Category VI: Malignant

8

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Background

The majority of cytologic studies of material obtained from the lower respiratory tract are performed for investigating whether a pulmonary lesion detected on imaging studies is or is not a malignancy. Classically, cytologic study of respiratory lesions involved sputum cytology followed by bronchial brushings and washings. More recently, fine-needle aspiration specimens obtained under computerized tomographic or endoscopic ultrasound guidance have become popular. The latter technique is preferable for centrally located lesions, while the CT transthoracic approach is optimal for peripheral nodules. Endobronchial FNA can also be used for staging of pulmonary carcinoma by sampling hilar and peribronchial lymph nodes.

The lungs are the site of a large number of primary and metastatic malignancies as well as a smaller number of benign neoplasms and localized nonneoplastic lesions.

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L. J. Layfield, Z. Baloch (eds.), *The Papanicolaou Society of Cytopathology System for Reporting Respiratory Cytology*,
https://doi.org/10.1007/978-3-319-97235-0_8

These pulmonary and pleural-based lesions present with a wide variety of morphologic patterns. Historically, cytologic evaluation only required the separation of primary pulmonary carcinomas into small cell and non-small cell varieties. Before the advent of targeted therapies, this limited stratification was acceptable for clinical management. Patients with non-small cell carcinomas were potential candidates for curative surgery, while patients with small cell carcinomas were nonsurgical candidates best treated by chemotherapy. Johnston and Frable [1] demonstrated good accuracy in typing carcinomas with a 92% accuracy for the recognition of squamous cell carcinoma, an 86% accuracy for adenocarcinoma, and an 88% accuracy for the diagnosis of small cell carcinoma. Other investigators have demonstrated an accuracy of subtyping between 75% and 77% [2–4]. Based on these data, it was standard of care to cytologically separate pulmonary carcinomas into small cell and non-small cell types not further classified; more specific classification was not attempted. With the advent of targeted therapies especially for adenocarcinomas, the cytologic diagnosis of adenocarcinoma versus squamous cell carcinoma has assumed new importance. Molecular testing of all primary pulmonary adenocarcinomas has become the standard of practice with testing for identification of molecular abnormalities in EGFR, ALK, and ROS-1 being routinely performed. While the initial World Health Organization classification for pulmonary carcinomas was based predominately on resection specimens and did not address issues relating to small biopsies or cytology specimens, more recent updates by the World Health Organization, the International Association for the Study of Lung Cancer, the American Thoracic Society, and the European Respiratory Society have addressed the use of small biopsy specimens and cytologic preparations [5]. These updated recommendations include information on a number of issues for the cytologic diagnosis of lung cancer with implications for the World Health Organization classification. These recommendations now include support for increased usage of immunohistochemistry for subclassification of lung carcinomas as well as recommending management schemes for the use of pathologic specimens in molecular studies [5]. A number of recent studies have shown improved subclassification of non-small cell carcinomas into squamous and adenocarcinoma subtypes using current morphologic criteria and immunohistochemical staining strategies [6, 7]. For cytologic specimens, the use of cytomorphologic criteria coupled with immunohistochemistry (p63, p40, napsin A, and TTF-1) allows accurate separation of non-small lung carcinomas into squamous and adenocarcinoma subtypes in the majority of cases [5]. The utilization of immunohistochemistry for subclassification of non-small cell carcinomas as well as molecular testing of primary pulmonary carcinomas is further discussed in Chap. 9. Adenocarcinoma appears to still require resection specimens for measurement of overall size and the presence or absence of invasion. Resection specimens are required for complete subtyping. The distinction of adenocarcinoma in situ, minimally invasive adenocarcinoma, and invasive lepidic pattern adenocarcinomas relies on both overall size of the carcinoma and size of the invasive component. Such measurements cannot be achieved by evaluation of only cytologic specimens or small biopsies. Occasional authors have attempted to grade adenocarcinomas in cytologic specimens [8].

Cytologic specimens assigned to the positive for malignancy category should be typed whenever possible [9–11]. Immunohistochemistry can be particularly useful in this process [9]. With current requirements morphologic analysis of Papanicolaou or

Diff-Quik® stained specimen appears to be insufficient for consistently accurate subclassification [9, 11]. The malignant category contains a number of morphologically low-grade malignancies including well-differentiated neuroendocrine tumors (formerly carcinoids) and intermediate-grade neuroendocrine tumors (atypical carcinoids). Other low-grade malignancies include adenoid cystic carcinoma and mucoepidermoid carcinomas. Additionally well-differentiated adenocarcinomas with a lepidic pattern (formerly bronchiolar-alveolar carcinomas) are assigned to the malignant category but must be cytologically separated from other neoplasms including sclerosing pneumocytoma.

In many clinical series, a majority of malignant pulmonary nodules represent metastatic disease from extrathoracic sites [12, 13]. Sites of origin for these metastases include the gastrointestinal tract, breast, bladder, head and neck, prostate, and gynecological tract. Sarcomas and melanomas also represent a significant component for metastatic neoplasms. Some of these metastases may appear years if not decades, after resection of the primary.

Age and gender of the patient are useful in narrowing the differential diagnosis, and ancillary studies, especially immunohistochemistry, are helpful in the workup of these lesions as discussed in the chapter on ancillary testing.

Malignancy risk for the malignant category is approximately 90% in the limited amount of published data [14].

Definition

Positive for malignancy aspirates represent a group of neoplasms that unequivocally display malignant cytologic features including squamous cell carcinoma, adenocarcinoma, small cell carcinoma, large cell neuroendocrine carcinoma, giant cell carcinoma, large cell carcinoma, lymphoma, adenoid cystic carcinoma, and mucoepidermoid carcinoma. Also included are mesotheliomas and rare primary sarcomas of the lung and pleura. Metastatic carcinomas and sarcomas to the lung are included in this category. The majority of carcinomas sampled by FNA are non-small cell carcinomas, and distinction of squamous cell carcinomas from adenocarcinomas is necessary for appropriate treatment. A number of cytomorphologic features are helpful in this separation (Table 8.1).

Criteria

Cytomorphologic Features of Squamous Cell Carcinoma

Cytomorphologic Features of Squamous Cell Carcinoma [15, 16] (Figs. 8.1, 8.2, 8.3, and 8.4)

- Background: Necrotic debris with acute inflammatory cells. The foci of necrosis may be found closely associated with viable tumor cells (aka clinging necrosis).

Table 8.1 Cytomorphologic features helpful in distinguishing squamous cell carcinoma from adenocarcinoma

Cytomorphologic feature	Squamous cell carcinoma	Adenocarcinoma
Background	Necrotic debris with acute inflammatory cells. The foci of necrosis may be found closely associated with viable tumor cells (aka clinging necrosis)	Clean and granular (tumor necrosis may be seen in high-grade tumors). Mucinous in well-differentiated carcinoma with mucin production
Cell pattern	Single cells, strips, and irregular clusters	Papillary, spherical, or acinar-like structures
Cell shape	Great variability in cell shape and size. Cell shapes vary from round to oval to spindle-shaped	Relatively uniform cell shapes and sizes, occasionally columnar
Nuclear shape and size	Often marked variation in nuclear shape varying from round to oval to spindle-shaped. Marked variation in nuclear size	Limited variability in nuclear size. Most nuclei are round to oval, often eccentrically located in the cell
Nucleoli	Nucleoli often inconspicuous when present may be eccentrically located	Often prominent, nucleoli may be single or multiple and may be eccentric
Nuclear chromatin	Often marked hyperchromasia Pyknosis relatively common	Finely granular Usually only slight hyperchromasia
Cell grouping	Single cells, strips and irregular clusters	Papillary, spherical, or acinar-like structures
Cytoplasm	May show keratinization	Cytoplasm often abundant, small fine cytoplasmic vacuoles more often than large ones

- Cell pattern: Single cells and flat sheets with well-defined cell borders and minimal nuclear overlap (highly dependent on degree of tumor differentiation).
- Cytoplasm: Polygonal, oval, spindled, and irregular cell contours with dense or keratinized cytoplasm.
- Nuclei: Oval, rectangular in shape with irregular contours, centrally situated in cytoplasm, coarse to pyknotic dark chromatin.
- Nucleoli often inconspicuous.

Cytomorphologic Features of Adenocarcinoma Not Otherwise Specified

Cytomorphologic Features of Adenocarcinoma Not Otherwise Specified [17, 18] (Figs. 8.5, 8.6, and 8.7)

- Background: Usually clean, tumor diathesis can be seen in high-grade tumors.
- Cell pattern: Mostly flat to three-dimensional aggregates (including spheres, acini, and papillary-like structures) and variable numbers of singly scattered tumor cells.

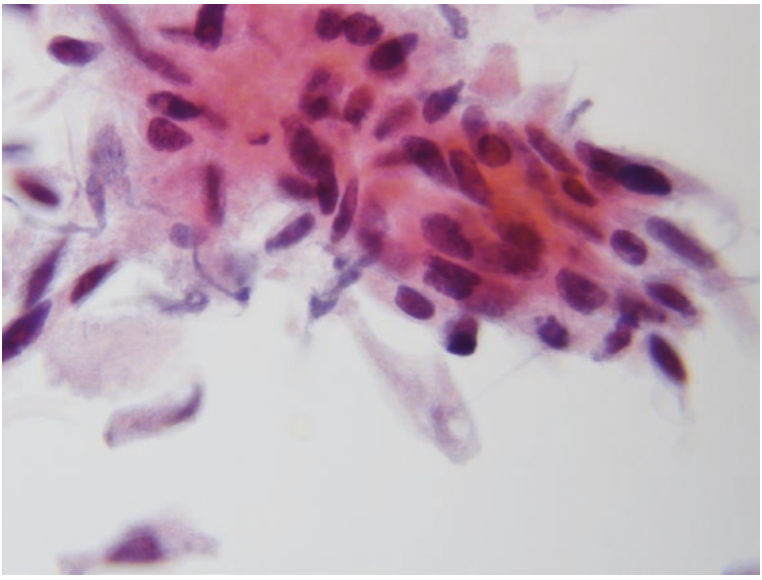

Fig. 8.1 Cluster of atypical spindle-shaped cells with metaplastic cytoplasm surrounding hyperchromatic nuclei of variable shapes and sizes characteristic of well- to moderately differentiated squamous cell carcinoma (Papanicolaou stain)

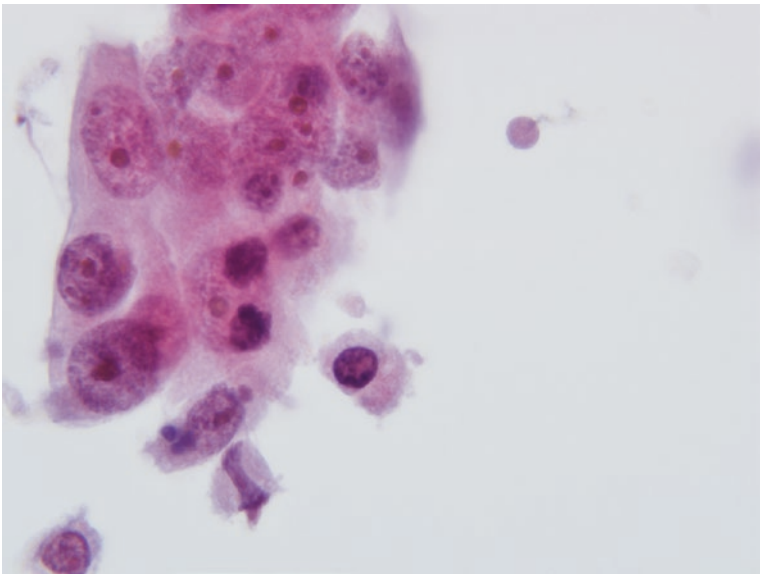

Fig. 8.2 Cluster of pleomorphic polygonal to short spindle-shaped cells with marked variability in nuclear size characteristic of poorly differentiated squamous cell carcinoma (Papanicolaou stain)

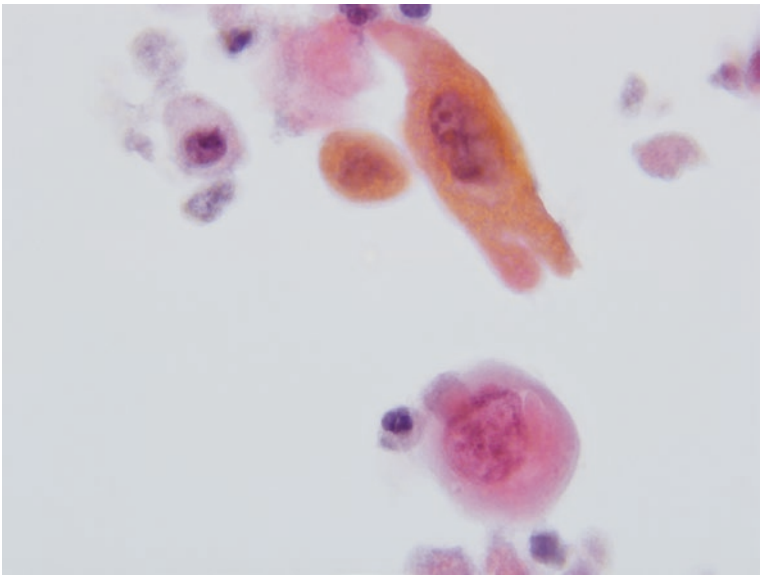

Fig. 8.3 Round and spindle-shaped cells with well-developed keratinization and marked variability in cell size and shape (Papanicolaou stain)

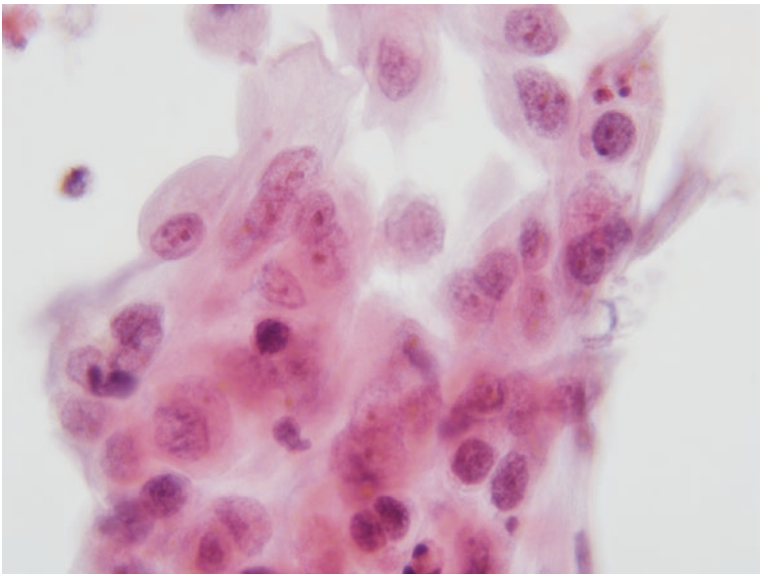

Fig. 8.4 Sheet of atypical cells with moderate to abundant amounts of cytoplasm surrounding variably sized nuclei with a wide range of shapes. Nucleoli tend to be small or indistinct as is common in squamous cell carcinomas (Papanicolaou stain)

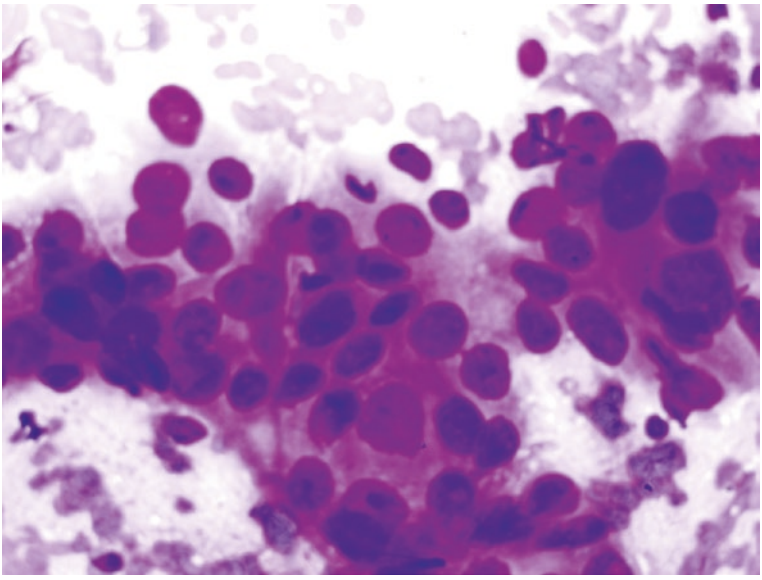

Fig. 8.5 Material aspirated from an adenocarcinoma. The cells are relatively uniform in shape and size. The cytoplasm is pale and focally foamy. Occasional acinar structures are present composed of rings of nuclei surrounding pale central cytoplasm (Diff-Quik® stain)

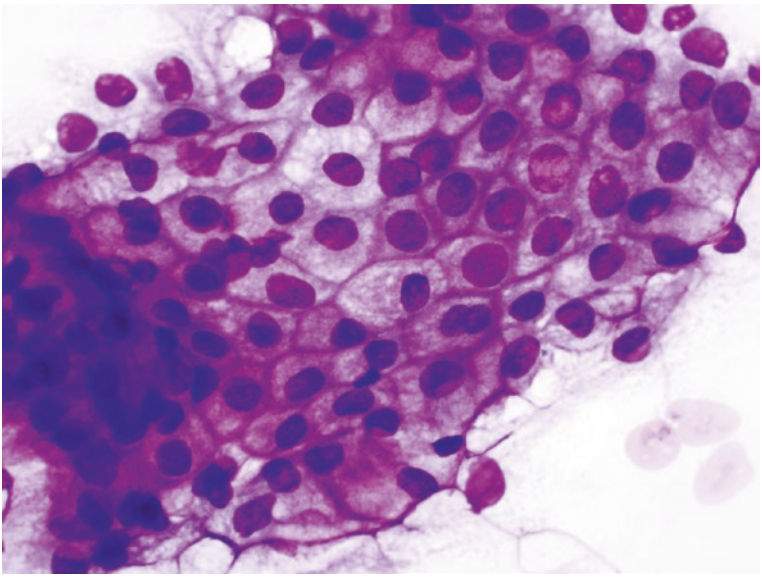

Fig. 8.6 Sheet of atypical cells with a "honeycomb" pattern. The nuclei are relatively uniform in size and shape. The cytoplasm is pale to foamy as is characteristic of an adenocarcinoma (Diff-Quik® stain)

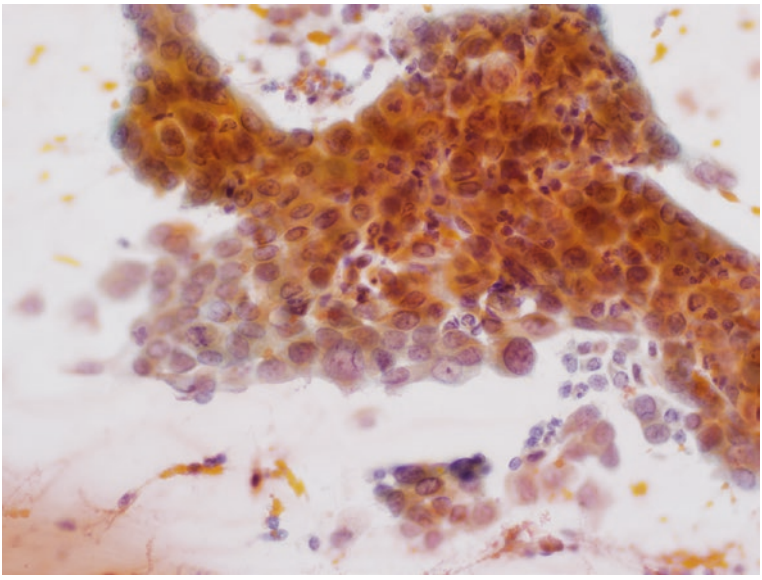

Fig. 8.7 Sheet of polygonal to cuboidal cells with modest amounts of cytoplasm surrounding large nuclei with a vesicular chromatin pattern and distinct nuclei characteristic of adenocarcinoma. Note palisading of cells along one edge of cell sheet (Papanicolaou stain)

- Acinar (nuclei polarized to one side of cell) and glandular (true central lumens) structures in aggregates.
- Cytoplasm: Delicate, granular to vacuolated; mucin vacuoles may be readily evident.
- Nuclei: Eccentric round to oval structures with minor membrane irregularities and fine vesicular chromatin.
- Prominent nucleoli (single or multiple).

Subtypes of Adenocarcinomas

Adenocarcinoma with Lepidic Pattern

Adenocarcinoma with Lepidic Pattern [19, 20] (Figs. 8.8, 8.9, and 8.10)

These carcinomas were termed as bronchioloalveolar carcinomas in the 2004 WHO classification but due to a rather favorable prognosis were reclassified as adenocarcinoma in situ, minimally invasive adenocarcinoma, or invasive adenocarcinoma with lepidic pattern. Distinction of these three entities depends on review of resections and identification and measurement of an invasive focus where present. Such evaluation cannot be performed on cytologic specimens. Therefore, the general term adenocarcinoma should be used and further subtyping is not applicable for cytologic samples.

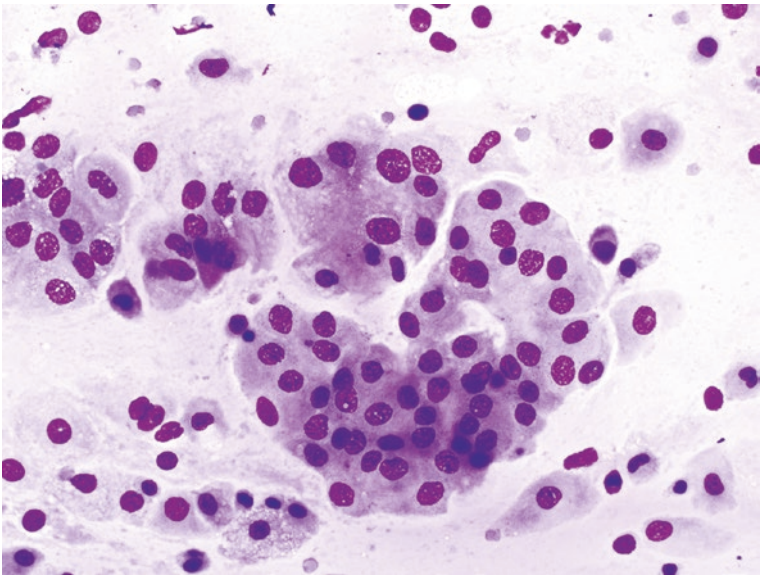

Fig. 8.8 Cell clusters obtained by FNA from a well-differentiated lepidic pattern adenocarcinoma. The tumor cells have abundant cytoplasm and round to oval nuclei with a bland chromatin. The nuclei are slightly larger than a red blood cell (Diff-Quik® stain)

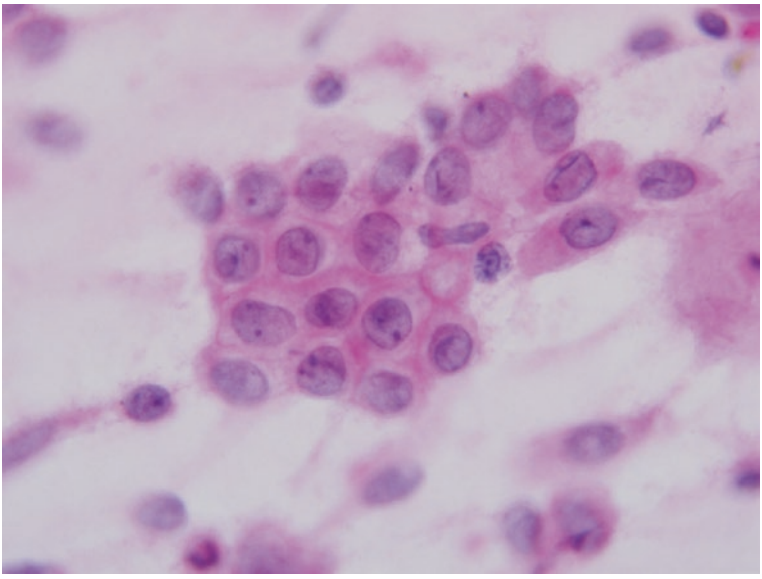

Fig. 8.9 The nuclei of cells obtained from lepidic pattern adenocarcinomas are slightly larger than a red blood cell, have small nucleoli, and may have nuclear membrane grooves or even intranuclear cytoplasmic pseudoinclusions (Hematoxylin and Eosin stain)

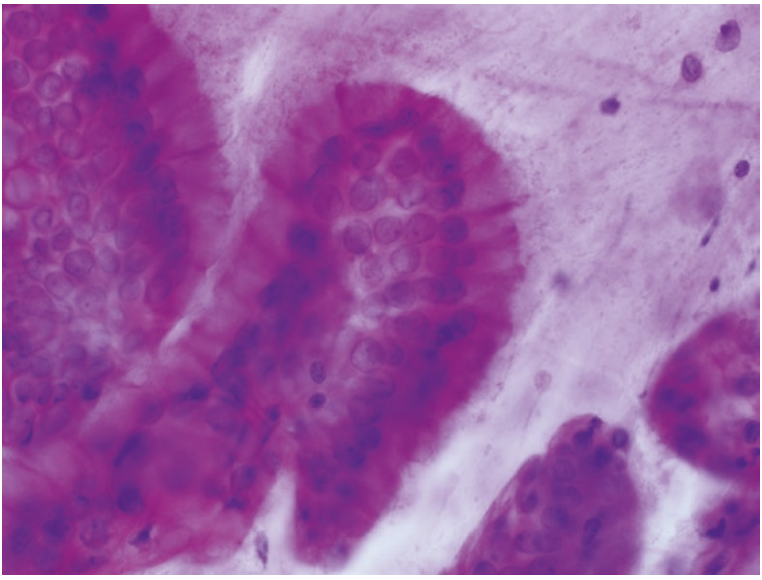

Fig. 8.10 The cell clusters of lepidic pattern adenocarcinomas may show peripheral palisading with nuclei polarized away from the outer edge of the cell group (Diff-Quik® stain)

Criteria

- Background: Mucinous material can be seen in some cases.
- Moderately cellular specimens.
- Cell pattern: Small flat sheets or three to four cell strips of relatively bland cells.
- Round nuclei slightly larger than a red blood cell.
- Distinct nucleoli.
- Nuclear grooves and/or cytoplasmic nuclear pseudoinclusions.
- Scant cytoplasm.
- Lack of demonstrable mucin.

Fetal Adenocarcinoma

Fetal Adenocarcinoma [21]

Low-grade fetal adenocarcinoma is important to recognize as it is associated with a favorable prognosis. It has characteristic features cytologically.

Criteria

- Homogenous round bland nuclei
- Inconspicuous nucleoli
- Glycogen-rich subnuclear vacuoles
- Focal tigroid background
- Component of small aggregates of somewhat larger cells with central bland nuclei (squamoid morules)

Mucinous Adenocarcinoma

Mucinous Adenocarcinoma [22, 23] (Figs. 8.11, 8.12, and 8.13)

This category consists of adenocarcinomas formally designated mucinous bronchioloalveolar carcinoma. Their cytologic appearance has been described.

Criteria

- Background: Easily demonstrable mucinous material (can be challenging to recognize in monolayer preparations).
- Cell pattern: Cells are arranged in flat sheets with mild loss of polarization (drunken honeycomb pattern).
- Columnar or elongated cells with voluminous vacuolated cytoplasm.
- Eccentrically placed bland nuclei.
- Finely granular chromatin.
- Moderate amounts of mucin in background of smears.
- CK7 positive (can be negative), CK20 positive, usually TTF-1 negative, and CDX2 and MOC31 variably positive.

Adenocarcinoma with Colloid Pattern

Adenocarcinoma with Colloid Pattern [24]

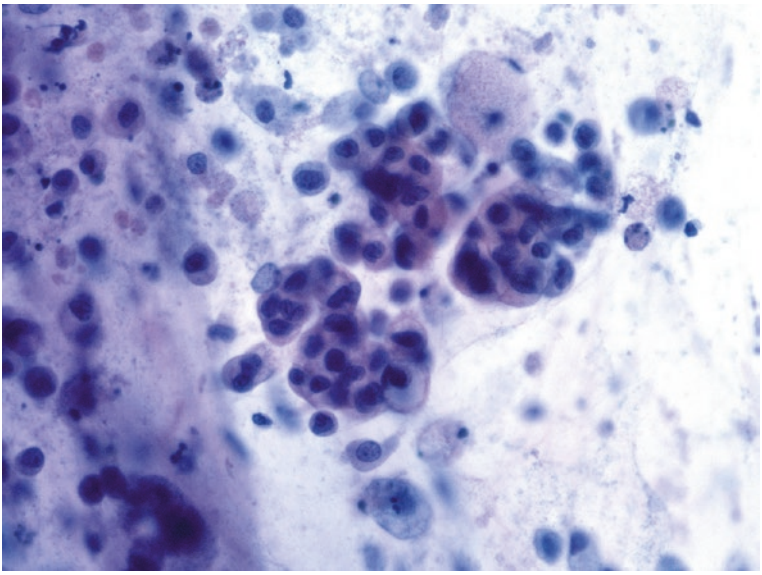

Fig. 8.11 Mucinous adenocarcinoma with clusters of cells lying in a mucin-rich background. Many cells have abundant mucin-rich cytoplasm (Papanicolaou stain)

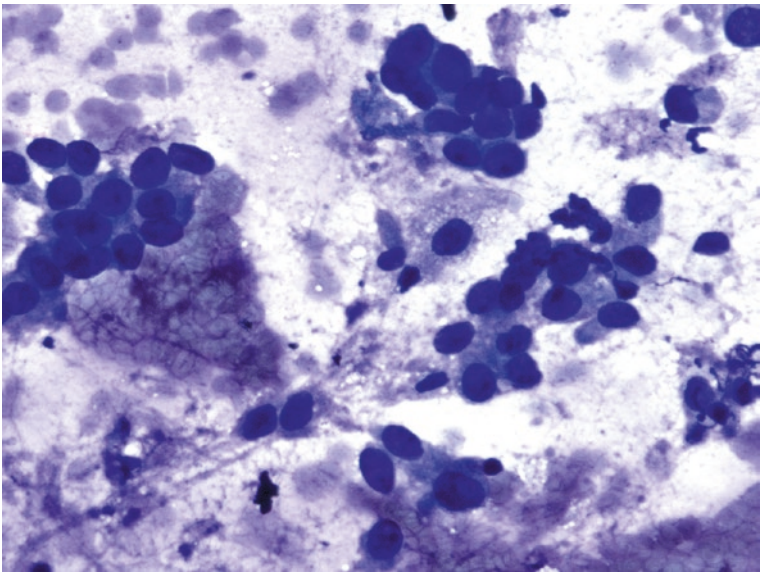

Fig. 8.12 Clusters of round to polygonal cells with thick aggregates of mucin in the background (Papanicolaou stain)

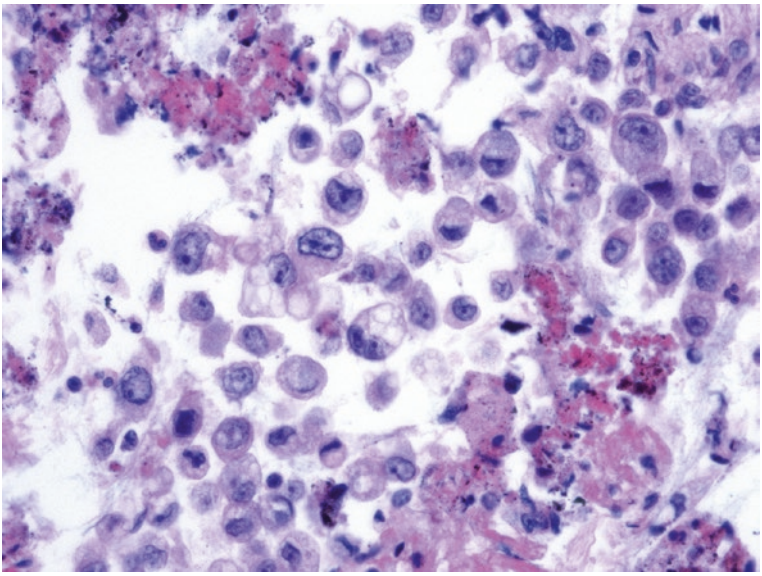

Fig. 8.13 Cell block material from a mucinous adenocarcinoma demonstrating round to polygonal cells with vacuolated mucin-rich cytoplasm (Hematoxylin and Eosin)

- Background: Abundant thick extracellular mucin.
- Cell pattern: Low cellularity specimen with single cells or small clusters of cells with large atypical nuclei.
- Anisonucleosis may be prominent.
- Nuclear size ranges from three to ten times the size of a red blood cell.
- CDX2-positive, TTF-1-negative, mucicarmine stain can readily highlight the extra- and intracellular mucin.

Adenocarcinoma with Signet Ring Features

Adenocarcinoma with Signet Ring Features [25] (Figs. 8.14 and 8.15)

- Background: Abundant thick extracellular mucin.
- Cell pattern: Low cellularity specimen with single cells or small clusters of cells with large atypical nuclei.
- Anisonucleosis may be prominent.
- Nuclear size ranges from three to ten times the size of a red blood cell.
- CDX2-positive, TTF-1-negative, mucicarmine stain can readily highlight the extra- and intracellular mucin.

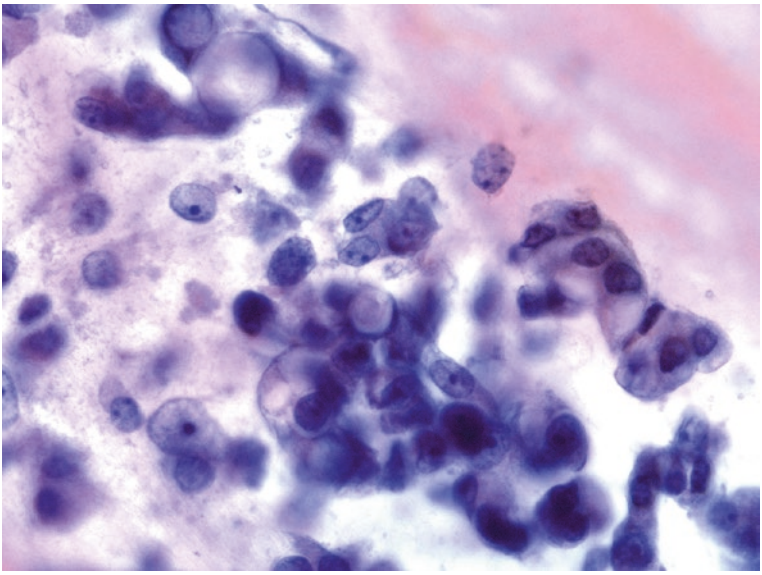

Fig. 8.14 Clusters of round to oval cells with single large cytoplasmic vacuoles pushing the nucleus to one side are characteristic of signet ring adenocarcinoma (Papanicolaou stain)

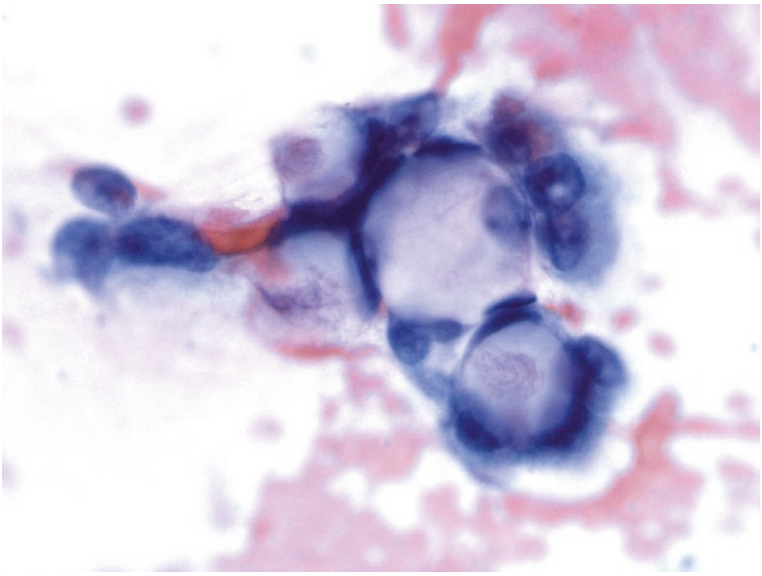

Fig. 8.15 Signet ring adenocarcinomas are characterized by cells with single large vacuoles displacing the cell nucleus to one side (Papanicolaou stain)

Adenocarcinoma with Clear Cell Features

Adenocarcinoma with Clear Cell Features [26] (Figs. 8.16, 8.17, and 8.18)

- Variably cellular specimens.
- Clear polygonal cells with abundant cytoplasm.
- Cytoplasm may have micro-vacuoles (Romanowsky).
- Small amounts of mucin may be present.
- Nuclei are of variable size but usually between three and ten times the size of a red blood cell.
- Eccentric nuclei with prominent nucleoli.
- TTF-1 negative and CK7 positive.

Adenosquamous Carcinoma

Adenosquamous Carcinoma [27] (Figs. 8.19, 8.20, and 8.21)

- Background: Abundant necrotic debris.
- Cell pattern: Solitary cancer cells admixed with cohesive cell clusters and loose cell groups.
- Minority of keratinizing cells scattered among large polygonal cells some of which contain multivacuolated cytoplasm; at times, mucin is obvious.

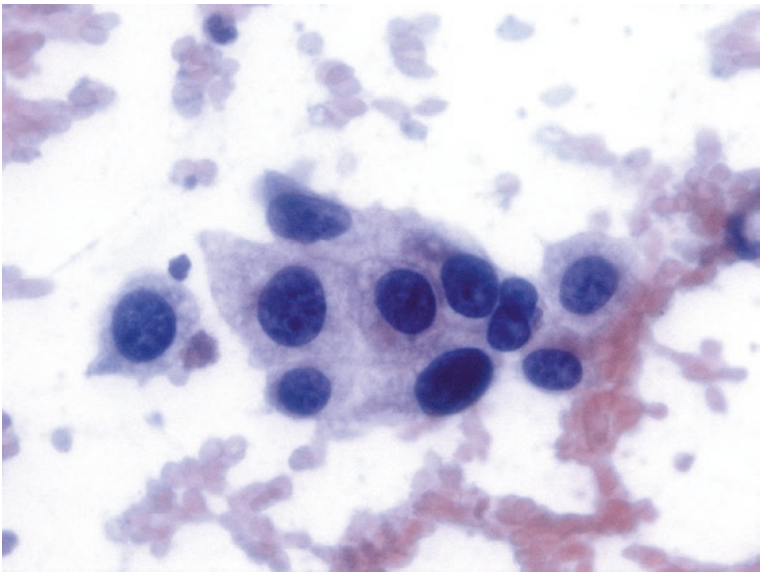

Fig. 8.16 Clear cell adenocarcinomas are composed of cells with moderate to abundant amounts of foamy cytoplasm surrounding enlarged hyperchromatic nuclei (Papanicolaou stain)

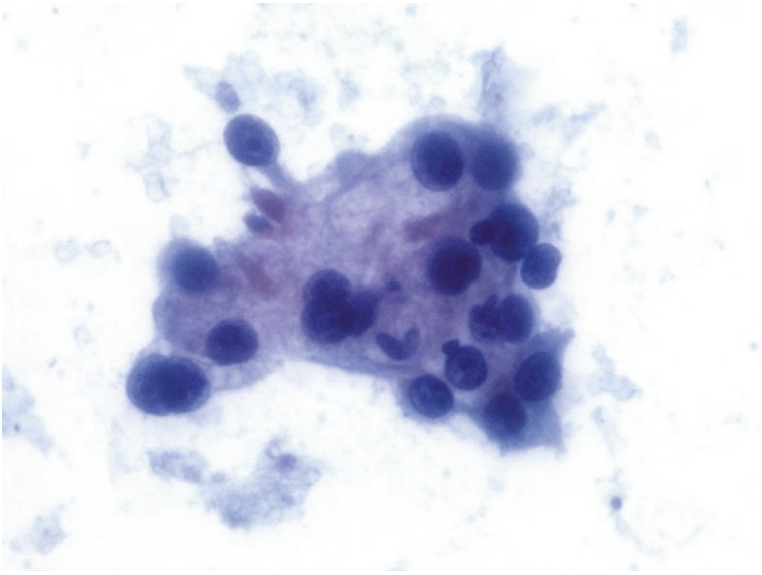

Fig. 8.17 Cluster of cells obtained from a clear cell adenocarcinoma. The cells have abundant pale foamy cytoplasm (Papanicolaou stain)

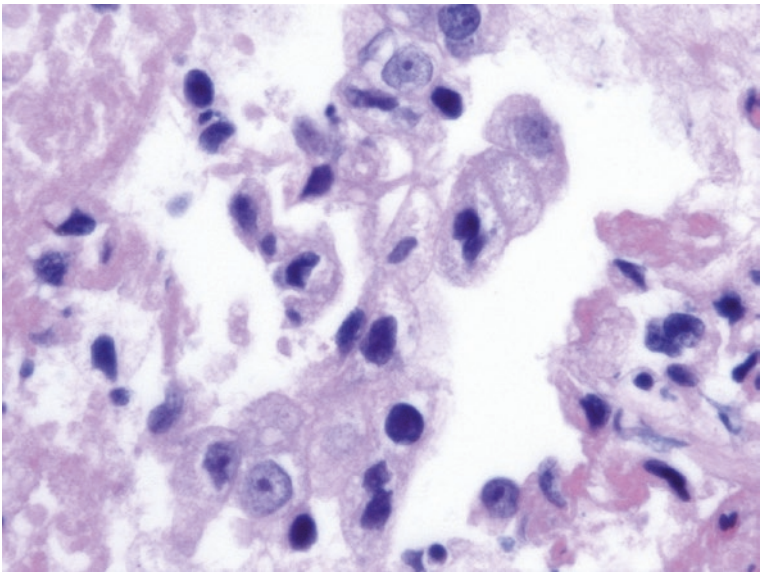

Fig. 8.18 Cell block material containing clusters of cells with pale, foamy, or clear cytoplasm characteristic of clear cell carcinoma (Hematoxylin and Eosin stain)

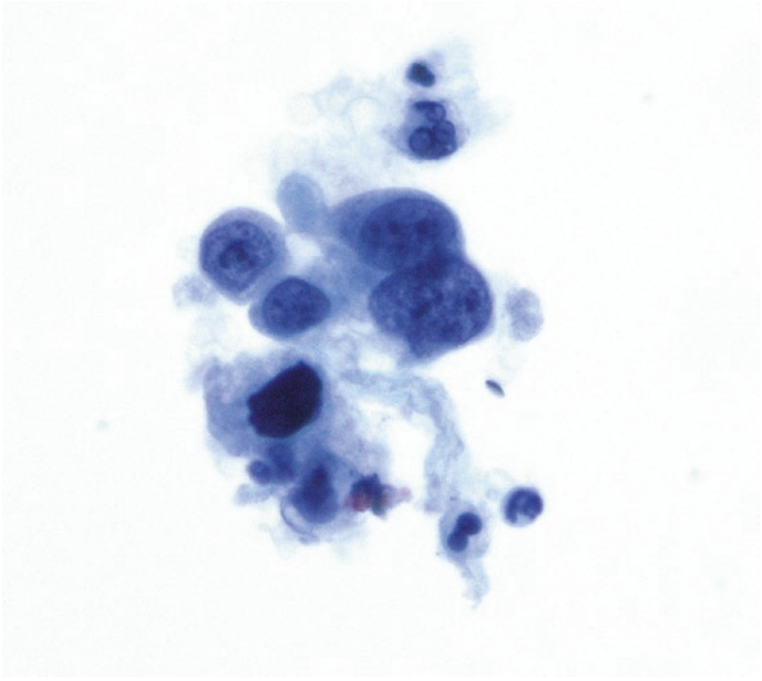

Fig. 8.19 Adenosquamous carcinoma composed of a mixed population of cells with squamous or glandular features (Papanicolaou stain)

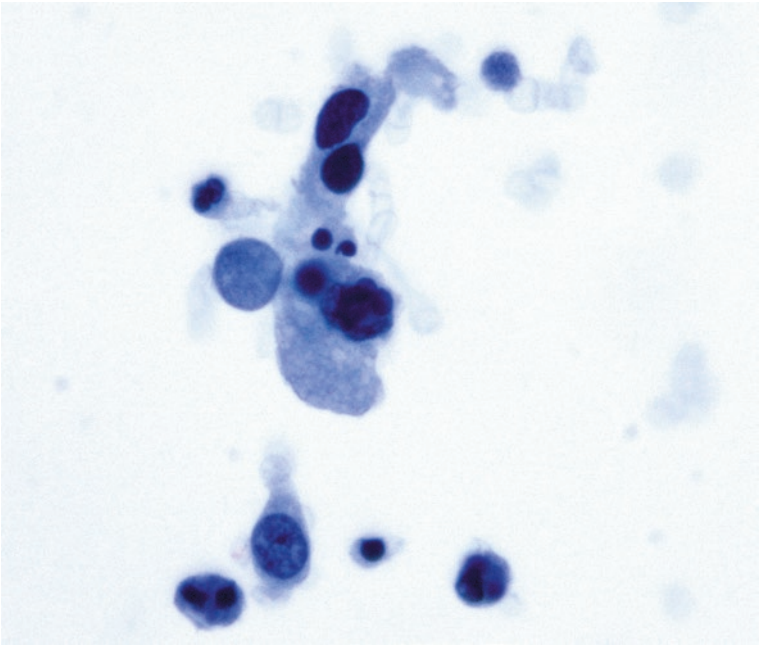

Fig. 8.20 Cluster of cells obtained from an adenosquamous carcinoma showing both squamous and glandular differentiation (Papanicolaou stain)

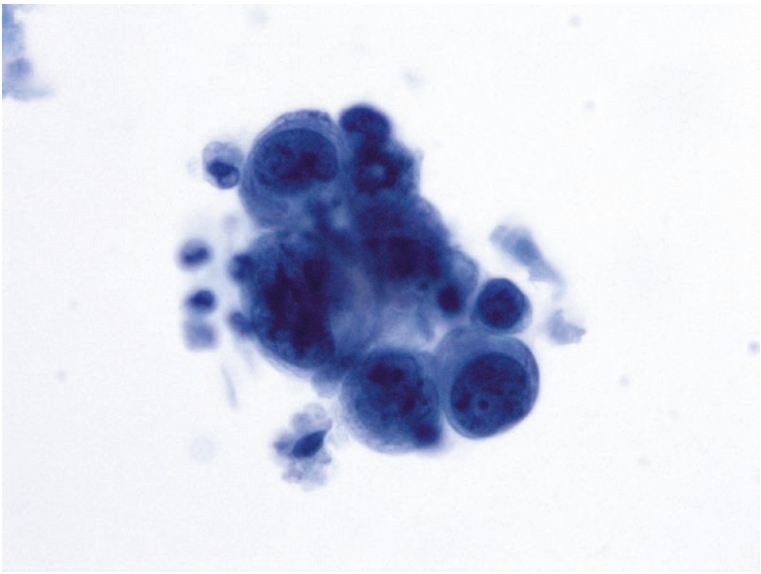

Fig. 8.21 Adenosquamous carcinoma is characterized a mixture of features with both squamous and glandular differentiations (Papanicolaou stain)

- Large atypical nuclei often with distinct nucleoli.
- Scattered pyknotic nuclei.
- p63 positive (focal, in some adenocarcinomas), TTF-1 positive (focal), and p40 positive (focal in squamous population).

Explanatory Notes

- Together squamous cell carcinoma and adenocarcinoma make up the majority of primary carcinomas of the lung.
- The well-differentiated squamous cell carcinomas are characterized by distinct keratin production and often show the presence of anucleated keratinocytes.
- Well-differentiated adenocarcinomas characteristically demonstrate papillary, acinar, or duct-like structures, and the individual cells may have small cytoplasmic vacuoles or even show mucin production.
- Distinction of moderately and poorly differentiated squamous cell carcinomas from adenocarcinomas is more challenging. Certain cytomorphologic features (Table 8.1) including variability of cell shape and size along with position of the nucleus, chromatin pattern, and prominence of the nucleoli are helpful in separating adenocarcinoma from squamous cell carcinoma.
- Squamous cell carcinomas demonstrate greater degrees of variability in cell size as well as shape.
- The nuclei of poorly differentiated squamous cell carcinomas may vary in size by fourfold or greater in a single cell group, while adenocarcinomas are more uniform in cell and nuclear size. Whereas, well-differentiated squamous cell carcinoma demonstrates high variability in cell and nuclear shape varying from oval to spindle-shaped.
- Nuclear membranes are often very irregular in squamous cell carcinomas. While many squamous cell carcinomas have small or absent nucleoli, the nucleoli of adenocarcinomas are often prominent. When distinction cannot be made by cytomorphology alone, immunohistochemical stains for TTF-1, p40, p63, and napsin are helpful.
- The presence of squamous cells in an FNA specimen from the lung is abnormal, but squamous cells can be seen in both metaplastic and neoplastic conditions. Squamous metaplasia and dysplasia are seen with some frequency in brushing and washing specimens obtained from patients who smoke, but the degree of atypia is less than that seen in true squamous cell carcinoma.
- Markedly atypical squamous cells can be seen in reactive metaplasia surrounding fungal abscess cavities, and such cells are a cause of false-positive diagnoses of cancer. Clinical and imaging findings are helpful in separating these atypical metaplasias from carcinoma. Cytopathologically, atypical squamous metaplasia associated with fungal abscesses will be accompanied by numerous inflammatory cells. However, some cases of squamous cell carcinoma may also have a rich inflammatory background.

- The current World Health Organization (WHO) classification requires histologic subtyping of pulmonary carcinomas [5].
- Certain carcinomas can only be diagnosed on resection specimens, and cytology reports should reflect this. The distinction of adenocarcinoma in situ, minimally invasive adenocarcinoma, and invasive adenocarcinoma requires a resection specimen to determine the overall size of the carcinoma and the size of the invasive focus.
- Cytology alone cannot make these distinctions, and the cytology report must acknowledge this fact [5]. Thus, the diagnostic terminology for small biopsy and cytology specimens must be modified from the standard WHO system.
- For cytology specimens, appropriate diagnostic terms include (1) adenocarcinoma (describe identifiable patterns when present), (2) adenocarcinoma with lepidic pattern (state that an invasive component cannot be excluded), (3) invasive mucinous adenocarcinoma, (4) colloid adenocarcinoma, (5) fetal pattern adenocarcinoma, (6) adenocarcinoma with enteric pattern, and (7) non-small cell carcinoma favor adenocarcinoma.

Sarcomatoid Carcinoma

Sarcomatoid Carcinoma [28]

- Population of poorly cohesive or non-cohesive spindle-shaped or pleomorphic cells.
- Lack of metachromatic stromal component.
- Cells lie singly or in small cohesive groups.
- Variable nuclear atypia but anaplastic giant cells may be present.
- Vimentin positive and keratin variably positive.

Cytomorphologic Features of Carcinoid Tumors

Cytomorphologic Features of Carcinoid Tumors [29, 30] (Figs. 8.22, 8.23, and 8.24) (Table 8.2)

- Background: Clean to granular and no necrotic debris.
- Cell pattern: Single cells and aggregates which include palisaded sheets, acini, trabeculae (especially anastomosing), branching clusters, and vascularized tissue fragments.
- Capillaries may be present, rarely traversing between cell groups.
- Monotonous population of tumor cells, which are round, oval, or spindled (the latter especially in lung periphery).
- Nuclei: Round to oval with smooth thin membranes, distinctly granular chromatin, and inconspicuous nucleoli. Bare nuclei may be frequent.

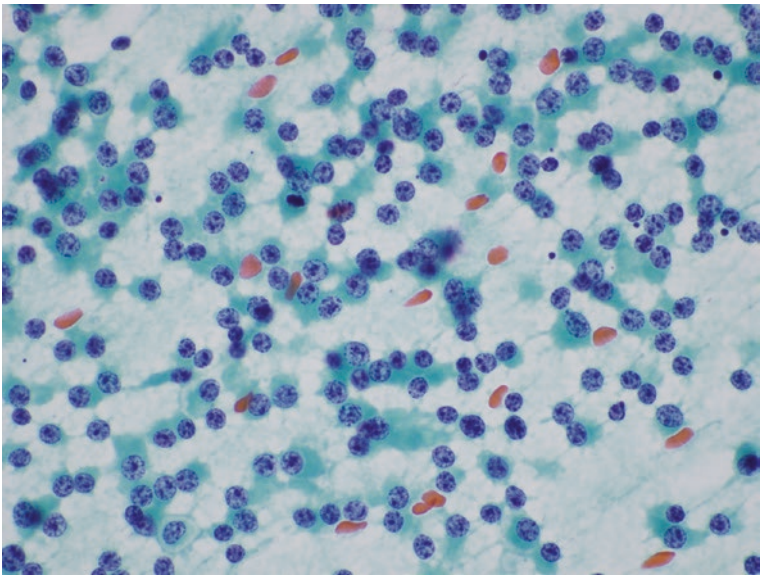

Fig. 8.22 Smears of carcinoid tumors are characterized by high cellularity and a dispersed cell pattern. Many cells lie as single cells or in small clusters. The nuclei have a neuroendocrine chromatin pattern (AKA salt and pepper). The individual cells may be round, ovoid, plasmacytoid, or spindle-shaped (Papanicolaou stain)

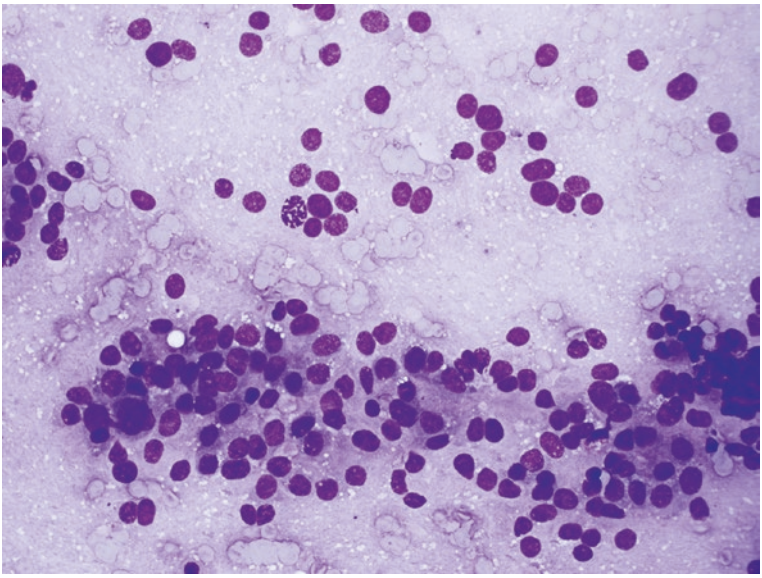

Fig. 8.23 The cells in specimens obtained by FNA from a carcinoid tumor often have scant cytoplasm. They may form sheets, acini, or trabeculae (Diff-Quik® stain)

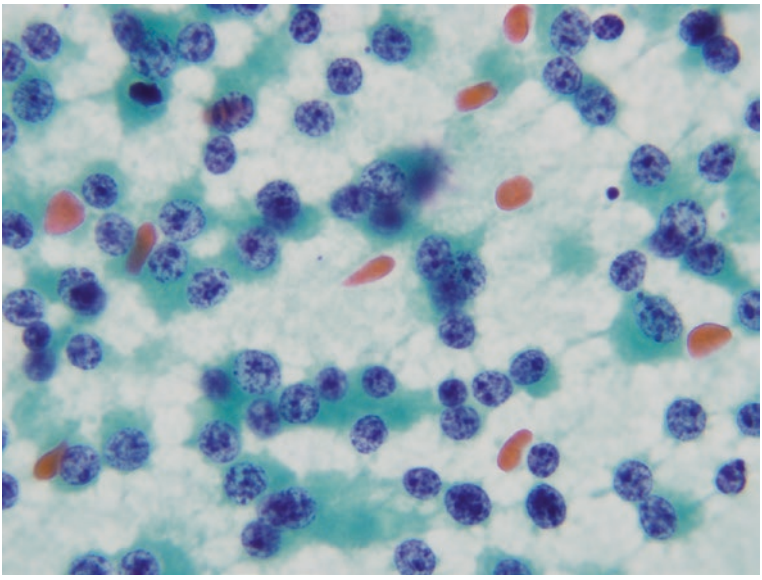

Fig. 8.24 While many cells obtained from carcinoid tumors will have an oval shape with scant cytoplasm, others will have a plasmacytoid appearance (Papanicolaou stain)

- Cytoplasm: Scant to moderate in volume, basophilic, may be distinctly granular; molding generally absent.
- Single cells may have a plasmacytoid appearance with round eccentric nuclei in moderate amounts of basophilic cytoplasm.
- Binucleation may occur.
- Distinction between typical and atypical carcinoid tumors is usually not possible by cytologic evaluation.
- Synaptophysin positive, chromogranin variably positive, and cytokeratin positive.

Explanatory Notes

- While carcinoid tumors have traditionally been considered benign or neoplasms of low malignant potential, the current WHO categorization considers them to be malignancies and are so categorized in the PSC Scheme.
- Both typical and atypical carcinoid tumors are generally recognizable as neuroendocrine neoplasms but are not separable by cytologic evaluation alone. Mitotic counts and Ki-67 index are helpful in histologic grading. Histologically, typical carcinoids are those with less than two mitotic figures per 2 mm^2 and lacking necrosis. Atypical carcinoids have 2–10 mitotic figures per mm2 and may have necrosis.

Table 8.2 Cytomorphologic features of well-differentiated neuroendocrine carcinoma aka carcinoids and small cell carcinoma

Cytomorphologic feature	Carcinoid	Small cell carcinoma
Background	Clean to granular	Necrotic cell debris, extruded nuclear material giving rise to "tangles" and mitotic figures
Cell pattern	Single cells, strips, and irregular clusters	Many singly dispersed tumor cells and loosely cohesive aggregates
Cell shape	Monotonous population of tumor cells are round, oval, or spindled	Variable cell size with very high N/C ratios
Nuclear shape	Round to oval with smooth thin membranes. Bare nuclei may be frequent. Binucleation may be present	Oval to spindle-shaped, often distorted in smear preparations
Nucleoli	Often indistinct when present may be eccentrically located giving rise to plasmacytoid appearance	Indistinct (especially in exfoliative samples), coarse basophilic chromatin may mimic nucleoli
Nuclear chromatin	Distinctly granular chromatin	Darkly stained that varies from distinctly granular (salt and pepper) to smudged
Cell grouping	Single cells and aggregates which include palisaded sheets, acini, trabeculae (especially anastomosing), branching clusters, and vascularized tissue fragments Fragments of capillaries may be present, rarely traversing between cell groups	Within cells aggregates, molding of adjacent nuclei and rosette-like structures
Cytoplasm	Scant to moderate in volume, basophilic, may be distinctly granular	Cytoplasmic blue bodies (commonly seen in the Diff-Quik®-stained smears), reminiscent of extruded DNA, apoptosis

- By immunohistochemistry the typical carcinoid/well-differentiated neuroendocrine carcinoma can show focal to no immunostaining with TTF-1 as compared to atypical carcinoid and small cell carcinoma which are strongly positive for TTF-1. In crushed specimens, carcinoid tumors may be difficult to separate from small cell carcinomas and a lymphoid neoplasm. Immunohistochemical staining of cell block material may be helpful as small cell carcinomas have a Ki-67 labeling index of greater than 50%, while carcinoid tumors have a labeling index of less than 10–20%. Rare cytologic specimens will display a carcinoid morphology but have a high mitotic count. Such specimens should be classified as large cell neuroendocrine carcinomas.

Cytomorphologic Features of Small Cell Carcinoma

Cytomorphologic Features of Small Cell Carcinoma [31–34] (Figs. 8.25, 8.26, 8.27, and 8.28) (Table 8.2)

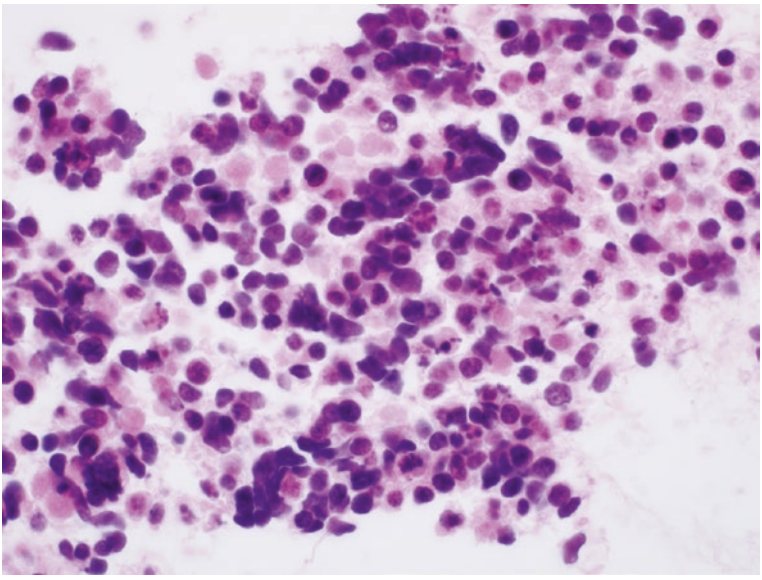

Fig. 8.25 Smears of aspirates obtained from small cell carcinomas are usually cellular and contain many singly dispersed tumor cells. Necrosis is often prominent (Diff-Quik® stain)

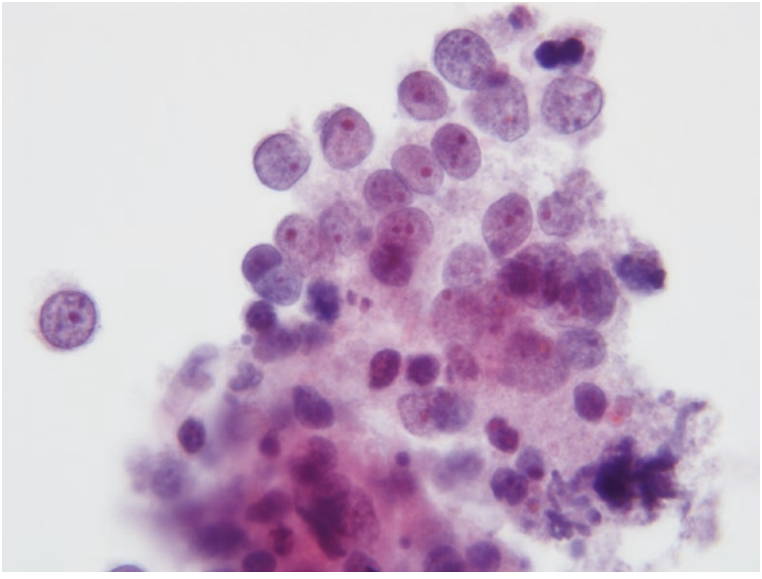

Fig. 8.26 Small cell carcinomas form loosely cohesive aggregates composed of small cells with scant cytoplasm and nuclei with a dark chromatin. Necrotic debris is frequently present (Papanicolaou stain)

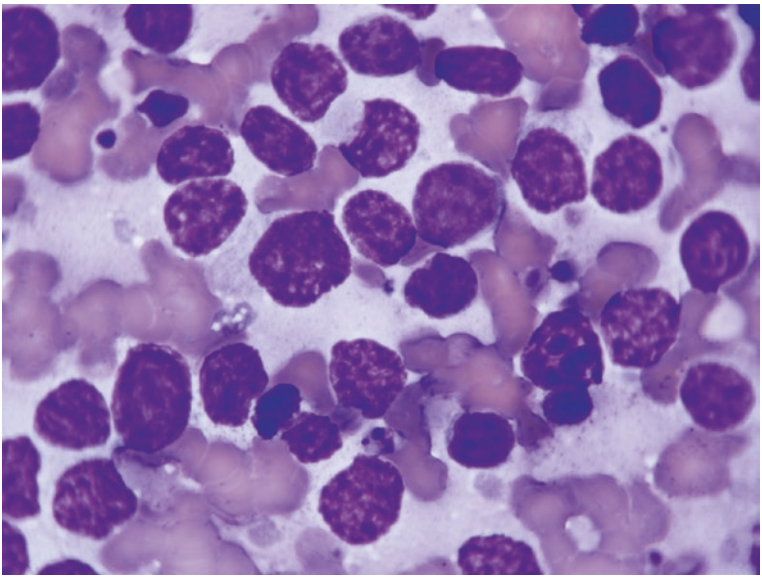

Fig. 8.27 The nuclei of small cell carcinomas contain a hyperchromatic chromatin. Nuclear molding is often seen (Diff-Quik® stain)

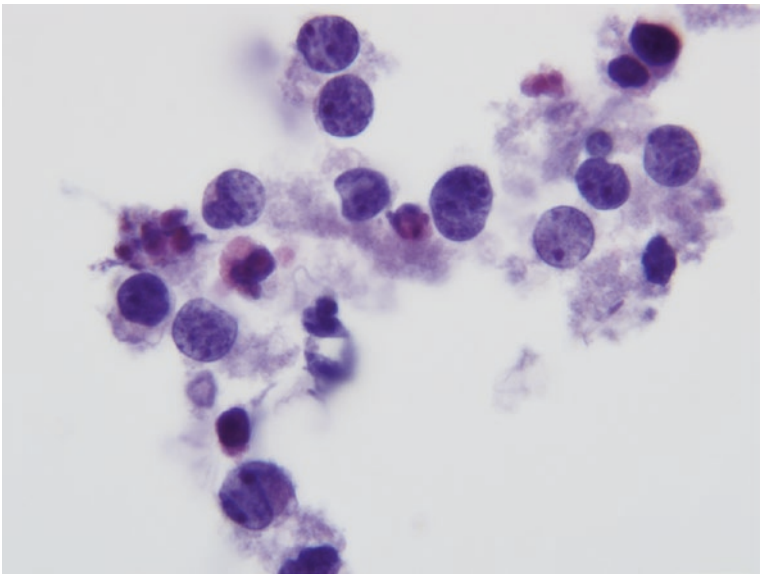

Fig. 8.28 Small cell carcinomas usually display a salt and pepper chromatin (Papanicolaou stain)

- Background: necrotic cell debris, extruded nuclear material giving rise to "tangles" and mitotic figures.
- Cell pattern: cellular samples with many singly dispersed tumor cells and loosely cohesive aggregates.
- Within cell aggregates, molding of adjacent nuclei and rosette-like structures
- Homogeneous small cell size with very high N/C ratios.
- Solitary nuclei with darkly stained chromatin that varies from distinctly granular (salt and pepper) to smudged.
- Indistinct nucleoli (especially in exfoliative samples); coarse basophilic chromatin may mimic nucleoli.
- Cytoplasmic blue bodies with the Diff-Quik® stain (reminiscent of extruded DNA, apoptosis).
- Synaptophysin and TTF-1 often positive, chromogranin rarely positive, and cytokeratin often positive.

Cytomorphologic Features of Large Cell Neuroendocrine Carcinoma

Cytomorphologic Features of Large Cell Neuroendocrine Carcinoma [35–37]

- Background: Clean or tumor diathesis.
- Cell pattern: Hypercellular specimens with numerous single cells with scattered cell clusters and rare large tissue fragments.
- Large cells with abundant cytoplasm.
- Generally low nuclear/cytoplasmic ratio.
- Many naked nuclei.
- Nuclear molding often prominent.
- Fine chromatin with distinct nucleoli.
- Nuclear crush artifact may be present.
- Rosette structures may be present.
- Synaptophysin and TTF-1 positive, chromogranin variably positive, and cytokeratin positive.

Cytomorphologic Features of Primary Pulmonary Lymphoma

Cytomorphologic Features of Primary Pulmonary Lymphoma (Figs. 8.29 and 8.30)

- Background: Granular with lymphoglandular bodies.
- Cell pattern: High cellularity smears with many singly scattered cells (large cell lymphomas can show tissue fragments and mimic small cell carcinoma).
- Monomorphous appearance of cell population.

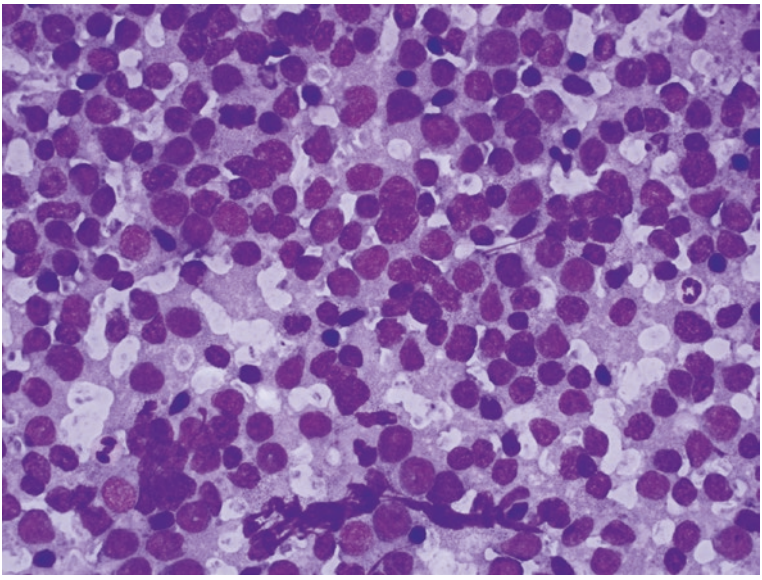

Fig. 8.29 Large cell lymphomas arising in the lung are characterized by a monomorphous population of non-cohesive cells with generally scanty cytoplasm and large hyperchromatic nuclei (Diff-Quik® stain)

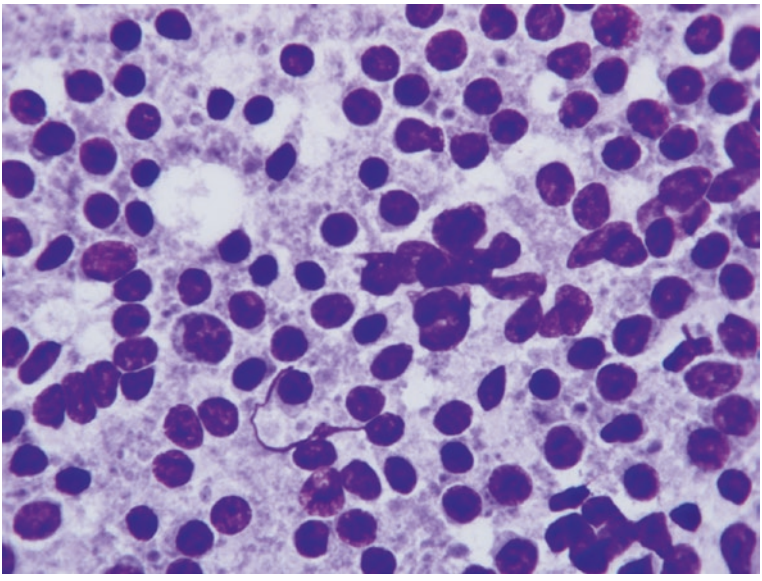

Fig. 8.30 Malignant lymphoma composed of a monomorphous population of atypical lymphoid cells. The background contains a large number of lymphoglandular bodies characteristic of proliferating lymphocytes (Diff-Quik® stain)

- Cells have modest amounts of cytoplasm.
- Monoclonality and specific typing by flow cytometry.

Sample Reports

Example of Cytologic Interpretations for Positive (Malignant) Category

Example 1

Adequacy: Satisfactory for evaluation
Diagnostic category: Malignant
Squamous cell carcinoma

Note: To list immunocytochemical findings and their correlation with morphologic interpretation. This note may include a comment regarding the % of viable tumor cells either in the rinse (if applicable) or cell block for molecular studies.

Example 2

Adequacy: Satisfactory for evaluation
Diagnostic category: Malignant
Lymphoma (please see attached flow cytometry report for subclassification)

Example 3

Adequacy: Satisfactory for evaluation
Diagnostic category: Malignant
Carcinoma present, compatible with metastatic renal cell carcinoma. see note.

Note: The immunoprofile is compatible with the cytologic interpretation.

Example 4

Adequacy: Satisfactory for evaluation
Diagnostic category: Malignant
Small cell carcinoma: See note.

Note: Malignant cells are immunoreactive for cytokeratin, TTF-1 synaptophysin, and occasionally chromogranin. The proliferative index by Ki-67 immunostaining is 60%.

Example 5

Adequacy: Satisfactory for evaluation
Diagnostic category: Malignant
Well-differentiated neuroendocrine neoplasm (carcinoid): see note.

Note: Neoplastic cells are immunoreactive for cytokeratin, synaptophysin, and chromogranin and are negative or focally positive for TTF-1. The proliferative index by Ki-67 immunostaining is 1%.

Example 6

Adequacy: Satisfactory for evaluation
Diagnostic category: Malignant, adenocarcinoma; see note.

Note: Specimen (if possible specify rinse or cell-block) is submitted for molecular testing including ALK, EGFR, and ROS-1.

Explanatory Notes

- Correlation of cytologic, imaging, and clinical findings preferably performed in a multidisciplinary conference is strongly advised to formulate patient follow-up and management decisions.
- While the malignancy risk/specificity of a malignnat cytologic diagnosis is high (90%), it is less than that published for the thyroid and pancreaticobiliary system classifications [13]. Given this degree of specificity, correlation with clinical and imaging findings is mandatory before radical surgery.
- While the cytomorphologic distinction of non-small cell from small cell carcinomas is highly accurate by morphology alone, the available data indicates that ancillary testing (immunohistochemistry) is to be strongly encouraged for the separation of non-small cell carcinomas into squamous carcinomas and adenocarcinomas [9–11]. It is encouraged that immunohistochemistry markers should also be validated in cytologic preparations.
- Further subtyping of adenocarcinomas and grading of adenocarcinomas while generally accurate appears to be imperfect [8].
- Definitive subclassification of adenocarcinomas probably requires resection specimens to carefully evaluate size of lepidic pattern carcinomas and to assess the size of their invasive component before final classification. While immunohistochemistry may be necessary for separation of squamous and adenocarcinomas, it must be born in mind that adequate material must be preserved for molecular analysis of mutation status in a variety of molecular markers including EGFR, ALK, and ROS-1.
- Newer markers are being developed which are important for selection of targeted therapies including PDL-1 and CMET. Careful triage of material is important to ensure adequate material for these molecular tests.
- ROSE is helpful in selecting appropriate transport media for ancillary testing including culture of infectious agents, flow cytometry, and molecular testing. Because metastatic lesions represent a high percentage of pulmonary nodules, review of patient history is important for appropriate interpretation of specimens. Ancillary testing, especially immunohistochemistry, also plays a role in defining site of origin for metastatic lesions.

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