Respiratory System

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The respiratory tract consists of the sinonasal region, larynx, trachea, and the lungs, which contain mainstem bronchi and their progressively smaller divisions of bronchi, bronchioles, and respiratory acini. The airways largely consist of cartilaginous rings supported by fibrous and smooth muscle mesenchymal elements to allow a passageway of air to the lungs. The lungs are paired organs of the respiratory system that function to extract environmental oxygen into the bloodstream via gas exchange. On gross examination, the right lung is divided into three lobes (upper, middle, and lower) while the left lung is divided into two lobes (upper and lower). The left lung also contains a rudimentary appendage, the lingula, which is derived from the left upper lobe and is considered an analogous structure to the right middle lobe. The lobes are further divided into nine and ten bronchopulmonary segments within the left and right lung, respectively. Each segment is partitioned such that fibrous septa derived from the pleura encapsulate each segment, prohibiting free flow of air as well as pathologic processes between bronchopulmonary segments. Lobules are the smallest unit of the lung that can be appreciated grossly and are bound by interlobular septa. A lobule is composed of various numbers of acini, which are the basic units for air exchange. An acinus is composed of respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli.

Histologically, the bronchus is organized into respiratory epithelium, under which lies a thin layer of smooth muscle, circumferential cartilage, and submucosal seromucous glands (Fig. 2.1a). The respiratory epithelium consists predominantly of pseudostratified tall columnar cells with cilia admixed with goblet cells, basal cells, neuroendocrine cells, and Clara cells. Neuroendocrine cells and Clara cells are inconspicuous on hematoxylin & eosin (H & E) stains (Fig. 2.1b). As the airways subdivide into smaller subunits, some morphologic changes can be identified. The membranous bronchiole lacks cartilaginous rings and shows fewer goblet cells with a proportionally increased number of Clara cells (Fig. 2.1c, d). The alveoli are lined mainly by type I pneumocytes, which are inconspicuous in routine H & E-stained sections but cover 90% of the alveolar surface, and type II pneumocytes (Fig. 2.1e). Although the type II pneumocytes are a small subset of the alveolar lining cells, they are the precursor cells for type I pneumocytes and are the main proliferating cells following alveolar injury (Fig. 2.1f). Within the alveolar spaces, alveolar macrophages are frequently seen and are often pigmented in smokers (Fig. 2.1g). The pigment seen in a smoker's macrophages is red-brown in color and is believed to be a ceroid-like granular autofluorescent pigment in the lysosomes [1]. Incidental findings that can be seen in the lung not too uncommonly include corpora amylacea (Fig. 2.1h), interstitial megakaryocytes (Fig. 2.1i), cytoplasmic Mallory-like hyaline bodies (Fig. 2.1j), carcinoid tumorlets (Fig. 2.1k), and meningothelial-like nodules (Fig. 2.1l). If sampled, these structures may be infrequently encountered in cytology samples.

Evaluation of different types of cytology specimens has played an important role in the diagnosis and staging of lung lesions. Routinely collected cytology specimens include exfoliative samples (sputum, bronchial brushing, bronchial washing, and bronchoalveolar lavage) and fine needle aspiration (FNA) samples obtained via transbronchial (Wang needle), endobronchial ultrasound (EBUS)-guided, endoscopic ultrasound (EUS)-guided transesophageal, or computed tomography (CT)-guided percutaneous approaches. The specimens are often processed using one or multiple preparations, including Diff-Quik- and/or Papanicolaou-stained conventional smears, liquid-based preparations (LBP) such as ThinPrep or SurePath, and cytospins, as well as H & E-stained cell block material. The Papanicolaou Society of Cytopathology previously developed a set of guidelines for pulmonary cytology, including indications and technical recommendations for obtaining the aforementioned lung cytology samples, terminology and classification scheme,

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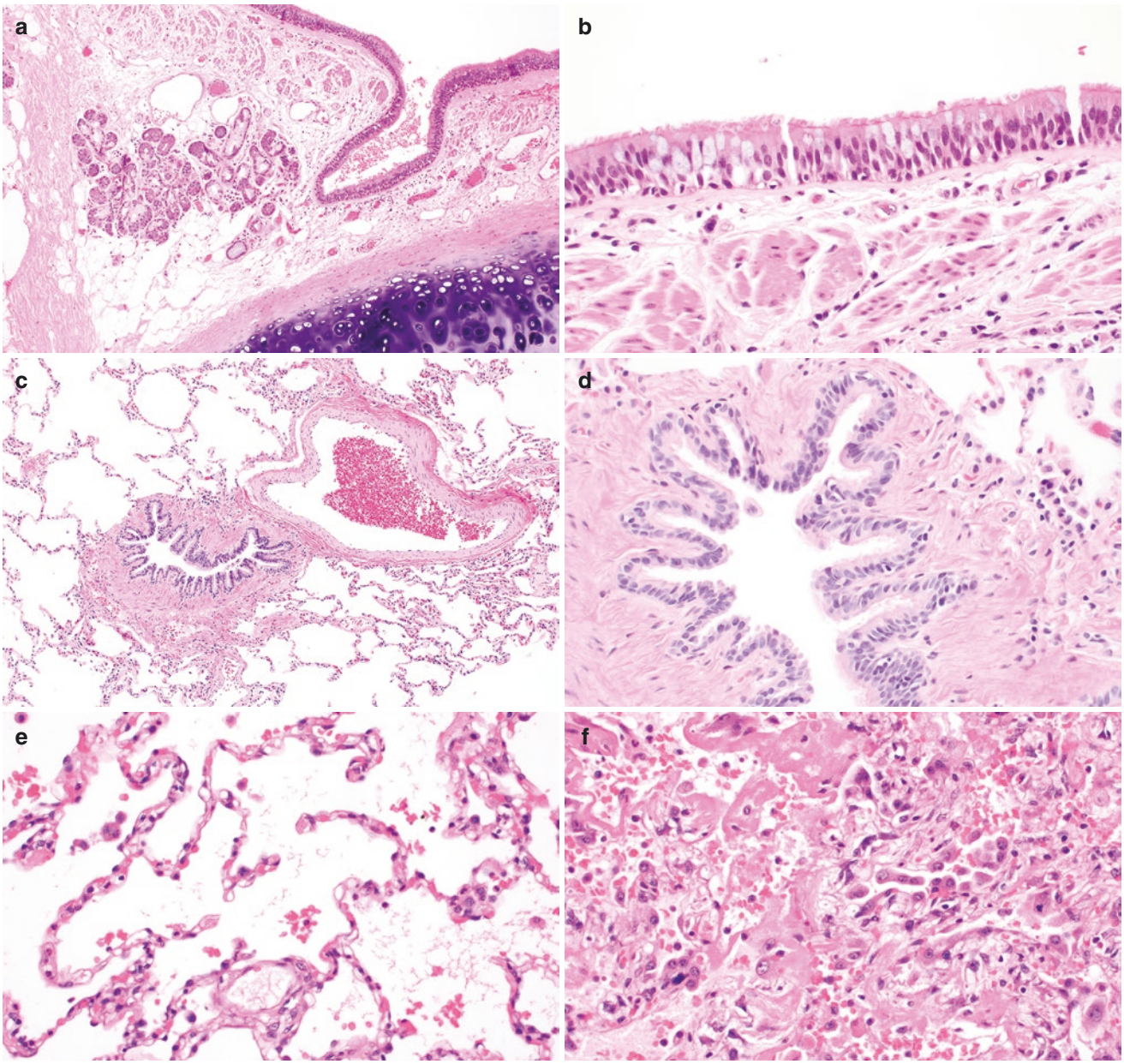

Fig. 2.1 (**a**) A bronchus is shown and composed of respiratory epithelium, an underlying thin layer of smooth muscle, submucosal seromucous glands, and hyaline cartilage. (**b**) The respiratory epithelium lining the bronchus is composed of pseudostratified tall columnar cells including ciliated cells, goblet cells, and basal cells. (**c**) In contrast, the membranous bronchiole does not have a cartilaginous ring and (**d**) is lined by pseudostratified columnar cells with prominent apical cilia, but without obvious goblet cells. (**e**) Type II pneumocytes with a hobnail-like appearance are shown lining the alveoli. (**f**) Type II pneumocytes proliferate to replace damaged cells following acute lung injury (such as acute diffuse alveolar damage) and can show marked cytologic atypia. (**g**) Respiratory bronchiolitis characterized by lightly pigmented alveolar macrophages clustered within the lumina of distal airways and peribronchiolar air spaces is a common finding in smokers. (**h**) Corpora amylacea are shown as amphophilic rounded structures with concentric lamellae within alveolar spaces. (**i**) A megakaryocyte (black arrow) is shown, characterized by a dark staining (on hematoxylin & eosin), irregularly shaped, and elongated nucleus. They should not be misinterpreted as malignant cells or cells with viral cytopathic changes. (**j**) Reactive and proliferating type II pneumocytes in the context of organizing diffuse alveolar damage show cytoplasmic accumulation of brightly eosinophilic amorphous material resembling Mallory's hyaline seen in liver diseases. (**k**) A carcinoid tumorlet focus (<5 mm) of neuroendocrine cells is shown associated with patchy fibrosis. These are often found in close proximity to bronchioles. The cells are cytologically bland with characteristically finely dispersed chromatin, inconspicuous nucleoli, and granular cytoplasm. (**l**) A meningothelial-like nodule is shown composed of a population of cytologically bland oval cells arranged in a nested pattern and distributed randomly within the interstitium. Lung resection, hematoxylin & eosin, 10× (**a**, **c**, **k**), 20× (**l**), and 40× (**b**, **d**–**j**) magnification

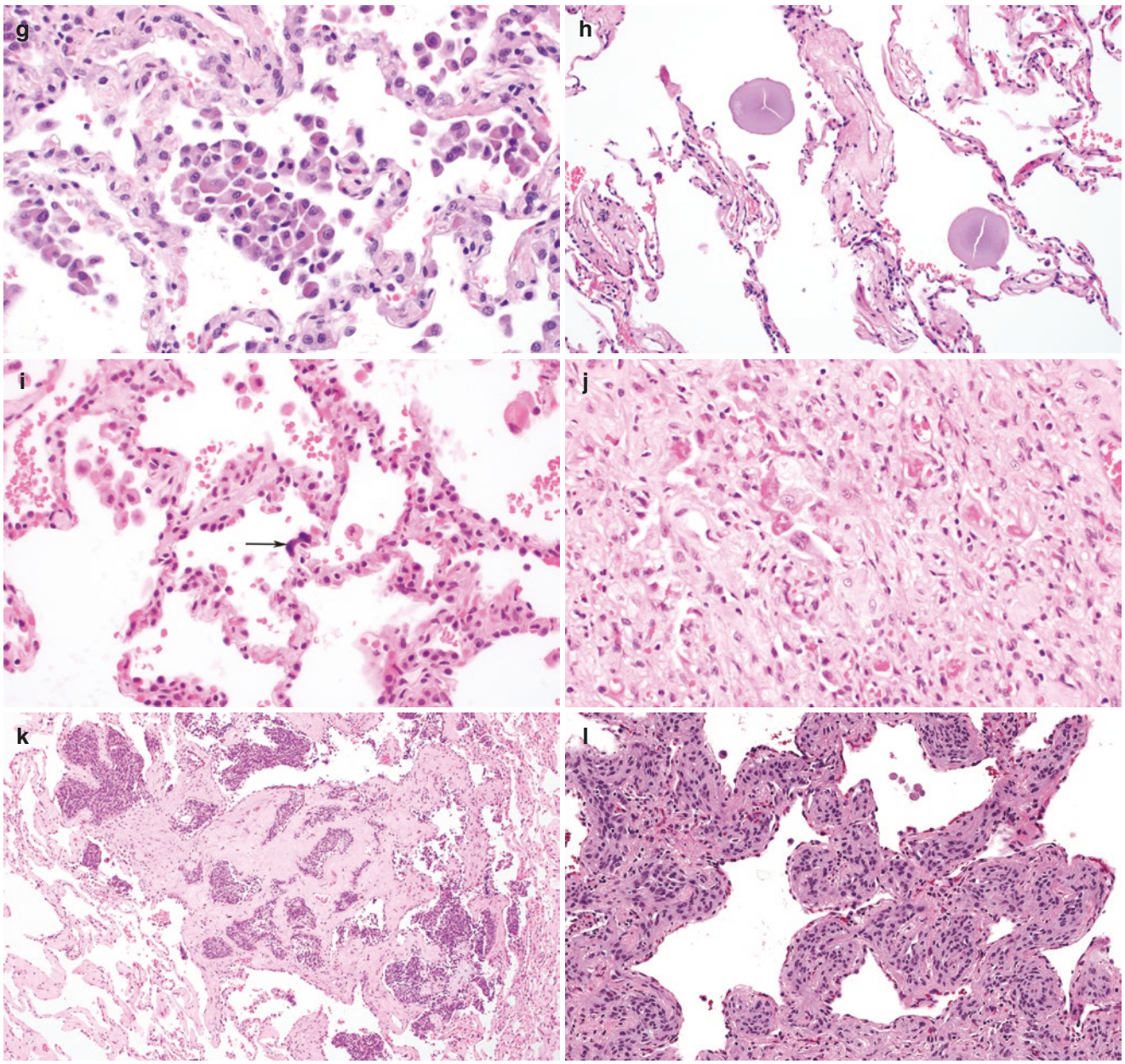

Fig. 2.1 (continued)

recommendations for ancillary testing, as well as recommendations for post-cytologic diagnosis management and follow-up [2–4]. More recently, the WHO System for Reporting Lung Cytopathology has been developed to guide standardized diagnostic categorization while leveraging the use of available ancillary studies.

The various cell types of the respiratory tract can be readily identified on cytologic specimens. Supporting mesenchymal elements (smooth muscle, cartilage, fibrous tissue) and resident pulmonary macrophages can be identified as well. While neuroendocrine or Kulchitsky or K cells may also be present on cytologic specimens, their definitive identification often requires the use of special stains, immunohistochemistry, or electron microscopy. While less common elements may be clearly delineated in histologic examination of biopsies and resection specimens, it is typically uncommon and/or difficult to recognize singly dispersed endocrine cells, type I pneumocytes, myocytes, and fibroblasts in cytologic specimens [5]. The most frequently encountered normal cellular elements in cytology preparations include squamous cells, ciliated columnar cells, goblet cells, basal or reserve cells, type II pneumocytes, and macrophages [6].

Squamous cells line the upper airway from the sinonasal region to the larynx and can be identified as normal components in sputum, bronchial brushing, bronchial washing, and bronchoalveolar lavage specimens. Squamous metaplasia can be seen as a result of reactive changes associated with various etiologies of airway irritation and injury, such as

cigarette smoking, air pollution, infection, or chronic airway disease. Metaplastic squamous cells have dense cytoplasm and bland, round nuclei with smooth nuclear contours, even chromatin distribution, and variably distinct nucleoli [7]. However, some immature metaplastic cells can display cytologic atypia in the form of enlarged, hyperchromatic nuclei. As such, it is important to consider squamous metaplasia as an etiology of atypical squamous cells to avoid a false positive diagnosis of a squamous cell carcinoma.

In cytology preparations, ciliated columnar cells of the trachea and bronchial epithelium are frequently seen in well-spaced monolayer sheets, cohesive clusters with variable nuclear overlapping, or as singly dispersed cells. Like their histologic appearance, they display round or ovoid nuclei with smooth nuclear contours, fine chromatin distribution, and variably distinct nucleoli. When longitudinally oriented, the basal localization of the nucleus is more evident. Most characteristically, the luminal surfaces of bronchial ciliated columnar cells have numerous cilia attached to thick terminal bars (Fig. 2.2a–c). Of note, these cells can display significant morphologic changes in reactive conditions, including nuclear enlargement, multinucleation, coarse chromatin distribution, and large nucleoli.

Caution should be taken to avoid misinterpretation of a well-differentiated neuroendocrine tumor as benign columnar bronchial cells. The spindled- and plasmacytoid-appearing neuroendocrine tumor cells may mimic bronchial columnar cells under low power magnification. However, the tumor cells are often arranged as single cells and lack cilia and terminal bars. The centrally or eccentrically located nuclei of tumor cells have "salt and pepper" chromatin and small but distinct nucleoli (Fig. 2.3a–c).

Tightly clustered benign bronchial cells can also be misinterpreted as well-differentiated adenocarcinoma, particularly in the case of Creola bodies. Creola bodies, first described by Naylor, are well-defined, compact clusters of ciliated columnar cells with peripheral palisading. They are frequently seen in the clinical setting of asthma and are the result of excessive shedding of epithelial cells of the lower respiratory tract [8]. Creola bodies may contain over 100

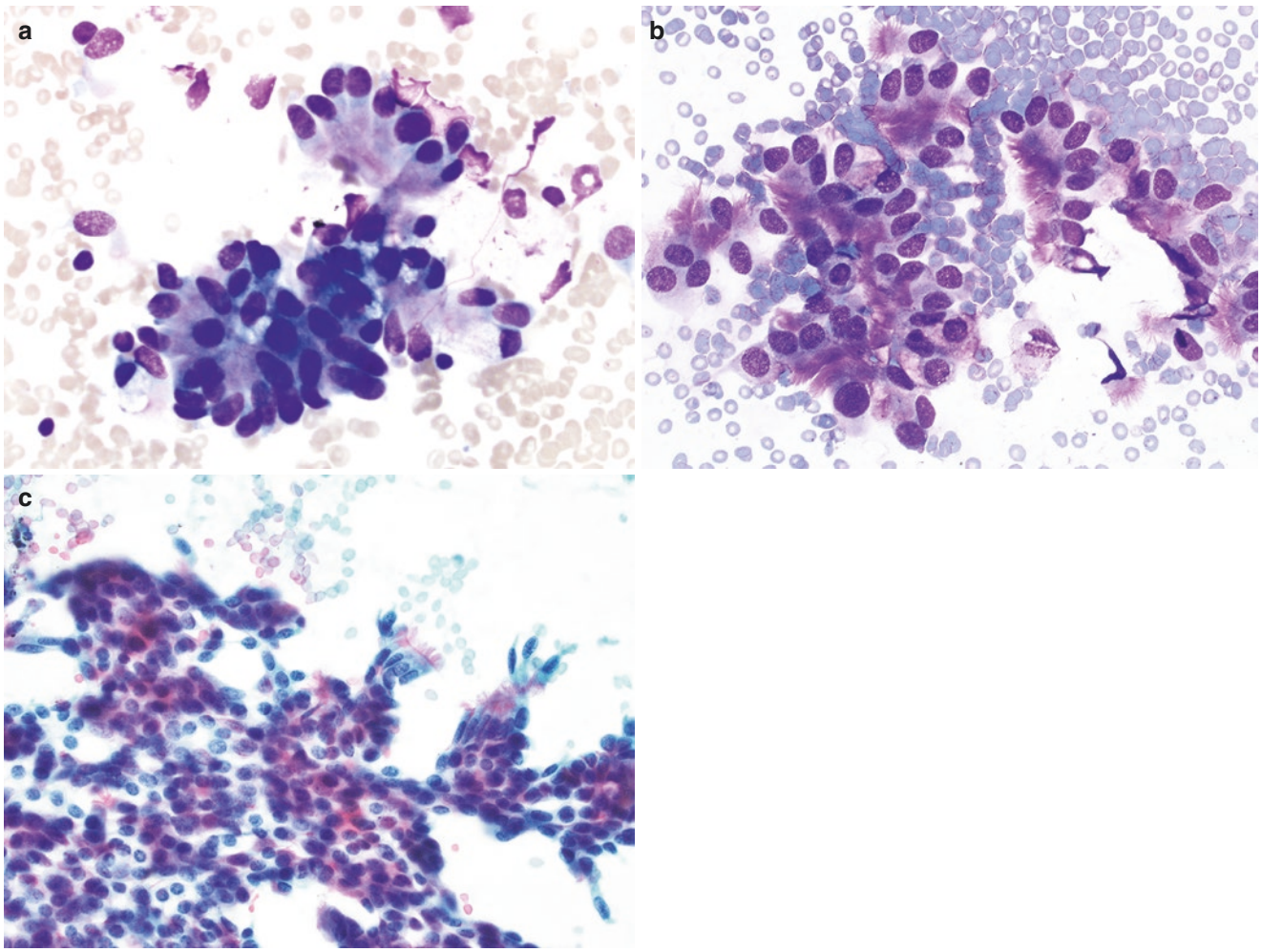

Fig. 2.2 Various bronchial epithelial cells. (**a**) A cohesive cluster of columnar bronchial lining cells with mild nuclear overlapping is shown. The cells have round or oval nuclei, smooth nuclear membranes, fine chromatin distribution, and distinct nucleoli. (**b**, **c**) Cilia attached to thick terminal bars are evident. Lung, air-dried smear preparation, Diff-Quik, 40× magnification (**a**, **b**). Lung, alcohol-fixed smear preparation, Papanicolaou stain, 40× magnification (**c**)

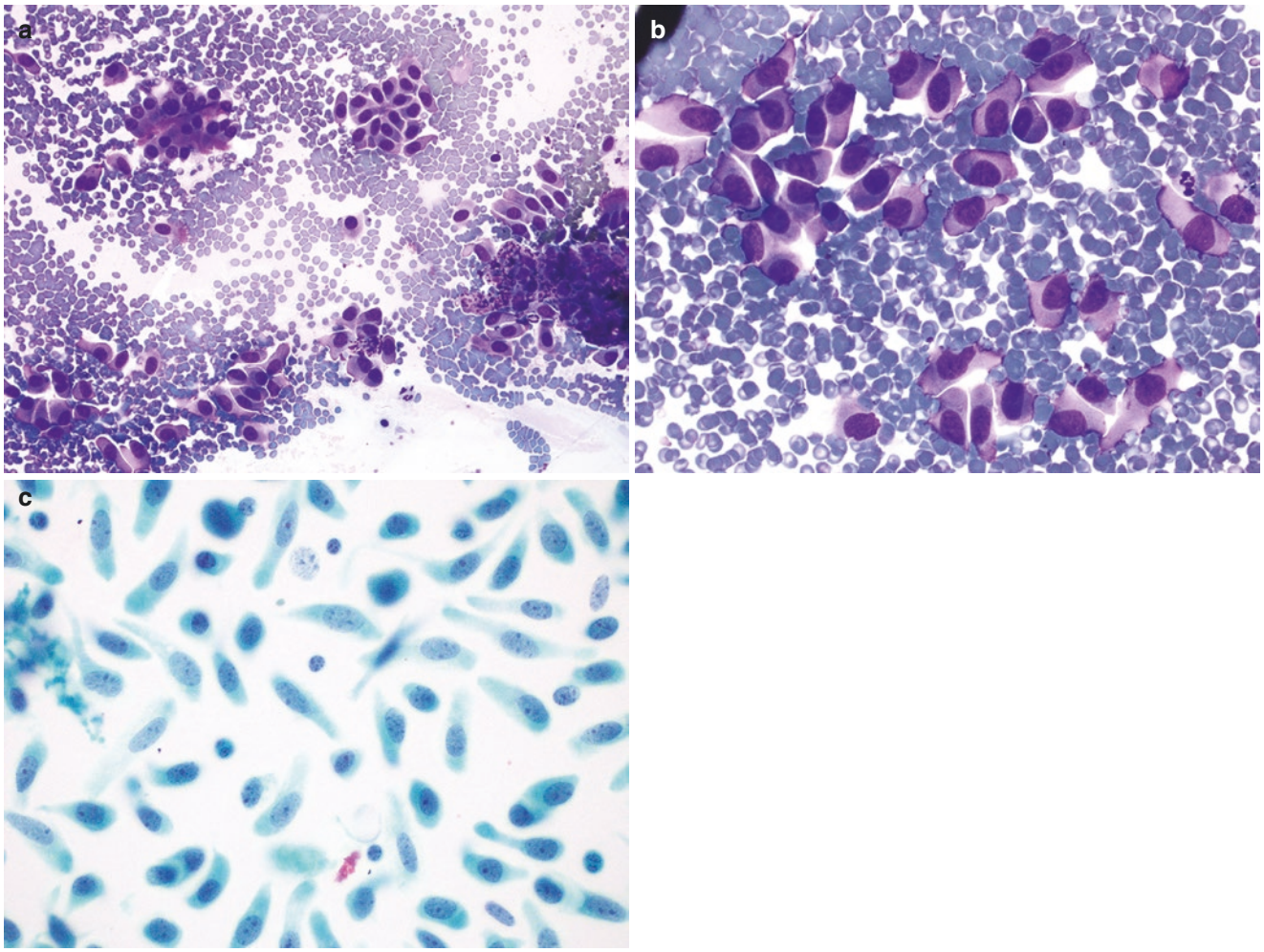

Fig. 2.3 (**a**) Unlike benign bronchial cells (left upper), spindled or plasmacytoid well-differentiated neuroendocrine tumor cells do not have cilia and terminal bars. (**b**, **c**) Neuroendocrine tumor cells are often arranged as single cells or in loosely cohesive clusters. The nuclei are centrally or eccentrically located. "Salt and pepper" chromatin distribution and distinct nucleoli are frequently appreciated. Lung, air-dried smear preparation, Diff-Quik, 20× (**a**) and 40× (**b**) magnification. Lung, alcohol-fixed smear preparation, Papanicolaou stain, 60× magnification (**c**)

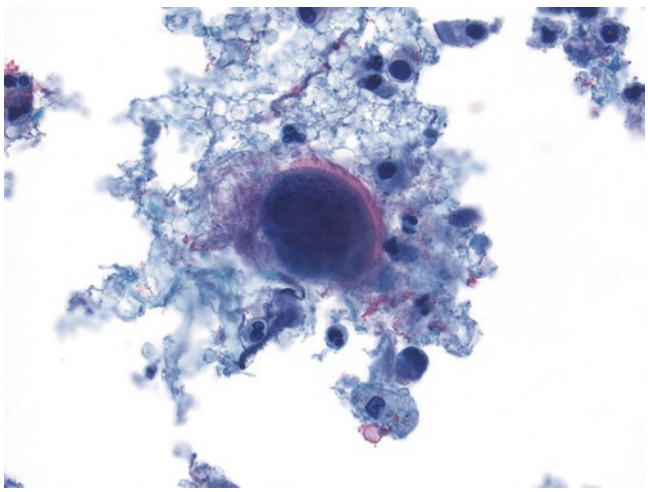

Fig. 2.4 Creola body presenting as a well-defined, compact cluster of uniform, ciliated columnar cells with peripheral palisading. ThinPrep preparation, 60× magnification

columnar cells, are round in shape and may display a papillary architecture. The cytoplasm of the columnar cells can also be vacuolated. However, the presence of cilia (which may be more difficult to identify in tight cell clusters), relatively uniform nuclear size and shape, and peripheral palisading of the papillary groups are helpful features to differentiate Creola bodies from adenocarcinoma (Fig. 2.4).

Another commonly encountered respiratory epithelial component in cytology specimens is the mucin-secreting goblet cell. Goblet cells are interspersed with ciliated columnar cells in a ratio of approximately 1 goblet cell:5–6 columnar cells. Goblet cells contain a single large or multiple smaller cytoplasmic mucin vacuoles, which push nuclei to the periphery. The nuclei have smooth nuclear contours and fine chromatin distribution. Clusters of goblet cells may indicate goblet cell metaplasia and/or hyperplasia associated with reactive processes [7]. Caution should be taken to avoid misinterpretation of goblet cell metaplasia/hyperplasia as a mucin-producing adenocarcinoma (Fig. 2.5a, b). While this

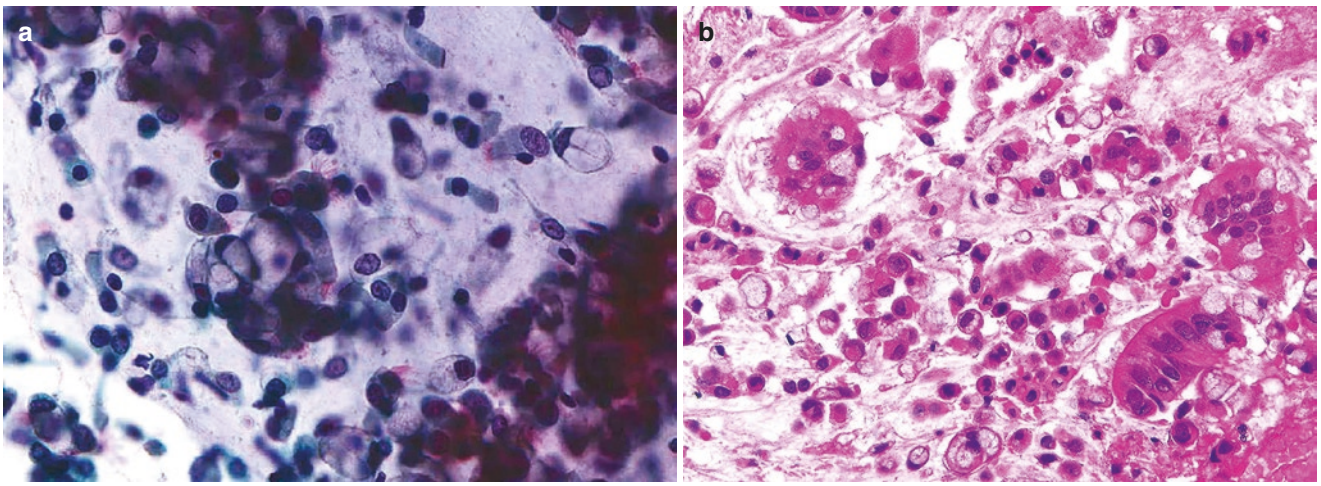

Fig. 2.5 (**a**) Goblet cells with cytoplasmic mucin vacuoles and peripheral nuclei are arranged singly and in clusters. Their nuclei have smooth nuclear membranes and fine chromatin distribution. (**b**) Cell block preparation of a bronchoalveolar lavage shows goblet cells admixed with ciliated bronchial cells. Lung, alcohol-fixed smear preparation, Papanicolaou stain, 40× magnification (**a**). Bronchoalveolar lavage, cell block, hematoxylin & eosin, 40× magnification (**b**)

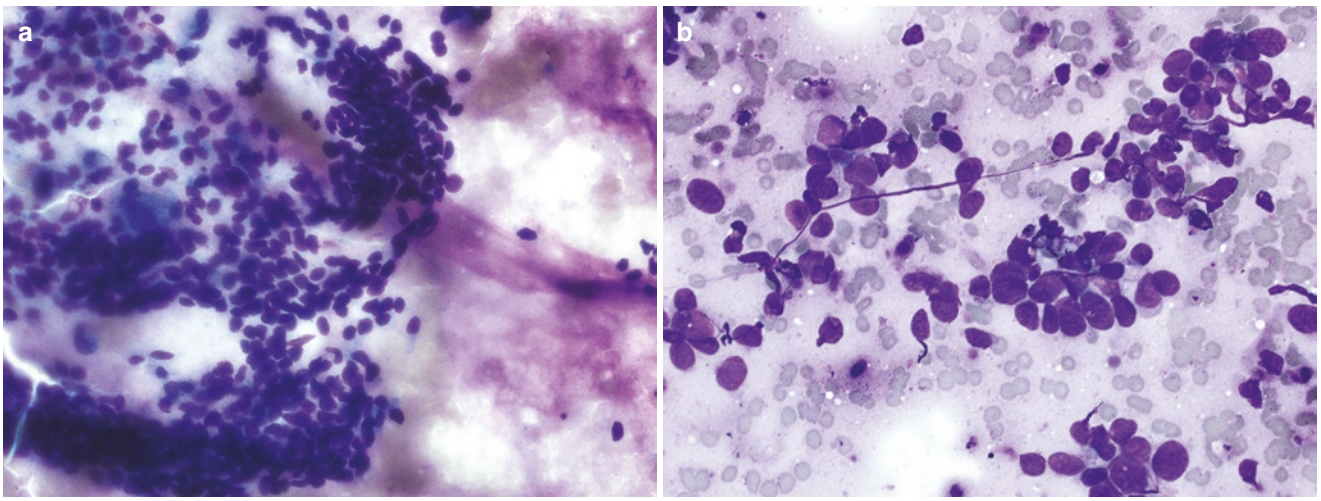

Fig. 2.6 (**a**) Reserve cells are shown as small oval or cuboidal cells with high nuclear:cytoplasmic ratios arranged in clusters that may demonstrate nuclear overlapping and molding. Their nuclei are monotonous. The background contains mucin but there is no associated necrosis. (**b**) Small cell carcinoma cells show cellular disruption, nuclear molding, nuclear enlargement with high nuclear:cytoplasmic ratios, irregular nuclear membranes, and "salt and pepper" chromatin distribution. Note the admixed apoptotic cells in the sample. Lung, air-dried smear preparation, Diff-Quik, 40× magnification (**a**, **b**)

differential may be difficult to distinguish on morphology alone, goblet cell metaplasia will often have a lower quantity of mucin-producing cells in a given sample. Additionally, clinical correlation is key in the identification of long-standing airway disease that could give rise to a reactive process such as goblet cell metaplasia.

Similar to squamous cell metaplasia, reserve or basal cell hyperplasia is also seen as a response to a local insult [7]. In cytology specimens, reserve cells are small columnar or cuboidal cells with high nuclear:cytoplasmic ratios arranged in variably crowded groups which can also contain ciliated columnar cells. They may show mild nuclear molding in which the nuclei compress each other and distort their shapes, potentially raising concern for small cell carcinoma. However, nuclei of reserve cells are monotonous, and the background of reserve cell hyperplasia lacks necrosis and apoptotic debris (Fig. 2.6a). Also in contrast to small cell carcinoma (Fig. 2.6b), mitotic figures are not readily identified.

In cytology specimens, type II pneumocytes are infrequently encountered, but when identified have round nuclei and vacuolated cytoplasm. Their morphology may be hard to distinguish from macrophages when they are singly dispersed. Type II pneumocyte hyperplasia follows injury to the more distal epithelial cells lining the alveolar walls. These are seen as grouped or individual polygonal or rectangular cells, which may show cytoplasmic vacuolization, smooth nuclear membranes, fine chromatin distribution, and distinct nucleoli. No cilia are attached to these cells. The

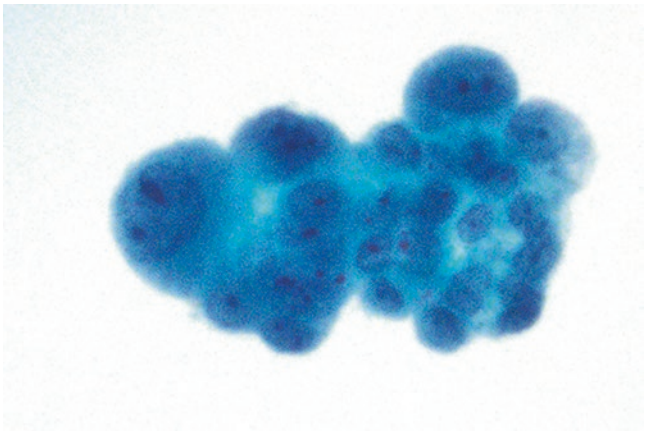

Fig. 2.7 A cluster of type II polygonal pneumocytes with variably sized nuclei, finely vacuolated cytoplasm, smooth nuclear membranes, fine chromatin distribution, and distinct nucleoli. Bronchoalveolar lavage, ThinPrep preparation, 40× magnification

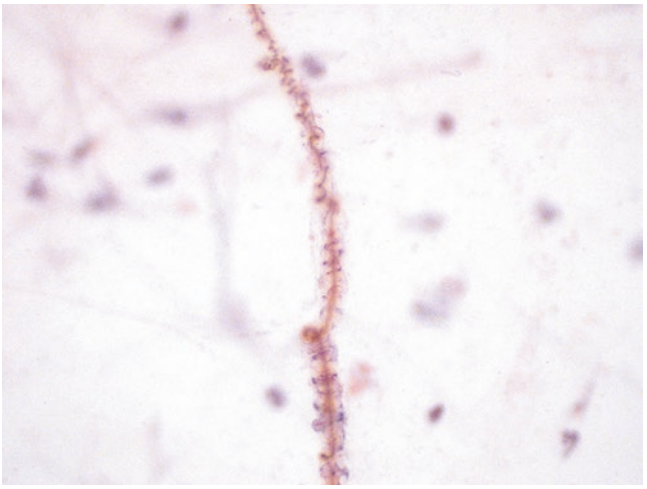

Fig. 2.9 Coiled Curschmann spiral. Lung, alcohol-fixed smear preparation, Papanicolaou stain, 60x magnification

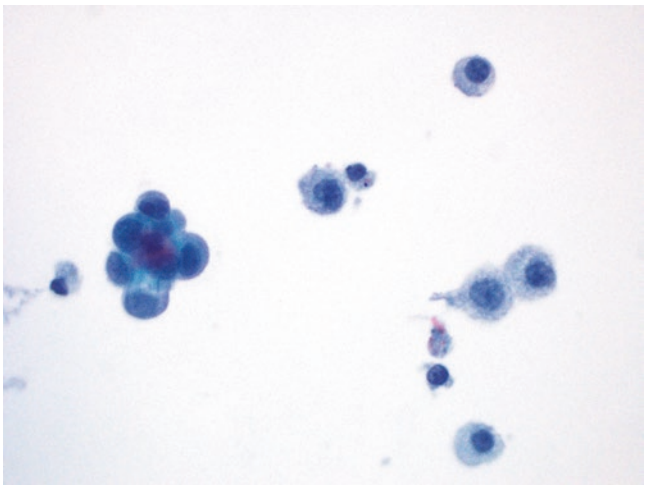

Fig. 2.8 Pulmonary macrophages are arranged as single cells and characterized by abundant pale, vacuolated cytoplasm and small, round nuclei. Bronchoalveolar lavage, ThinPrep preparation, 60× magnification

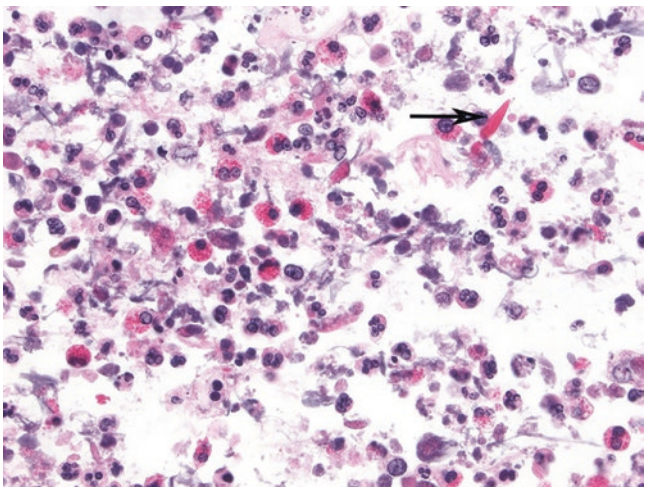

Fig. 2.10 A Charcot-Leyden crystal is present characterized by needle-shaped eosinophilic structure (black arrow). Many eosinophils are seen in the background. Cell block, hematoxylin & eosin, 40× magnification

features of type II pneumocyte hyperplasia may be misinterpreted as adenocarcinoma (Fig. 2.7). As such, in the clinical context of a patient with acute respiratory distress and radiologic findings of diffuse pulmonary infiltrates, one must be cautious to prevent misinterpreting reactive conditions as malignant.

The presence of pulmonary macrophages is required for sputum and bronchoalveolar lavage specimens to be deemed adequate for evaluation. Similar to macrophages seen elsewhere, pulmonary macrophages are characterized by an abundant amount of pale, vacuolated cytoplasm and small round, ovoid, or occasionally indented nuclei (Fig. 2.8). Multinucleation can be seen. Pulmonary macrophages commonly contain cytoplasmic pigment granules which may include finely granular golden brown tobacco pigment, dark and coarse yellow-brown hemosiderin pigment, or black anthracotic pigment from carbon products.

Noncellular components may be encountered in cytology specimens prepared from the respiratory tract, including Curschmann spirals, Charcot-Leyden crystals, asbestos bodies, and corpora amylacea [7, 9]. Curschmann spirals are clumps and casts of mucus from smaller airways, forming coiled structures characterized by densely staining central cores and a lighter staining periphery that can be identified in various chronic respiratory diseases (Fig. 2.9).

Charcot-Leyden crystals are rhomboid or needle-shaped eosinophilic to orangeophilic granule concretions. They may be present in the clinical setting of asthma or any other condition resulting in eosinophilia with degranulation in the respiratory tract (Fig. 2.10).

Asbestos bodies represent asbestos fibers which have been coated by a proteinaceous iron coat derived from alveolar macrophages. Asbestos bodies have a distinguishing thin, transparent fibrous core (Fig. 2.11).

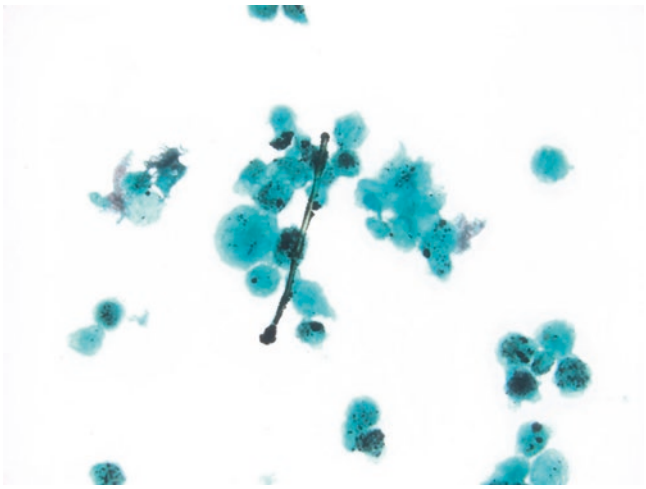

Fig. 2.11 Asbestos body with a thin, transparent fibrous core. Smear preparation, Gomori-methenamine silver (GMS) stain, 40× magnification

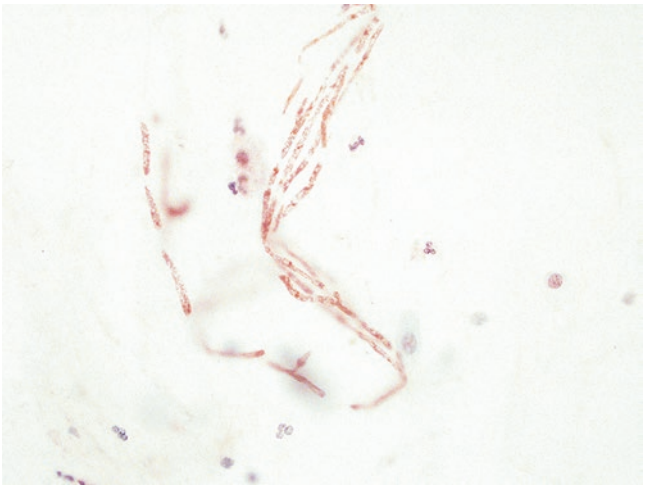

Fig. 2.13 Vegetable matter comprised of degenerated plant cells. Alcohol-fixed smear preparation, Papanicolaou stain, 60× magnification

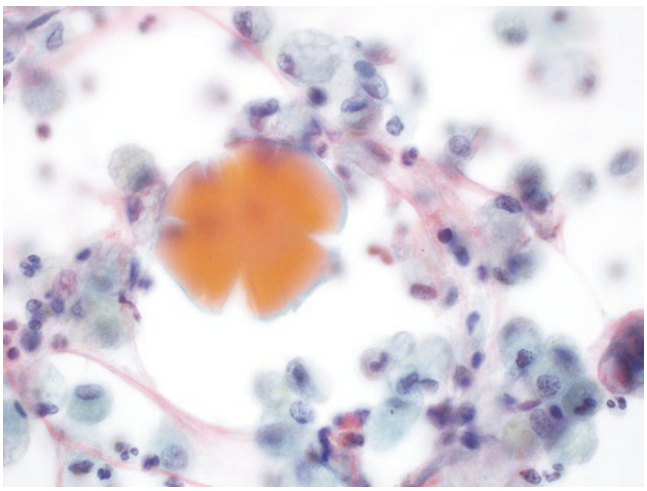

Fig. 2.12 Corpora amylacea, as shown in this image, are noncalcified concentric lamellated structures with a glass-like appearance. Alcohol-fixed smear preparation, Papanicolaou stain, 60× magnification

Corpora amylacea may be seen in bronchoalveolar lavage and sputum cytology specimens as variably sized, noncalcified, round to oval concentrically lamellated structures, sometimes showing a glass-like appearance. The presence of corpora amylacea in sputum smears is related to age, being more frequent in older people, and in nonneoplastic lung diseases such as chronic obstructive pulmonary disease (Fig. 2.12).

In addition, pollen, calcifications, amyloid, and vegetable matter may be rarely seen (Fig. 2.13).

In lung fine needle aspirations, it is important to be cognizant of potential contaminants from other anatomic sites to avoid misinterpretation. In percutaneous approaches to lung lesions, contaminating sheets and clusters of mesothelial cells may be identified and misinterpreted as lesional cells. However, the sheet-like arrangements of mesothelial cells as well as the presence of slit-like spaces between these cells should provide morphologic clues to their mesothelial origin. Similarly, hepatocytes may be seen in specimens collected through a transdiaphragmatic approach or inadvertent sampling when targeting a right lower lobe lung lesion. In addition, transbronchial approaches can result in the sampling of submucosal seromucinous glands and cartilage of the respiratory tract. The morphologic features of seromucinous glands are similar to those of salivary gland acini. While these seromucinous cells often appear bland, it is important to note that, similar to salivary gland acinar cells, they can undergo oncocytic metaplasia that can be misinterpreted as lesional tissue. Stripped individual chondrocytes that get incidentally picked up when placing a needle through bronchial hyaline cartilage may sometimes be mistaken for keratinizing squamous cells.

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