cytokeratin, S100
Desmoplastic small round cell tumor	Cytokeratin, CD99, desmin, WT1 (N-terminus)
Alveolar soft part sarcoma	TFE3
Rhabdomyosarcoma	Myogenin, desmin, MyoD1
Smooth muscle tumor	SMA, MSA, desmin, calponin
Ewing sarcoma/PNET	NKX2.2, CD99, Fli-1
Myxoid and round cell liposarcoma	NY-ESO-1
Low-grade fibromyxoid sarcoma	MUC4
Epithelioid sarcoma	CD34, loss of INI1
Atypical lipomatous tumor	MDM2 (MDM2 by FISH is a more sensitive and specific test), CDK4
Histiocytosis X	CD1a, S100
Angiomyolipoma	HMB45, SMA, PNL2
Gastrointestinal stromal tumor	CD117, DOG1
Solitary fibrous tumor	STAT6, CD34, Bcl2, CD99
Myoepithelial carcinoma	Cytokeratin and myoepithelial markers. May lose INI1
Myeloid sarcoma	CD43, CD34, MPO
Follicular dendritic cell tumor	CD21, CD35
Mast cell tumor	CD117, tryptase

Note: *ADC* adenocarcinoma, *TTF1* thyroid transcription factor 1, GATA3 GATA binding protein 3, *ER* estrogen receptor, *GCDFP-15* gross cystic disease fluid protein 15, *TFF1* trefoil factor 1, *MGB* mammaglobin, *UP* uroplakin, *S100P* placental S100, *CK* cytokeratin, *SOX* sex-determining region Y-box, *RCC* renal cell carcinoma, *PAX* paired box gene, *RCCma* renal cell carcinoma marker, *pVHL* von Hippel-Lindau tumor suppressor, *CD* cluster of differentiation, *KIM-1* kidney injury molecule 1, *P504S* alpha-methylacyl-CoA racemase, *TFE3* transcription factor E3, *HCC* hepatocellular carcinoma, *Arg-1* arginase-1, *HepPar-1* hepatocyte paraffin-1, *AFP* alpha-fetoprotein, *Mart-1* melanoma-associated antigen recognized by T cells 1, *SF-1* steroidogenic factor 1, *HMB45* human melanoma black 45, *MiTF* microphthalmia-associated transcription factor, *PNL2* melanoma-associated antigen PNL2, *MCPyV* Merkel cell polyomavirus, *WT1* Wilms' Tumor 1, *D2-40* podoplanin, *GI* gastrointestinal, *CDH17* cadherin-17, *CDX2* caudal-type homeobox 2, *SATB2* special AT-rich sequence-binding protein 2, *CAIX* carbonic anhydrase IX, *MUC* mucin, maspin mammary serine protease inhibitor, *IMP3* IMP3 insulin-like growth factor II messenger RNA-binding protein 3, *NET* neuroendocrine tumor, *PR* progesterone receptor, *PDX1* pancreatic duodenal homeobox 1, *PSA* prostate-specific antigen, *PSAP* prostate-specific acid phosphatase, *ERG* ETS-related gene, *NKX3.1* NK3 homeobox 1, *HNF-1B* hepatocyte nuclear factor 1 beta, *CEA* carcinoembryonic antigen, *HPV* human papilloma virus, *MIB-1* mindbomb homolog 1, *AR* androgen receptor, *SALL4* sal-like protein 4, *LIN28* lin-28 homolog A, *OCT4* octamer-binding transcription factor 4, *NANOG* NANOG homeobox, *Beta-HCG* Beta human chorionic gonadotropin, *FOXL2* forkhead box L2, *Fli-1* friend leukemia virus integration-1, *TLE1* transducin-like enhancer of split 1, *MyoD1* myogenic differentiation 1, *SMA* smooth muscle actin, *MSA* muscle-specific actin; *PNET* primitive neuroectodermal tumor, *NKX2.2* NK2 homeobox 2, *NY-ESO-1* cancer/testis antigen 1B; *INI1* integrase interactor 1, *MDM2* mouse double minute 2 homolog, *FISH* fluorescence in site hybridization, *CDK4* cyclin-dependent kinase 4, *DOG1* discovered on GIST-1, *Bcl2* B-cell CLL/lymphoma 2, *MPO* myeloperoxidase

Table 1.4 Tumors that frequently or rarely co-express cytokeratin and vimentin

Carcinomas that frequently express both	Mesenchymal tumors that frequently express both	Carcinomas that rarely express both
RCC	Synovial sarcoma	Breast carcinoma
Anaplastic thyroid carcinoma	DPSRCT	Ovarian carcinoma
Endometrial carcinoma	Epithelioid sarcoma	GI carcinoma
Thyroid carcinomas	Epithelioid angiosarcoma	Small-cell carcinoma
Sarcomatoid carcinoma	Malignant rhabdoid tumor	Lung non-small-cell carcinoma
Mesothelioma	Leiomyosarcoma	Prostatic carcinoma
Myoepithelial carcinoma	Chordoma	
Metaplastic breast carcinoma	Adamantinoma	

Note: *RCC* renal cell carcinoma, *DPSRCT* desmoplastic small round cell tumor, *GI* gastrointestinal

Table 1.5 Expression of hematopoietic markers in non-hematopoietic neoplasms

Marker	Diagnosis
CD5	Thymic carcinoma, cholangiocarcinoma, pancreatic carcinoma
CD30	Embryonal carcinoma
CD138	Carcinoma of lung, cholangiocarcinoma, UC
CD10	RCC, HCC, ESS, choriocarcinoma
CD15	Carcinoma of lung and other organs; renal oncocytoma
CD56	Neuroendocrine carcinomas and thyroid carcinomas

Note: *CD* cluster of differentiation, *UC* urothelial carcinoma, *RCC* renal cell carcinoma, *HCC* hepatocellular carcinoma, *ESS* endometrial stromal sarcoma

Review of Selected Antibodies

The following selected antibodies are either recently described or frequently used in identifying a tumor of uncertain origin/undifferentiated neoplasm, especially a carcinoma. As well documented, every antigen can demonstrate an aberrant expression in a certain tumor. Table 1.6 summarizes the common application of these antibodies. The antibody information for the frequently used antibodies is summarized in Table 1.7.

Recommended Diagnostic IHC Panels

As aforementioned, this section will focus on carcinomas of uncertain origin, which can be separated into four main diagnostic groups: CK7+/CK20-, CK7+/CK20+, CK7-/CK20+, and CK7-/CK20-.

Table 1.6 Summary of commonly used antibodies

Marker	Common application
TTF1	Lung ADC, 80–85% Carcinoma of thyroid (follicular, papillary, and medullary carcinomas), >90% Small-cell carcinoma of lung, >90% Small-cell carcinoma of prostate, 40% Small-cell carcinoma of bladder, 40%
Napsin A	Lung ADC, 75–80% Papillary RCC, 60% Clear cell RCC, 30% Clear cell carcinoma of the ovary, >90%
CEA	Lung ADC, >90% Colorectal ADC, >90% Gastric ADC, >90% Pancreatic ADC, >90% Breast carcinoma >50% Urothelial carcinoma, 25% Medullary carcinoma of thyroid, nearly 100% Endocervical ADC, <10%
CDX2	Colorectal ADC, >90% Small intestinal ADC, >90% Neuroendocrine neoplasm of GI tract, variable Ovarian mucinous ADC, >90% Upper GI ADC, 40–50% Pancreas/biliary ADC, 10%
SATB2	Colorectal ADC, >90% Colorectal NET >70% Upper GI, pancreas, lung ADCs, <10% Osteogenic sarcomas, >90%
GATA3	Breast carcinoma, >85% Urothelial carcinoma, >80% Salivary ductal carcinomas and mammary analogue secretory carcinomas, >90% Metastatic paraganglioma, 80% 7% of anal SCCs and 19% of uterine cervical SCCs, focally positive Parathyroid neoplasm, >90% Choriocarcinoma and yolk sac tumor, >90%
ER	Breast ductal carcinoma, >80% Breast lobular carcinoma, >95% Ovarian serous carcinomas, >90% Ovarian clear cell carcinomas, >80% Endometrial ADCs, >90%
pVHL	RCCs, >90% Intrahepatic cholangiocarcinoma, 70% Salivary oncocytoma, >90% Clear cell carcinoma of the ovary, 70% Clear cell carcinoma of the uterus, 70% Normal ducts in pancreatobiliary tract, 100%
S100	Melanoma, >90% Carcinoma/breast/renal/lung, variable Neural tumors: Neurofibroma, >90% Schwannoma, >90%, diffuse and strong MPNST, 50–60%, focal staining only Granular cell tumor, >90% Liposarcoma, >90%, weak, focal Chondrosarcoma, >90%, weak Myoepithelioma, >90%

(continued)

Table 1.6 (continued)

Marker	Common application
SOX10	Melanoma, >90% Neurogenic tumor, >90% Myoepithelial cells, >90% Some salivary gland tumors, variable
HMB45	Malignant melanoma, 80–90% Epithelioid schwannoma, >90% Clear cell sarcoma of soft part, >90% Clear cell (sugar) tumor of lung, >90% Angiomyolipoma, 80–90%, focal Lymphangioleiomyomatosis, >90% Cardiac rhabdomyoma, >90%
SALL4	Nearly all germ cell tumors, >90% Hepatoid carcinoma of GI tract, >90%
OCT4	Seminoma, >90% Embryonal carcinoma, >90% Yolk sac tumor, <5%
Arginase-1	Normal liver, 100% Benign liver lesion/tumor, 100% HCC, >85% Some hepatoid carcinomas, 60%
Glypican-3	HCC, 80% Yolk sac tumor, >80% Hepatoid carcinoma, 70% Normal liver and benign hepatocellular lesions, usually negative
PAX8	Thyroid carcinomas (follicular and papillary), >90% Renal cell carcinomas, >90% Ovarian serous carcinomas, >90% Ovarian clear cell carcinomas, >80% Endometrial ADCs, >90% Nephrogenic adenomas, >80% Thymic tumors, 70% Pancreatic NET, 50%
INI1 (loss)	Malignant rhabdoid tumors, >90% Atypical teratoid/rhabdoid tumors of CNS, 90% Medullary carcinoma of the kidney, >90% Epithelioid sarcoma, 90% Epithelioid MPNST, 50% Myoepithelial carcinoma, 30–40%

Note: *TTF1* thyroid transcription factor 1, *ADC* adenocarcinoma, *RCC* renal cell carcinoma, *CEA* carcinoembryonic antigen, *CDX2* caudal-type homeobox 2, *GI* gastrointestinal, *SATB2* special AT-rich sequence-binding protein 2, *NET* neuroendocrine tumor, *GATA3* GATA binding protein 3, *SCC* squamous cell carcinoma, *ER* estrogen receptor, *pVHL* von Hippel Lindau tumor suppressor, *RCC* renal cell carcinoma, *MPNST* malignant peripheral nerve sheath tumor, *SOX10* sex-determining region Y-box 10, *HMB45* human melanoma black 45, *SALL4* sal-like protein 4, *OCT4* octamer-binding transcription factor 4, *HCC* hepatocellular carcinoma, *PAX8* paired box gene 8, *IN1* integrase interactor 1

- *Differential diagnosis of CK7+ and CK7+/focal CK20+ carcinomas.*
  When working on a tumor of unknown primary, CK7+ or CK7+/CK20+ carcinomas are nearly always included in the diagnostic consideration. The differential diagnosis usually encompasses a broad spectrum of organs and entities, such as the breast, lung, ovary, uterus, urinary bladder, upper GI tract, pancreatobiliary tract, thyroid, kidney (papillary RCC), and mesothelioma. Table 1.8 summarizes the frequently used markers in the differential diagnosis of these common entities. A significant portion of these CK7+ carcinomas also express ER, and the major differential diagnosis of CK7+/ER+ carcinomas, including tumors from the breast and gynecologic tract, is summarized in Table 1.9.
  In real practice, each individual case will have a unique presentation; therefore, it is impractical and impossible to create a specific IHC panel for every diagnostically challenging case here. However, a few potentially useful IHC panels are recommended in Tables 1.10, 1.11, 1.12, 1.13, 1.14, and 1.15.
  Caution should be taken when using a polyclonal antibody to napsin A. A significant percentage of esophageal ADCs and some pancreatic ADCs can be positive for napsin A. Less than 10% of pancreatic ADCs may show focal positivity for GATA3.
  In addition to the entities mentioned in Tables 1.9, 1.10, 1.11, 1.12, 1.13, and 1.14, many other entities can present as CK7+ or CK7+/focally CK20+ carcinomas, including anal/rectal ADCs, ampullary ADCs, common bile duct ADCs, gallbladder ADCs, small bowel ADCs, renal collecting duct carcinomas, renal medullary carcinomas, medullary thyroid carcinomas, thymic carcinomas, salivary gland carcinomas, ovarian mucinous carcinoma, and SCCs of the uterine cervix.
- *Differential diagnosis of CK7+/ER+ carcinomas.*
  ER is one of the most critical immunomarkers when working on a tumor of uncertain origin or undifferentiated neoplasm, especially in a woman. ER is frequently positive in breast carcinomas and gynecologic primaries. Therefore, ER itself plays a limited role in differential diagnosis among these carcinomas. Table 1.9 includes the most common ER-positive carcinomas when working on a tumor of uncertain origin. GATA3 and TFF1 are two recently described sensitive markers for identifying a breast origin, which is rarely positive in other gynecologic carcinomas, including endometrial ADCs, endocervical ADCs, ovarian serous carcinomas, and clear cell carcinomas. TFF1 is expressed in 80% and 90% of breast and colorectal carcinomas, respectively, whereas other carcinomas, including those of the lung, endometrium, and ovary, are rarely positive. Vimentin is expressed in 90% of endometrial ADCs and negative in other gynecologic carcinomas, with the exception in ovarian endometrioid ADCs, which showed immunoreactivity for vimentin in over 90% of cases. p16 is a useful marker in distinguishing between endometrial ADC and endocervical ADC; it tends to be diffusely and strongly positive in endocervical ADC (nearly every tumor cell) with only patchy immunoreactivity in endometrial ADC. Human papilloma virus (HPV) in situ hybridization (ISH) demonstrated positivity in the majority of endocervical ADCs. pVHL and hepatocyte nuclear factor 1 beta

Table 1.7 Summary of antibody information

Antibody	Catalog no	Vendor	Clone	AR/Temp/Time	Dilution	pH	Loc
CAM5.2	349,205	BD Biosciences	CAM5.2	CC1/95/36	1:4	8	C
CK7	307 M-95	Cell Marque	OV-TL 12/30	CC1/95/36	1:200	8	C
CK20	790-4431	Ventana	SP33	CC1/95/64	Predilute	8	C
CK5/6	790-4554	Ventana	D5 + 16B4	CC1/95/64	Predilute	8	C
p40	PC373	Millipore	Polyclonal	CC1/95/64	1:2000	8	N
S100	790-2914	Ventana	4C4.9	CC1/95/36	Predilute	8	N + C
LCA	M0701	Dako	2B11 + PD7/26	CC1/95/36	1:80	8	C
Vimentin	790-2917	Ventana	V9	CC1/95/36	Predilute	8	C
TTF1	790-4398	Ventana	8G7G3/1	CC1/95/36	Predilute	8	N
Napsin A	AC-0191	Epitomics	EP205	CC1/95/36	1:100	8	C
ER	790-4324	Ventana	SP1	CC1/95/36	Predilute	8	N
GATA3	CM405	Biocare Medical	L50-823	CC1/95/64	1:400	8	N
TFF1	E100004-RUO	Epitomics	EPR3972	CC1/95/36	1:2000	8	C
CDX2	235R-16	Cell Marque	EPR2764Y	CC1/95/36	1:600	8	N
CDH17	AC-0095RUO	Epitomics	EP86	CC1/95/36	1:100	8	M
SATB2	SC-81376	Santa Cruz	SATBA4B10	CC1/95/64	1:20	8	N
Arg-1	5222-1	Epitomics	EPR6672(B)	CC1/95/36	1:500	8	C + N
Glypican-3	261 M-98	Cell Marque	1G12	CC1/95/36	Predilute	8	C
PAX8	CP379AK	Biocare Medical	Polyclonal	CC1/95/36	1:20	8	N
SALL4	CM384C	Biocare Medical	6E3	CC1/95/64	1:100	8	N
OCT4	309 M-18	Cell Marque	MRQ-10	CC1/95/36	Predilute	8	N
pVHL	SC5575	Santa Cruz	Polyclonal	Protease 1/37/8	1:150	8	M + C
WT1	RB-9267P	Neomarkers/ Thermo	Polyclonal	CC1/95/36	1:200	8	N

Note: *AR* antigen retrieval, *Loc* localization, *CC1* cell conditioning solution 1 (Ventana), *C* cytoplasmic, *N* nuclear, *M* membranous, *CK* cytokeratin, *LCA* leukocyte common antigen, *TTF1* thyroid transcription factor 1, *ER* estrogen receptor, *GATA3* GATA binding protein 3, *TFF1* trefoil factor 1, *CDX2* caudal-type homeobox 2, *CDH17* cadherin-17, *SATB2* special AT-rich sequence-binding protein 2; *Arg-1* arginase-1, *PAX8* paired box gene 8, *SALL4* sal-like protein 4, *OCT4* octamer-binding transcription factor 4, *pVHL* von Hippel-Lindau tumor suppressor, *WT1* Wilms' tumor 1
Vendor Information: BD Biosciences, BD Biosciences**,** San Jose, CA; Biocare, Biocare Medical, Inc., Concord CA; Cell Marque, Cell Marque Corporation, Rocklin CA; Dako, Dako North America, Inc., Carpinteria, CA; Epitomics, Epitomics, an Abcam Company, Burlingame, CA; Millipore, EMD Millipore, Corp., Billerica, MA; Neomarkers/Thermo, Thermo Scientific, Waltham, MA; Santa Cruz, Santa Cruz Biotechnology, Inc., Santa Cruz CA; Ventana, Ventana Medical Systems, Tucson AZ

Table 1.8 Summary of CK7+ and CK7+/CK20+ epithelial neoplasms

Marker	LADC	BADC	UGI	PADC	ICC	UC	PRCC	PTC	SADC	MS
CK7	+	+	+	+	+	+	+	+	+	+
CK20	−	−	−/+	−/+	−/+	+/−	−	−	+/−	−/+
CK5/6	−	−	−	−	−	+	−	−	−	+
p40	−	−	−	−	−	+	−	−	−	−
GATA3	−	+	−	−	−	+	−	−	−	−
ER	−	+	−	−	−	−	−	−	−	−
TTF1	+	−	−	−	−	−	−	+	−	−
Napsin A	+	−	−	−	−	−	+	−	−	−
CDH17	−	−	+/−	+/−	+/−	−	−	−	+	−
CDX2	−	−	−/+	−/+	−/+	−	−	−	+	−
PAX8	−	−	−	−	−	−	+	+	−	−
RCCma	−	−	−	−	−	−	+	−/+	−	−
pVHL	−	−	−	−	+	−	+	−	−	−
Calretinin	−	−	−	−	−	−	−	−	−	+
Vimentin	−	−	−	−	−	−	+/−	+	−	+/−

Note: *CK* cytokeratin, *LADC* lung adenocarcinoma, *BADC* breast carcinoma, *UGI* upper gastrointestinal tract, *PADC* pancreatic adenocarcinoma, *ICC* intrahepatic cholangiocarcinoma, *UC* urothelial carcinoma, *PRCC* papillary renal cell carcinoma, *PTC* papillary thyroid carcinoma, *SADC* small bowel adenocarcinoma, *MS* mesothelioma, *GATA3* GATA binding protein 3, *ER* estrogen receptor, *TTF1* thyroid transcription factor 1, *CDH17* cadherin-17, *CDX2* caudal-type homeobox 2, *PAX8* paired box gene 8, *RCCma* renal cell carcinoma marker, *pVHL* von Hippel-Lindau tumor suppressor, "+" >75% of cases are positive, "−" <5% of cases are positive, "+/−" 50–75% of cases are positive, "−/+" – <50% of cases are positive

Table 1.9 Summary of CK7+/ER+ carcinomas and useful markers

Antibody	Breast CA	EMADC	ECADC	OSCA	OCCCA
GATA3	+	−	−	−	−
TFF1	+	−	−	−	−
PAX8	−	+	+/−	+	+/−
WT1	−	−	−	+	−
Vimentin	−	+	−	−	−
p16	−	Patchy +	Diffusely +	+	+/−
HPV in situ	−	−	+/−	−	−
pVHL	−	−	−	−	+/−
HNF-1B	+/−	+/−	+/−	−	+

Note: *CK* cytokeratin, *ER* estrogen receptor, *CA* carcinoma, *EMADC* endometrial adenocarcinoma, *ECADC* endocervical ADC, *OSCA* ovarian serous carcinoma, *OCCCA* ovarian clear cell carcinoma, *GATA3* GATA binding protein 3, *TFF1* trefoil factor 1, *PAX8* paired box gene 8, *WT1* Wilms' tumor 1, *HPV* human papilloma virus, *pVHL* von Hippel-Lindau tumor suppressor, *HNF-1B* hepatocyte nuclear factor 1 beta, "+" >75% of cases are positive, "-" <5% of cases are positive, "+/−" 50-75% of cases are positive, "−/+" <50% of cases are positive

Table 1.10 Lung ADC vs. breast carcinoma

Marker/diagnosis	Lung ADC	Breast carcinoma
TTF1	+	−
Napsin A	+	−
ER	−	+
GATA3	−	+
TFF1	−	+

Note: *ADC* adenocarcinoma; *TTF1* thyroid transcription factor 1, *ER* estrogen receptor, *GATA3* GATA binding protein 3, *TFF1* trefoil factor 1, "+" >75% of cases are positive, "−" <5% of cases are positive

Table 1.11 Lung ADC vs. mesothelioma

Antibody	Lung ADC	Mesothelioma
Calretinin	−	+
WT1	−	+
CK5/6	−	+
D2-40	−	+
CEA	+	−
MOC-31	+	−
TTF1	+	−

Note: *ADC* adenocarcinoma, *WT1* Wilms' tumor 1, *CK* cytokeratin, *D2-40* podoplanin, CEA carcinoembryonic antigen, *MOC-31* epithelial-related antigen clone MOC-31, *TTF1* thyroid transcription factor 1, "+" >75% of cases are positive, "−" <5% of cases are positive. In general, to render a diagnosis of mesothelioma, the tumor should be positive for at least 2 mesothelial markers and negative for 2 carcinoma markers

(HNF-1B) are helpful markers in distinguishing ovarian serous carcinoma from ovarian clear cell carcinoma. Additionally, p53 is usually diffusely and strongly positive or completely negative in serous carcinomas and only focally and weakly positive in ovarian clear cell carcinomas. KIM-1, which is not currently commercially available, is a sensitive and relatively specific marker for ovarian and uterine clear cell carcinomas; pVHL plays a

Table 1.12 Lung vs. upper GI, pancreatobiliary primary, and urinary bladder

Markers/diagnosis	Lung ADC	Upper GI ADC	Pancreatic ADC	UC
TTF1	+	−	−	−
Napsin A	+	−/+	−	−
CDH17	−	+/−	−/+	−/+
CDX2	−	−/+	−/+	−
CK20	−	−/+	−/+	+/−
MUC5AC	−	−/+	+/−	−
p40	−	−	−	+
GATA3	−	−	−	+

Note: *GI* gastrointestinal, *ADC* ADC, *UC* urothelial carcinoma, *TTF1* thyroid transcription factor 1, *CDH17* cadherin-17, *CDX2* caudal-type homeobox 2, *CK* cytokeratin, *MUC5AC* mucin 5 AC, *GATA3* GATA binding protein 3, "+" >75% of cases are positive, "−" <5% of cases are positive, "+/−" 50–75% of cases are positive, "−/+" <50% of cases are positive

Table 1.13 Breast carcinoma vs. upper GI, pancreatobiliary primary, and urinary bladder

Markers/diagnosis	Breast CA	Upper GI ADC	Pancreatic ADC	UC
ER	+	−	−	−
GATA3	+	−	−	+
CDH17	−	+/−	+/−	−/+
MUC5AC	−	−/+	+/−	−
p40	−	−	−	+
CK20	−	−/+	−/+	+/−
p63	−	−	−	+

Note: *GI* gastrointestinal, *CA* carcinoma, *ADC* adenocarcinoma, *UC* urothelial carcinoma, *ER* estrogen receptor, *GATA3* GATA binding protein 3, *CDH17* cadherin-17, *MUC5AC* mucin 5 AC, CK20 cytokeratin 20 "+" >75% of cases are positive, "−" <5% of cases are positive, "+/−" 50–75% of cases are positive, "−/+" <50% of cases are positive

Table 1.14 Lung ADC vs. gynecologic primaries

Markers/diagnosis	Lung ADC	OSC	EMADC	ECADC	OCCC
TTF1	+	−	−	−	−
Napsin A	+	−	−	−	+
PAX8	−	+	+	+	+/−
ER	−	+	+	+/−	+/−
Vimentin	−	−	+	−	−
WT1	−	+	−	−	−
pVHL	−	−	−	−	+/−
HPV in situ	−	−	−	+	−

Note: *ADC* adenocarcinoma, *OSC* ovarian serous carcinoma, *EMADC* endometrial ADC, *ECADC* endocervical ADC, *OCCC* ovarian clear cell carcinoma, *TTF1* thyroid transcription factor 1, *PAX 8* paired box gene 8, *ER* estrogen receptor, *WT1* Wilm's tumor 1, *pVHL* von Hippel-Lindau tumor suppressor, *HPV* human papilloma virus, "+" >75% of cases are positive, "−" <5% of cases are positive, "+/−" 50–75% of cases are positive. Clear cell carcinomas of the endometrium are frequently positive for napsin A as well

similar role. No reliable immunomarkers are available for differentiating a uterine serous carcinoma from an ovarian serous carcinoma.

- *Differential diagnosis of CK20+/CK7- carcinomas*.
  Predominately CK20+ carcinomas include colorectal ADC (CRADC), small intestinal ADC (SADC), bladder ADC (BADC), appendiceal ADC (APADC), Merkel cell carcinoma, and salivary gland small-cell carcinoma. Perinuclear dot-staining patterns are seen in both Merkel cell carcinoma and salivary gland small-cell carcinoma. Merkel cell polyomavirus (MCPyV) was detected in approximately 80% of Merkel cell carcinomas but not in other high-grade neuroendocrine carcinomas, including salivary gland small-cell carcinomas; therefore, MCPyV is a potentially sensitive and highly specific marker for identification of Merkel cell carcinoma of the skin. CK20+ carcinomas and useful markers are summarized in Table 1.16.
- *Differential diagnosis of CK7−/CK20- carcinomas*.
  The following neoplasms usually present as CK7−/CK20- carcinomas, and some relatively tissue-specific markers may be helpful in reaching a definitive diagnosis. These tumors include but are not limited to (1) medullary carcinomas of the colon, (2) some neuroendocrine neoplasms, (3) clear cell RCCs, (4) HCCs, (5) adrenal cortical neoplasm/carcinomas, (6) germ cell tumors, (7) prostatic ADCs, and (8) SCCs. A subset of small-cell carcinomas of the lung, gastric ADCs, esophageal ADCs, and mesotheliomas can be CK7−/CK20-.
  Medullary carcinoma of the colon frequently shows loss of microsatellite instability (MSI) markers, especially MutL homolog 1 (MLH1) and postmeiotic segregation increased 2 (PMS2), and is commonly positive for CDH17, SATB2, calretinin, trefoil factor (TFF)3, and mucin 4 (MUC4). CDX2 expression tends to be weak and focal. Focal positivity (<25% of the tumor cells stained) for neuroendocrine markers such as synaptophysin can be seen. Approximately 70% of medullary carcinomas of the large bowel can be positive for calretinin.
  Neuroendocrine neoplasms/carcinomas are positive for chromogranin, synaptophysin, and CD56. Chromogranin is expressed in well to moderately differentiated neuroendocrine neoplasms/carcinomas and tends to be only focally positive in poorly differentiated neuroendocrine carcinomas/small-cell carcinomas. CD56 is a highly sensitive marker for small-cell carcinoma; however, its expression is only seen in approximately 50% of pancreatic NETs. Additionally, CD56 can be positive in non-neuroendocrine carcinomas. More recently, preliminary studies showed that approximately 50% of pancreatic NETs showed loss of expression of anti-ATRX or anti-DAXX. The expression of ATRX/DAXX is usually present in NETs from other organs. To differentiate the tissue origin of a given neuroendocrine neoplasm/carcinoma, the following markers are useful and are summarized in Table 1.17.
  Germ cell tumors are frequently negative for CK7 and CK20. SALL4 and LIN28 are excellent screening markers for germ cell tumors, which are positive in nearly 100% of seminomas, embryonal carcinomas, and yolk sac tumors, 70% of choriocarcinomas, and 50% of teratomas. D2-40 and CD117 are specific markers for seminoma; AFP and glypican-3 are specific markers for yolk sac tumors; SOX2, NANOG, and CD30 are relatively specific markers for embryonal carcinomas, although NANOG is also positive in seminomas and SOX2 may be positive in yolk sac tumors; and CD10 and beta-human chorionic gonadotropin (B-HCG) are specific markers for choriocarcinomas. GATA3 are frequently positive in cho-

Table 1.15 PRCC vs. UC vs. CDC vs. PADC

Markers/diagnosis	PRCC	UC	CDC	PADC
PAX8	+	−	+	−
RCCma	+	−	+/−	−
GATA3	−	+	−	−
p40	−	+	−	−
S100P	−	+	−	−
P504S	+	−/+	−	+
PSA	−	−	−	+
CK7	+	+	+	−

Note: *PRCC* papillary renal cell carcinoma, *UC* urothelial carcinoma, *CDC* collecting duct carcinoma, *PADC* prostatic adenocarcinoma, *PAX 8* paired box gene 8, *RCCma* renal cell carcinoma marker, *GATA3* GATA binding protein 3, *S100P* placental S100, *P504S* alpha-methylacyl-CoA racemase, *PSA* prostate-specific antigen, *CK7* cytokeratin 7, "+" >75% of cases are positive, "−" <5% of cases are positive, "+/−" 50–75% of cases are positive, "−/+" <50% of cases are positive. Urothelial carcinoma of the renal pelvis may be focally positive for PAX8

Table 1.16 Summary of CK20+ carcinomas and useful markers

Marker/diagnosis	CRADC	SADC	BADC	Merkel	APADC	SSCC
CK7	−	−/+	−	−	−	−
CDX2	+	+	+	−	+	−
SATB2	+	−	N/A	N/A	+	N/A
CDH17	+	+	+	−	+	−
Beta-catenin (nuclear)	+	+/−	−	−	+/−	−
Synaptophysin	−	−	−	+	−	+
GATA3	−	−	+/−	−	−	−

Note: *CRADC* colorectal adenocarcinoma, *SADC* small intestinal adenocarcinoma, *BADC* bladder adenocarcinoma, *APADC* appendiceal adenocarcinoma, *SSCC* salivary gland small-cell carcinoma, *CK* cytokeratin, *CDX2* caudal-type homeobox 2, *SATB2* special AT-rich sequence-binding protein 2, *CDH17* cadherin-17, *GATA3* GATA binding protein 3, "+" >75% of cases are positive, "−" <5% of cases are positive, "+/−" 50–75% of cases are positive, "−/+" <50% of cases are positive

Table 1.17 Markers for NETs

Markers/ diagnosis	Lung	Pan	Stoma	Duo	Ileum	Appen	LC	Rec
CK7	+	+/−	+/−	−	−	−	−	−
CK20	−	−	−	−	+	+/−	+	−/+
CDX2	−	−	−	−	+	+	+/−	+/−
SATB2	−	−	−	−	−	−/+	+	+
CDH17	−	+	+	+/−	+	+	+	+
TTF1	−/+	−	−	−	−	−	−	−
PR	−	+/−	−	−	−	−	−	−
PAX8	−	+/−	−	−	−	−	−	−/+
PDX1	−	+	−	+	−	−	−	−

Note: *Pan* pancreas, *Stoma* stomach, *Duo* duodenum, *Appen* appendix, *LC* left colon, *Rec* rectum, *CK* cytokeratin, *CDX2* caudal-type homeobox 2, *SATB2* special AT-rich sequence-binding protein 2, CDH17 cadherin-17, *TTF1* thyroid transcription factor 1, *PR* progesterone receptor; *PAX8* paired box gene 8, *PDX1* pancreatic duodenal homeobox 1,"+" >75% of cases are positive, "−" <5% of cases are positive, "+/−" 50–75% of cases are positive, "−/+" <50% of cases are positive

Table 1.18 Markers for germ cell tumors

Marker	Seminoma	Embryonal CA	Yolk sac tumor	ChorioCA
SALL4	+	+	+	+/−
LIN28	+	+	+	+/−
OCT4	+	+	−	+/−
SOX2	−	+	−/+	−
NANOG	+	+	−	−
CD30	−	+	−	−
CD117	+	−	−	−
D2-40	+	−	−	−
Glypican-3	−	−	+	−
AFP	−	−	+	−
Beta-HCG	−	−	−	+
CD10	−	−	−	+

Note: *CA* carcinoma, *chorioCA* choriocarcinoma, *SALL4* sal-like protein 4, *LIN28* lin-28 homolog, *OCT4* octamer-binding transcription factor 4, *SOX2* sex-determining region Y-box 2, *NANOG* NANOG homeobox, *CD* cluster of differentiation, *D2-40* podoplanin, *AFP* alpha-fetoprotein, *beta-HCG* beta-human chorionic gonadotropin, "+" >75% of cases are positive,"−" <5% of cases are positive, "+/−" 50–75% of cases are positive, "−/+" <50% of cases are positive

riocarcinomas and yolk sac tumors. The useful markers are summarized in Table 1.18.

Over 90% of clear cell RCCs are negative for both CK7 and CK20. Co-expression of cytokeratin/vimentin is one of the important features for clear cell RCC. Five markers (PAX8, pVHL, RCCma, CD10, and KIM-1) are helpful in confirming the diagnosis of clear cell RCC. PAX8 is likely the most sensitive marker among these five markers; however, it also expresses in tumors from the thyroid, gynecologic tract, thymus, and others. RCCma has a low sensitivity of approximately 50% in detecting a high-grade clear cell RCC. Both pVHL and KIM-1 are also expressed in clear cell carcinomas of the uterus and ovary; however, KIM-1 is not commercially available yet. CD10 is a highly sensitive but not very specific marker for clear cell RCC. In general, a small panel of antibodies consisting of CAM5.2, vimentin, PAX8, pVHL, and RCCma can serve as an initial panel to confirm a metastatic clear cell RCC. When it comes to a sarcomatoid RCC, an extended panel of antibodies including cytokeratins, vimentin, PAX8, pVHL, RCCma, CD10, KIM-1, and alpha-methylacyl-CoA racemase (P504S) is recommended to increase the diagnostic sensitivity.

A majority of HCCs are negative for CK7 and CK20, with the exception of fibrolamellar HCC, which is usually CK7+/CK20-. Approximately 10% of HCCs may show positive staining for both CK7 and CK20. AE1/AE3 is only positive in approximately 30% of HCCs, whereas over 90% of HCCs are positive for CAM5.2 which contains keratin 8. Other cytokeratins such as 5D3, 5D3/LP34, and KL1 (containing both keratins 8 and 18) are good screening markers for HCC. Many markers are useful for identifying HCC, including arginase-1, glypican-3, HepPar-1, CD10, and polyclonal carcinoembryonic antigen (CEA). Arginase-1 is the most sensitive and specific marker for HCC, including poorly differentiated HCC, whereas HepPar-1 is a sensitive but not very specific marker for HCC since its immunoreactivity has been reported in many other carcinomas. The diagnostic sensitivity of both arginase-1 and HepPar-1 for identifying liver cell origin is over 90%. Glypican-3 is a good marker for both well-differentiated and poorly differentiated HCC, with a diagnostic sensitivity of approximately 85%. In addition, glypican-3 is not expressed in benign or reactive hepatocytes; in contrast, both arginase-1 and HepPar-1 are expressed in both benign and neoplastic hepatocytes. Both CD10 and polyclonal CEA demonstrate a canalicular staining pattern in HCC and benign liver. AFP has limited utility due to its low sensitivity of approximately 25%, but AFP is a highly sensitive marker for hepatoblastoma. Nearly all HCCs are negative for MOC-31.

Adrenal cortical neoplasm/carcinoma is another group of epithelial tumors which is usually negative for both CK7 and CK20. Mart-1, calretinin, SF-1, and inhibin-alpha are a group of sensitive and relatively specific markers for identifying adrenal cortical neoplasm/carcinomas. They are usually negative for hepatocellular markers (arginase-1, HepPar-1, and glypican-3) and RCC markers (PAX8, RCCma, CD10, CAIX, and pVHL) .

Over 90% of prostatic acinar ADCs are negative for CK7 and CK20, with the exception of prostatic ductal ADC, which is usually positive for CK7. Prostate-specific antigen (PSA) and prostate-specific acid phosphatase (PSAP) are highly sensitive and specific markers for identifying over 90% of metastatic prostatic ADCs. NK3 homeobox 1 (NKX3.1) is a highly sensitive and specific nuclear staining marker for both primary and metastatic prostatic ADCs