Pathology and Molecular Pathology of Lung Cancer

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20.1 Introduction

The new molecular markers and assays reviewed in this chapter are the first wave of an exciting new phase in lung cancer pathology and treatment. The optimal interpretation and clinical application of these remarkable advances requires proficiency in lung anatomy and histopathology. Hence, we begin with a brief overview of lung structure, followed by a discussion of histological types of lung cancer, and the principles of grading and staging. We then focus on the molecular abnormalities of lung cancer, targeted therapies and the molecular biomarkers that help identify patients likely to benefit from these targeted therapies, the basic molecular biology principles behind these therapies, selected molecular diagnostic techniques, and the pathological features correlated with molecular abnormalities in lung cancer. Lastly, we discuss predictive biomarkers and their corresponding drugs that are currently under investigation in various phases of clinical trials. The investigation and analysis of lung cancer for particular abnormalities expands the expertise of the pulmonary oncologic pathologist, who in addition to conventional pathologic analysis of surgical lung specimens will determine predictive biomarkers for lung cancer-targeted therapies [1–4].

20.2 Embryology and Development of the Lung

Human lung development begins with a budding longitudinal groove from the ventral side of the primitive foregut around 26 days after fertilization. This bud progressively bifurcates and grows on either side of the foregut as the embryonic lungs, with successive branching giving rise to the lobar, segmental, and other divisions of the airways. The developing lungs grow into the coelomic cavity, the mesothelial lining of which forms from the mesoderm early in gestation. After the development of the diaphragm and a pleuropericardial membrane, the lungs become confined to the pleural cavities.

The phases of lung development and the major events are assigned to: (1) an embryonic stage that lasts until 6 weeks' gestation; (2) a pseudoglandular stage in which the primitive air spaces are widely separated by abundant mesenchyme and feature a vacuolated, glycogen-rich columnar or cuboidal epithelium; after division of the conductive airways down to the terminal bronchiole is complete ($\sim$16 weeks) this phase ends; (3) a canalicular stage which includes appearance of air spaces with an attenuated lining epithelium. Although no true alveoli are yet

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present, respiration is possible near the end of the canalicular stage (~28 weeks). The development of true alveoli is first readily apparent at ~36 weeks' gestation, but it is worth noting that alveoli develop mainly, but not completely, in the first year of life. This process, along with remodeling of airways, continues slowly through childhood and possibly into adolescence.

We finish by noting that the relationship of the biology of lung development to tumorigenesis has long sparked interest. For example, the familiar tumor marker TTF-1 (Nkx2.1) has critical functions as a transcription factor in lung budding and development. Especially relevant to the molecular pathology focus of this chapter are recent reviews that explore both specific molecular pathways [5] and potential roles for normal/cancer stem cells [6].

20.3 Anatomy of the Lung

The main bronchi divide to five lobar bronchi, and subsequent divisions supply the 19 lung segments [7, 8]. It is certainly worthwhile to be familiar with their terminology/nomenclature so as to correlate pathology with input from radiologists and surgeons. However, for the pathologist, this is perhaps the perfect situation in which to apply Einstein's advice to "never memorize something that you can look up."

After multiple airway divisions (following asymmetrical dichotomy—two smaller but unequal branches), eventually respiratory bronchioles alveolar ducts and spaces are formed where gas exchange occurs. One instance of somewhat confusing anatomic terminology merits discussion. The lung parenchyma can be formally divided into primary and secondary lobules. Primary lobules in the original sense correspond to one acinus, the unit supplied by one terminal bronchiole. The secondary lobule is a visible portion of lung surrounded by fibrous septa that comprises several primary lobules. However, in practice the septation of secondary lobules varies and the ability to precisely define these structures is limited. The term lobule is commonly used to refer to either true primary or secondary lobules, and must be interpreted in the context of the discussion.

The vasculature of the lungs features both branches of the pulmonary artery that bifurcate along with the airways (the 'broncho-vascular bundle') and pulmonary veins that run in the interlobular septa at the periphery of the lungs but join the artery and airway more proximally near the hilum. Other components include the bronchial artery circulation derived from the aorta that supplies oxygenated blood to the larger airways, and the lymphatics. The latter include the valved channels that begin at the bronchiolar level and the pleural plexus that both ultimately drain into the hilar and mediastinal lymph nodes. The importance of the mediastinal lymph nodes to modern lung cancer staging is paramount. The precise mapping of the inferior, aortic, and superior groups of nodes is well-summarized and illustrated elsewhere [9, 10].

20.4 Histology of the Lung

The microscopic structure of the lung is beautifully complex, and we cannot do it justice in this cursory review. Especially, germane to lung cancer biology are: (1) pseudostratified ciliated columnar epithelium of the airways; (2) the abundant mucus-secreting goblet cells within airways and in the submucosal glands present more proximally in the larger airways; (3) the close proximity of rich lymphatic vasculature to the airway epithelium, facilitating invasion by malignant tumors. Newer microanatomy research in mice identifies specialized bronchoalveolar stem cells at the junction of the airways and alveoli. One hypothesis is that these cells may also act as cancer stem cells, but their role in human lung cancer development remains to be characterized [7].

20.5 Classification of Lung Cancer

Lung cancer is generally divided into two major categories: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). The recent tumor classification system (Table 20.1) issued

in 2011 [9] addresses the inadequacies of the 2004 classification and provides the foundation for tumor diagnosis and patient therapy and a critical basis for epidemiologic, molecular, and clinical studies [11]. The most important changes to the 2004 revised classification system were:

- eliminate bronchioloalveolar carcinoma,
- define the term adenocarcinoma in situ (AIS),
- define the term minimally invasive adenocarcinoma (MIA),
- revive the term "lepidic,"
- promote comprehensive histologic subtyping,

Table 20.1 WHO classification of lung tumors

Histologic type and subtypes
EPITHELIAL TUMORS
Adenocarcinoma
Lepidic adenocarcinoma
Acinar adenocarcinoma
Papillary adenocarcinoma
Micropapillary adenocarcinoma
Solid adenocarcinoma
Invasive mucinous adenocarcinoma
Mixed invasive mucinous and Non-Mucinous adenocarcinoma
Colloid adenocarcinoma
Fetal adenocarcinoma
Enteric adenocarcinoma
Minimally invasive adenocarcinoma
Non-Mucinous
Mucinous
Preinvasive lesions
Atypical adenomatous hyperplasia
Adenocarcinoma *in situ*
Non-mucinous
Mucinous
Squamous cell carcinoma
Keratinizing squamous cell carcinoma
Non-keratinizing squamous cell carcinoma
Basaloid squamous cell carcinoma
Pre-invasive lesion
Squamous cell carcinoma *in situ*
Neuroendocrine Tumors
Small cell carcinoma
Combined small cell carcinoma
Large cell neuroendocrine carcinoma
Combined large cell neuroendocrine carcinoma
Carcinoid tumors
Typical carcinoid tumor
Atypical carcinoid tumor
Pre-invasive lesion
Diffuse idiopathic pulmonary neuroendocrine Cell hyperplasia
Large cell carcinoma
Adenosquamous carcinoma
Sarcomatoid carcinomas
Pleomorphic carcinoma
Spindle cell carcinoma
Giant cell carcinoma
Carcinosarcoma
Pulmonary blastoma
Other and Unclassified carcinomas
Lymphoepithelioma-like carcinoma
NUT carcinoma
Salivary gland-type tumors
Mucoepidermoid carcinoma

Table 20.1 (continued)

Adenoid cystic carcinoma
Epithelial-myoepithelial carcinoma
Pleomorphic adenoma
Papillomas
Squamous cell papilloma
Exophytic
Inverted
Glandular papilloma
Mixed squamous and glandular papilloma
Adenomas
Sclerosing pneumocytoma
Alveolar adenoma
Papillary adenoma
Mucinous cystadenoma
Mucous gland adenoma
Mesenchymal Tumors
Pulmonary hamartoma
Chondroma
PEComatous tumors
Lymphangioleiomyomatosis
PEComa, benign
Clear cell tumor
PEComa, malignant
Congenital Peribronchial myofibroblastic tumor
Diffuse pulmonary lymphangiomatosis
Inflammatory myofibroblastic tumor
Epithelioid hemangioendothelioma
Pleuropulmonary blastoma
Synovial sarcoma
Pulmonary artery intimal sarcoma
Pulmonary myxoid sarcoma with EWSR1-CREB 1 translocation
Myoepithelial tumors
Myoepithelioma
Myoepithelial carcinoma
Lymphohistiocytic tumors
Extranodal marginal zone lymphomas of mucosa associated
Lymphoid tissue (MALT lymphoma)
Diffuse large-cell lymphoma
Lymphomatoid granulomatosis
Intravascular large B-cell lymphoma
Pulmonary Langerhans cell histiocytosis
Erdheim-Chester disease
Tumors of ectopic origin
Germ cell tumors
Teratoma, mature
Teratoma, immature
Intrapulmonary thymoma
Melanoma
Meningioma, NOS
Metastatic tumors

- emphasize and introduce the term micropapillary carcinoma,
- detach the term mucinous adenocarcinoma, and
- discourage use of the term NSCLC and subclassify the tumors in as much detail as possible.

The histological features of each are described separately below.

20.5.1 Non-small Cell Carcinoma

20.5.1.1 Adenocarcinoma

Clinical Features

Adenocarcinoma is the most frequent cell type of lung cancer, accounting for over 50 % of cancers in most recent series. To date, most validated and investigational predictive biomarkers have been

identified in adenocarcinoma as compared to other cell types and a new subtype classification of adenocarcinoma has been proposed by the International Association for the Study of Lung Cancer, American Thoracic Society and the European Respiratory Society that takes into account the molecular pathology of these tumors [9]. The current classification of lung adenocarcinoma by the World Health Organization recognizes several distinct morphologic subtypes of adenocarcinoma: papillary (Fig. 20.1), micropapillary (Fig. 20.2), acinar (Fig. 20.3), solid (Fig. 20.4), and lepidic (Fig. 20.5) [11]. The majority of lung adenocarcinomas exhibit combinations of morphologic patterns [12–14]. While the biologic basis for the histologic subtypes remains an area of active investigation [14], there is evidence that some subtypes may be associated with specific molecular alterations [14–19] or a better outcome [20–22].

Pathologic Features

Gross Findings

Grossly, adenocarcinoma typically has an irregularly lobulated configuration, with a gray-white cut appearance. As they are predominantly peripheral parenchymal masses, adenocarcinomas, in contrast to squamous cell carcinoma of the lung, are rarely associated with large airways. Anthracotic pigment is commonly entrapped in the tumor mass. Gross necrosis is uncommon except in larger masses. They may be found in association with fibrosis and pleural puckering. The penetration of the pleura may require additional studies such as elastic stains are important in tumor staging (see below).

Microscopic Findings

Adenocarcinoma in situ (AIS): AIS, formerly BAC, is an important subtype of pulmonary adenocarcinoma (Fig. 20.6). This cancer has received increasing attention in recent years owing to its increasing incidence and rate of sensitivity to epidermal growth factor–tyrosine kinase inhibitors [24]. AIS is a primary lung tumor with a peripheral location, well-differentiated cytology, lepidic growth pattern, and a tendency for both aerogenous and lymphatic spread. The key feature is preservation of the underlying architecture of the lung with no invasion.

Minimally invasive adenocarcinoma (MIA): MIA was introduced to define patients with a near 100 % 5-year disease-free survival. It is defined as a lepidic predominant tumor measuring 3 cm or less that has an invasive component

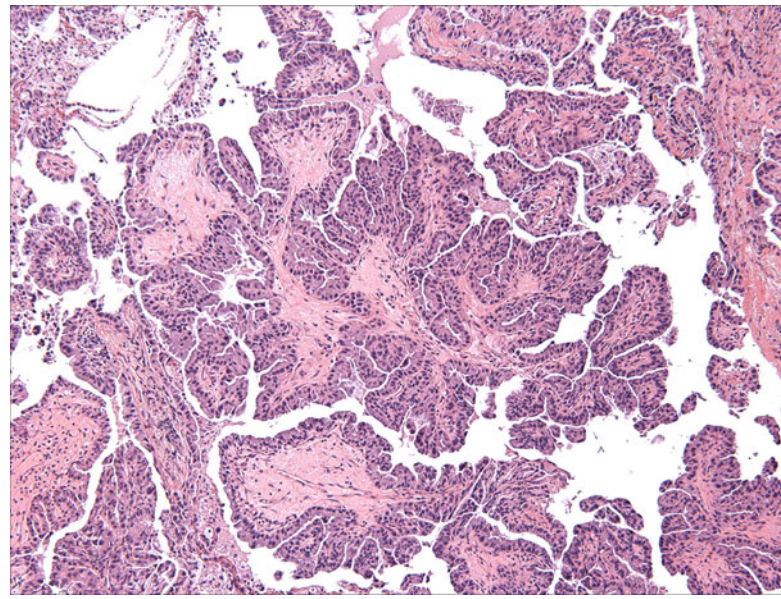

Fig. 20.1 Papillary adenocarcinoma is characterized by finger-like projections of tumor cells connected by a stromal core abundant in vascular structures, with tumor cells protruding to the outside of these core structures. Due to the spatial 3D arrangements, some of these papillae give the false impression of floating into the tumor spaces

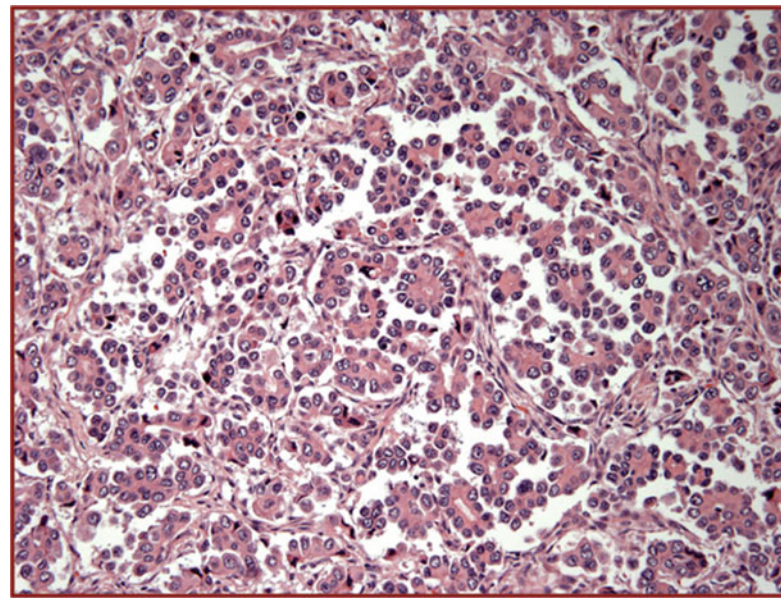

Fig. 20.2 Micropapillary adenocarcinoma is characterized by a piling up and clustering of tumor cells in the alveoli. These clusters miss the vascular cores, in contrast to papillary adenocarcinoma

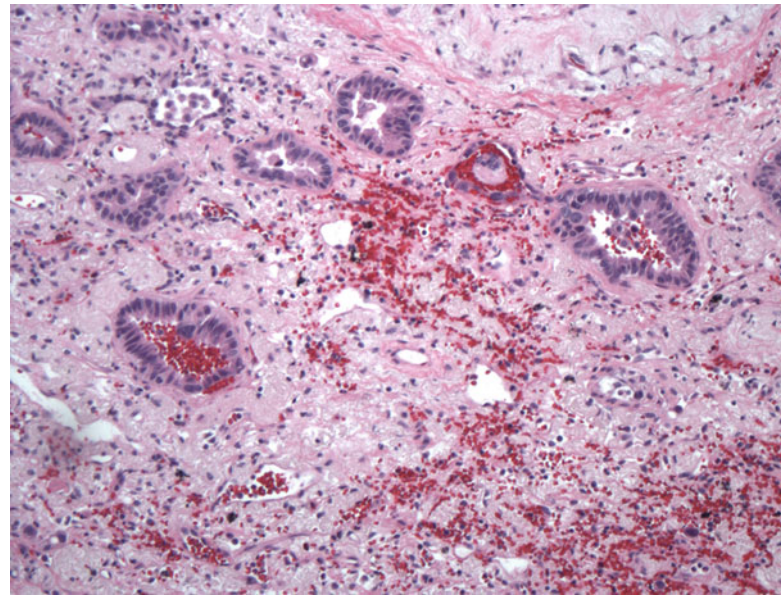

Fig. 20.3 Acinar adenocarcinoma is the classical pattern of adenocarcinoma with tumor cells arranged in tubular architectures also called acinar

of 5 mm or less [25]. MIA is characterized by a combination of ground glass opacity (GGO) and a central solid opacity, with the solid component measuring 5 mm or less. Nonmucinous MIA is more common than mucinous MIA and most often appears as a GGO. Mucinous MIA appears radiologically as a solid or part-solid nodule.

Invasive adenocarcinoma: Changes were inserted in the classification of invasive adenocarcinomas. The current classification of lung adenocarcinoma by the World Health Organization recognizes several distinct morphologic subtypes of adenocarcinoma: papillary, micropapillary, acinar, solid, and lepidic [11].

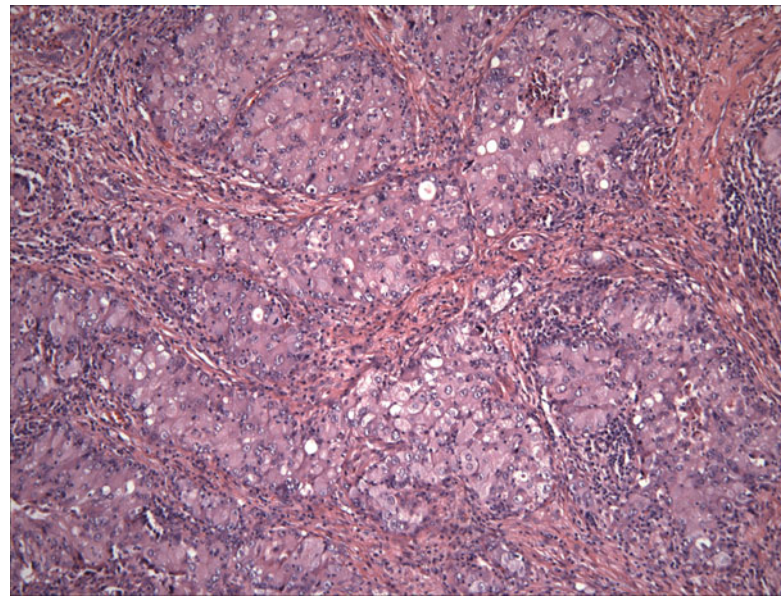

Fig. 20.4 Solid lung adenocarcinoma characterized by irregularly shaped islands of tumor cells sometimes separated by stroma (desmoplastic reaction). This architectural pattern has no particular shapes, and is considered one of the worst behaving and aggressive type of lung cancer

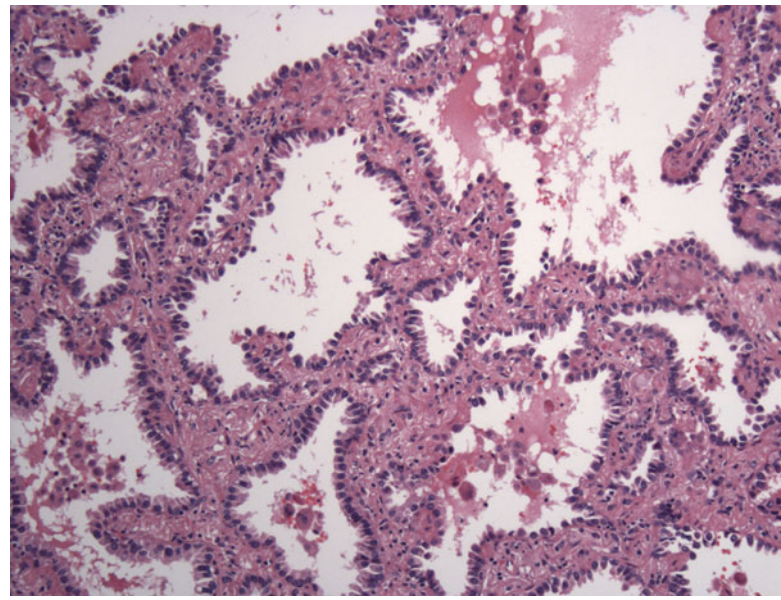

Fig. 20.5 The lepidic pattern characterized by the tumor cells missing any invasion into the stroma. In this architectural pattern, the tumor cells spread along the alveolar walls, replacing the normal pneumocytes type II. This pattern is most commonly associated with some of the other previously mentioned patterns

The majority of lung adenocarcinomas exhibit combinations of morphologic patterns [12–14]. While the biologic basis for the histologic subtypes remains an area of active investigation [14], there is evidence that some subtypes may be associated with specific molecular alterations [14–18] or a better outcome [20–23]. Overtly invasive adenocarcinomas are classified according to the predominant subtype after the use of comprehensive histologic subtyping to estimate the percentages of the various components in a semiquantitative fashion in 5–10 % increments. The adenocarcinoma patterns are: lepidic, acinar, papillary, micropapillary, and solid. The invasive adenocarcinoma variants are mucinous adenocarcinoma (Fig. 20.7), colloid, fetal, and enteric morphologies. The term lepidic predominant adenocarcinoma consists of mixed

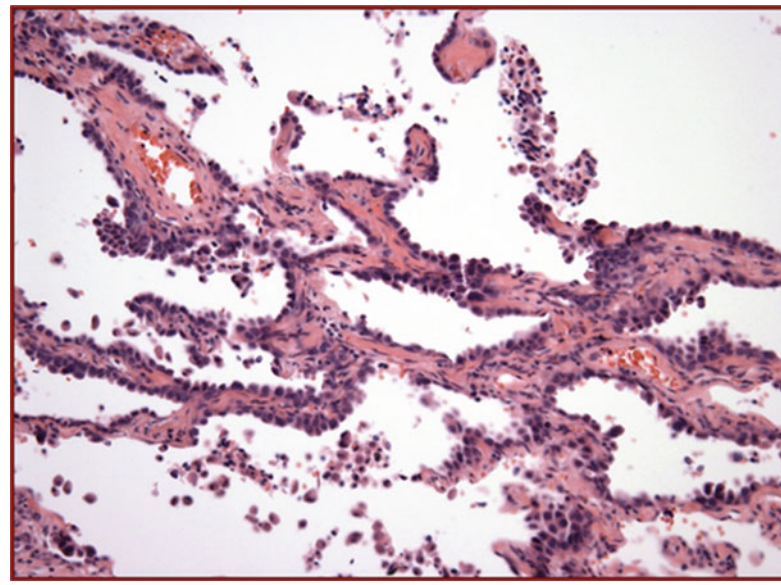

Fig. 20.6 Adenoma in situ (AIS) This tumor is composed only by the noninvasive lepidic pattern. No invasion is identified into stroma, lymphatics, or pleura. Patients with this type of in situ carcinoma are considered to be cured by simple surgical excision

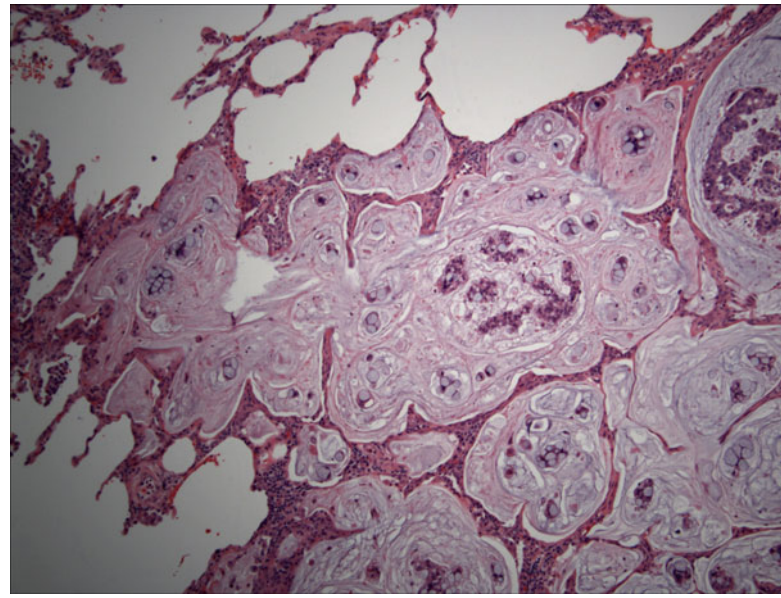

Fig. 20.7 Mucinous adenocarcinoma composed of tumor cells secreting large amounts of mucus in the alveolar spaces and stroma

subtype tumors containing a predominant lepidic growth pattern of type II pneumocytes and/or Clara cells (formerly known as nonmucinous BAC) that have an invasive component >5 mm. A micropapillary predominant subtype is added because it has been recognized as a poor prognostic category. Signet ring (Fig. 20.8) and clear cell carcinoma (Fig. 20.9) subtypes are now recorded as cytologic features whenever present with a comment about the percentage identified.

High-power examination reveals that the tumor cells are typically polygonal with large vesicular nuclei, prominent nucleoli, and moderately abundant cytoplasm. Unlike SCC, the cytoplasmic borders are often poorly defined or indistinct. In addition, a variety of cell types have

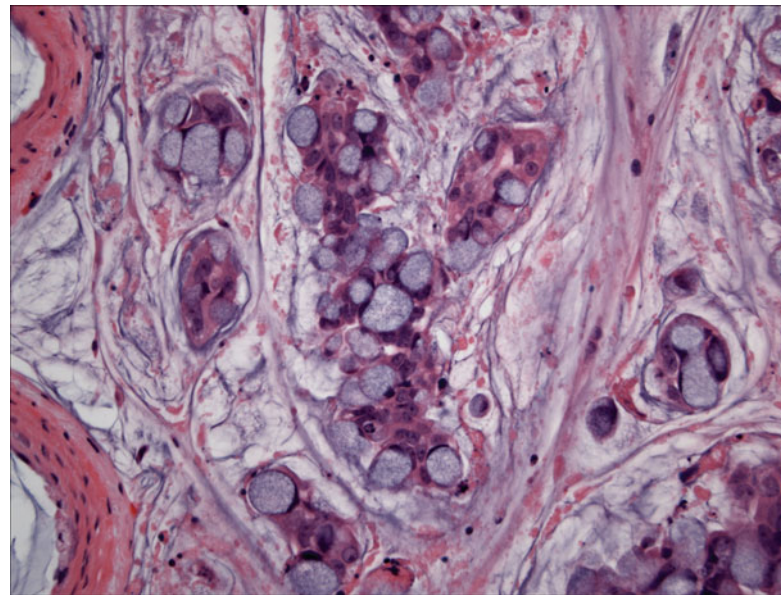

Fig. 20.8 Signet ring cell carcinoma are cells with a peculiar cytology: the mucin is collected in the cytoplasm, pushing the nucleus to the periphery of the cell, giving the cell a peculiar appearance resembling an archaic ring with an attached seal structure

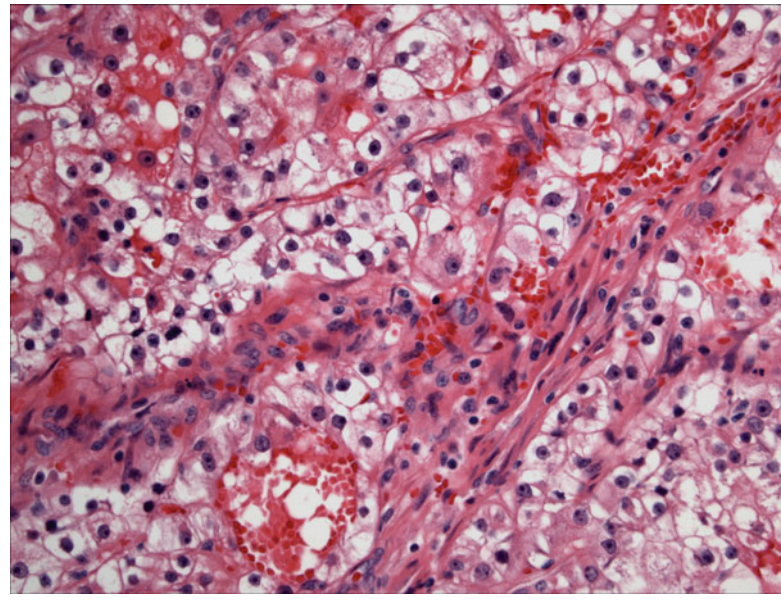

Fig. 20.9 Some of the carcinomas have a glycogenized (clear) cytoplasm. This particular type of cytology (clear cell cytology) has no particular prognostic or biological significance

been described, such as clear cell, mucinous, fetal, sarcomatoid, and signet-ring cell. Moreover, it is not uncommon for adenocarcinoma to be present in association with other types of lung carcinoma (e.g., combined with SCLC, or large-cell neuroendocrine carcinoma, or SCC, or sarcomatoid carcinoma).

20.5.1.2 Squamous Cell Carcinoma

Clinical Features

SCCs represent approximately 30 % of all NSCLC. Their incidence has been decreasing compared to adenocarcinomas, possibly due to changes in smoking habits. It is strongly linked to a history of cigarette smoking.

Pathologic Features

Gross Findings

Most SCCs arise centrally from the segmental or subsegmental bronchi. However, the incidence of SCC of the peripheral lung is increasing. Grossly, tumor masses are usually gray white to yellow tan. SCC is the most common type to give rise to a thick-walled irregular cavity with central necrosis. The texture can be firm or gritty and may be surrounded by areas of obstructive consolidation. Some of the proximal tumors have an exophytic, papillary, and endobronchial growth pattern. Because of their frequent central location (in the mainstem, lobar, or segmental bronchi), diagnosis by cytological examination of sputum, bronchoalveolar lavage (BAL), bronchial brushing and washing, or endoscopic biopsies can be performed with generally satisfactory results. Also, because of their central location, direct extension of the primary tumor mass into the adjacent hilar lymph nodes is common.

Microscopic Findings

SCC is a malignant epithelial tumor with keratinization and/or intercellular bridges (Fig. 20.10). SCC is graded as well differentiated if prominent keratinization, intercellular bridges, or pearl formation is present. They are moderately differentiated if these features are easily seen but not extensive. Poorly differentiated SCCs have only focal morphologic features of squamous differentiation. Keratinization may take the form of squamous pearls or individual cells with markedly eosinophilic dense cytoplasm. The presence of intracellular mucin in a few cells does not exclude tumors from this category. In situ SCC may be seen in the adjacent airway mucosa.

SCCs can present as histological variants which include: papillary, clear cell, small cell, and basaloid patterns. Rarely, these patterns are seen throughout the tumor, but more commonly, they are focal. Even though invasive growth is not identified, papillary SCC can be diagnosed if there is sufficient cytological atypia. However, small biopsy specimens that show a very well-differentiated papillary squamous epithelium should be interpreted with caution since separation of a papillary squamous carcinoma from a papilloma can be difficult. Furthermore, the squamous epithelium of a squamous papilloma may extend into the bronchial glands and should not be confused with invasion.

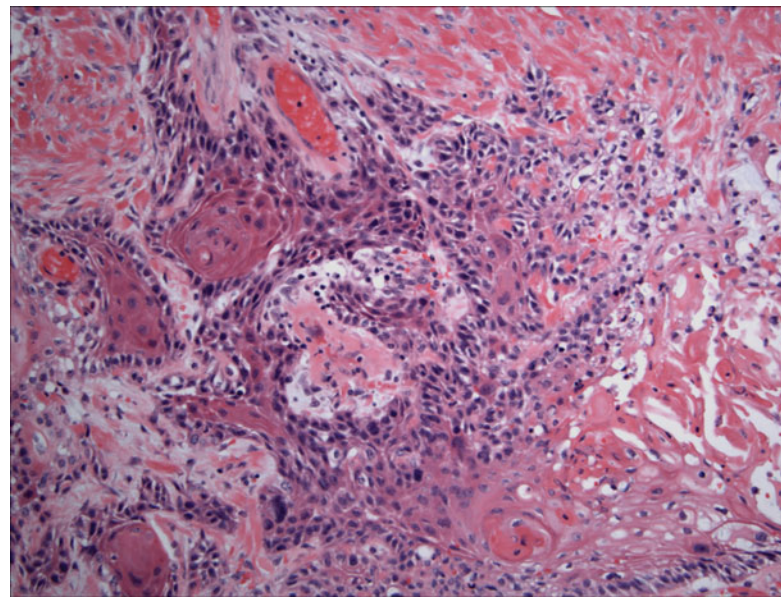

Fig. 20.10 Squamous cell carcinoma is a malignant epithelial tumor with keratinization and/or intercellular bridges

20.5.1.3 Large-Cell Carcinoma

Large-cell carcinoma (LCC) is an undifferentiated malignant epithelial tumor accounting for approximately 10 % of all lung cancers in many series. It is strongly associated with cigarette smoking. The lesion tends to occur in the periphery and grows rapidly. It usually presents at a late stage, resulting in a poor outcome.

Pathologic Features

Gross Findings

About half of the cases display a relationship to a large airway. Grossly, LCCs are frequently greater than 5 cm in size and have a white to gray or fish-flesh cut appearance. Gross tumor necrosis is commonly appreciable.

Microscopic Findings

LCC is a diagnosis of exclusion made after ruling out the presence of a component of SCC, adenocarcinoma, or SCLC. Because of this, the diagnosis is best made on resection specimens, and not on small biopsy specimens. In general, the cells typically have large nuclei, prominent nucleoli, and a moderate amount of cytoplasm. They are typically arranged in sheets or large nests, frequently revealing foci of necrosis.

Morphologically, they have lobular, trabecular, or palisading growth patterns surrounding typically centrally located comedo-type necrosis. These tumors consist of relatively small monomorphic cuboidal to fusiform cells with moderately hyperchromatic nuclei, finely granular chromatin, absent or only focal nucleoli, scant cytoplasm, and a high mitotic rate. Neither intercellular bridges nor individual cell keratinization are present. A high percentage of cases have associated carcinoma in situ. Immunohistochemical stains for adenocarcinoma, SCC, and neuroendocrine markers are negative. About half of the tumors with this histological pattern are pure basaloid carcinomas. Immunohistochemistry (IHC) reveals that these neoplasms commonly express cytokeratin, but do not express TTF-1 or p63. Ultrastructural analysis of LCCs usually shows evidence of squamous, glandular, or neuroendocrine differentiation, suggesting that these are, in fact, very poorly differentiated NSCLCs.

20.5.2 Small Cell Carcinoma

SCLC is defined as a neuroendocrine tumor with more than 10 mitoses per 2 mm^2 and small cell cytologic features. Cells have an oval or vaguely spindled shape and have scant cytoplasm. Nuclei are hyperchromatic and have absent or very small nucleoli (Fig. 20.11). Crush artifact may be prominent on small biopsies, but this is not pathognomonic for the diagnosis of SCLC. In larger core biopsies or resected specimens, the cells may appear slightly larger than in a transbronchial biopsy and may have distinct cytoplasm. Numerous prominent nucleoli and large cells should not be seen.

20.6 Lung Cancer Grading

Histological grading (well, moderately, and poorly differentiated adenocarcinomas) has prognostic significance and recently published analyses have been validated in clinical practice using histological and cytologic criteria [26].

20.7 Lung Cancer Staging

Stage is the most important prognostic and predictive factor for patients with lung neoplasms, and pathologists are expected to provide accurate staging information in lung-resection specimens. Separate staging systems have been proposed for patients with NSCLC and SCLC. The seventh edition of the published guidelines of the American Joint Commission on Cancer (AJCC) compiles the most recent clinical and pathologic staging of patients with lung cancer and other neoplasms (Table 20.1). The designation "T" refers to a primary tumor that has not been previously treated. The symbol "p" refers to the pathologic classification of the tumor/node/metastasis (TNM), as opposed to the clinical classification, and is based on gross and

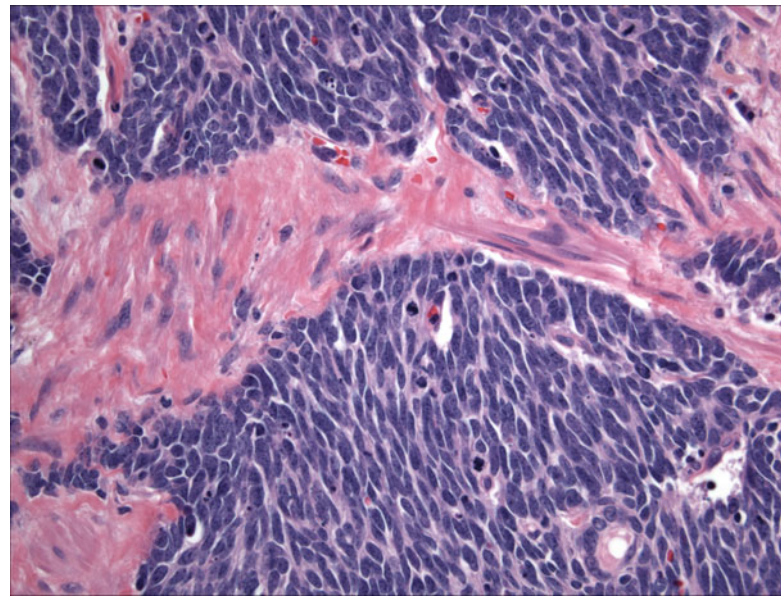

Fig. 20.11 Small cell carcinomas are extremely aggressive tumors with a neuroendocrine differentiation. Cells have an oval or vaguely spindle shape with very scant cytoplasm, nuclear molding with absent or very small nucleoli

microscopic examination. "pT" entails a resection of the primary tumor or biopsy adequate to evaluate the highest pT category, pN entails removal of nodes adequate to validate lymph node metastasis, and pM implies microscopic examination of distant lesions. Overall survival for lung cancer is 16 %; however, survival is stage-dependent. Overall survival rates for patients with stages I–IV NSCLC are 60–80, 25–50, 10–40, and 4:5 %, respectively.

Patients with Stage I usually undergo surgical resection with lobectomy, segmentectomy, or wedge resection. Usually for patients with stage II disease (and for those with Stage III disease diagnosed upon final pathology following resection), treatment should include anatomic surgical resection followed by adjuvant chemotherapy. Patients with resectable disease (Stages I and II) who have medical contraindications for surgery are candidates for curative radiation therapy. Patients with stage IIIA NSCLC receive neoadjuvant chemotherapy followed by surgical resection. Patients with Stage IIIB disease are treated with radiation therapy in combination with chemotherapy; those with stage IV disease receive this regimen, predominantly as palliative therapy.

20.8 Molecular Alterations and Precision Oncology in Lung Cancer

NSCLC is the second most common cancer diagnosed in the United States and the leading cause of cancer-related mortality, with an estimated 221,200 new cases and 158,040 deaths anticipated in 2015 [27]. Lung cancer was the leading cause of cancer death among men in 2012 [28]. Among women, lung cancer was the leading cause of cancer death in more developed countries, and the second leading cause of cancer death in less developed countries [28]. Globally, the overall lifetime risk of lung cancer is about 1 in 13 for men and 1 in 16 for women. The risk is significantly higher for smokers and lower for nonsmokers [29–31]. However, lung cancer rates in Chinese women (20.4 cases per 100,000 women) were higher than rates among women in some European countries despite a lower prevalence of smoking. This is thought to reflect indoor air pollution from unventilated coal-fueled stoves and cooking fumes [32]. Other known risk factors for lung cancer include

exposure to occupational and environmental carcinogens such as asbestos, arsenic, radon, and polycyclic aromatic hydrocarbons [33]. Recently, outdoor pollution has also been determined to cause lung cancer [34, 35]. More than one-half of the lung cancer deaths attributable to ambient fine particles were projected to have been in East Asian countries [36].

In the past years, given the development of new targeted therapies, tremendous efforts have been directed towards identifying potentially druggable molecular alterations, especially against known activating mutations. Although numerous mutations have been described in lung adenocarcinoma [37], the mutation status remains unknown in more than 50 % of cases [38]. So far, we can identify at the present moment therapeutic targets in only 20 % of lung cancers.

Molecular profiling has become the standard of care for advanced (metastatic) lung cancer. For nonsquamous NSCLC, which accounts for more than half of all lung cancer cases, routine testing for epidermal growth factor receptor (EGFR) mutations and anaplastic lymphoma kinase (ALK) rearrangements is recommended. In cases with identified EGFR (approximately 15 % of NSCLC) or ALK alterations (approximately 5 % of NSCLC), molecularly targeted therapy with EGFR-or ALK-targeting drugs is now the preferred initial approach to treatment [39].

20.8.1 Targeted Therapies in Lung Cancers with Epidermal Growth Factor Receptor (EGFR) Abnormalities

20.8.1.1 EGFR

Recognized mechanisms of EGFR gain of function in NSCLC include somatic activating mutations in the exons encoding the tyrosine kinase domain and EGFR gene amplification [40–42]. The EGFR mutation status is best determined by gene sequencing abnormalities of EGFR status may also be observed with gene copy number determined by fluorescence in situ hybridization (FISH) or chromogenic in situ hybridization (CISH), and protein expression determined by IHC with mutation-specific antibodies. Several mutations have been recently described in the tyrosine kinase domain of EGFR [40, 43]. EGFR is expressed in 50 % of NSCLCs, and its expression is correlated with poor prognosis [44]. These two factors make EGFR and its family members prime candidates for the development of targeted therapeutics [45]. EGFR kinase domain mutations target four exons [18–21], which encode part of the tyrosine kinase domain (the entire kinase domain is encoded by exons 18–24) and are clustered around the ATP-binding pocket of the enzyme [46–50].

EGFR gene amplification is detected in some EGFR-mutation-positive patients as well [51]. A subset of lung adenocarcinomas has activation of growth factor receptor (EGFR) by mutations and/or amplification but the interaction between them is complex and unclear. Some EGFR-amplified lung adenocarcinomas have distinct genetic alterations, unique clinicopathologic features, and worsened prognosis [51, 52]. Furthermore, EGFR amplification and EGFR mutations are heterogeneously distributed within any given tumor. These are novel and important findings with implications for the efficacy of treatment with tyrosine kinase inhibitors in patients with EGFR-mutant lung adenocarcinoma [51].

Recent discoveries described EGFR mutation-specific antibodies that could help in the rapid screening of lung cancers with EGFR mutations (Fig. 20.12) [52].

Mutations in the tyrosine kinase domain of the EGFR have prognostic significance since patients with EGFR-mutant NSCLC have prolonged disease-free survival compared with those with wild-type disease, regardless of the treatment received [2, 53, 54]. Although EGFR mutations are predictive of response to EGFR tyrosine kinase inhibitor (TKI) therapy, they do not appear to be predictive of a differential effect on survival [54].

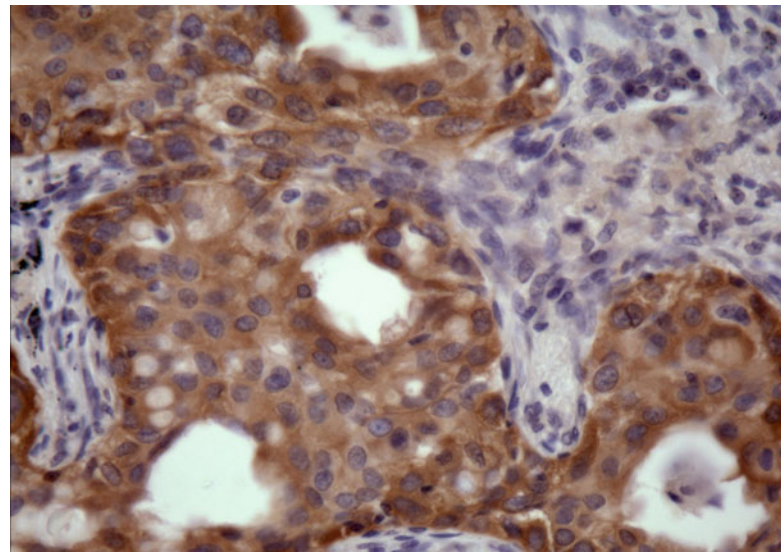

Fig. 20.12 Using exon 19 deletion-specific antibody recently described one is able to directly visualize the location of tumor cells with EGFR exon 19 deletion mutations and show heterogeneity in receptor overexpression among different tumor cells (Immunohistochemistry, exon 19 deletion-specific antibody, 200× magnification)

20.8.1.2 Targeted Agents Against Lung Cancer with EGFR Mutations

EGFR-mutant NSCLC generally refers to cases with sensitizing mutations in the EGFR kinase domain (exon 19 deletions or exon 21 L858R substitutions). These activating mutations result in constitutive activity of the EGFR kinase domain, generating survival and proliferative signals through the PI3 K-Akt-mTOR and Ras-Raf-MEK pathways. In these cases, EGFR inhibitors such as erlotinib, gefitinib, and afatinib in the first-line setting yield response rates in excess of 75 %, and overall survival exceeding 2 years [39]. In contrast, in NSCLC without actionable molecular alterations treated with conventional chemotherapy, response rates are approximately 30 %, median overall survival is 12 months. The first two TKI agents approved for use in lung cancer that target lung cancer with EGFR mutations were gefitinib (2002) and erlotinib (2003). EGFR mutation is a specific target for therapy by TKIs and is a validated biomarker of treatment response [42]. The clinical utility of this biomarker is supported by prospective clinical trials that have demonstrated a progression-free survival benefit of TKI as first-line therapy in EGFR-mutant patients [55]. Based on current data, predictive biomarker tests for EGFR should involve mutational analysis. Molecular profiling has become the standard of care for advanced (metastatic) lung cancer and routine testing for EGFR is recommended [56, 57]. In cases with identified EGFR alterations, molecularly targeted therapy with EGFR-targeting drugs is now the preferred initial approach to treatment.

Resistance to TKI therapy is associated with KRAS mutation and specific acquired EGFR mutations such as T790 M [58, 59]. These molecular events, as well as other genetic alterations in cMet (amplification), ERBB3 (overexpression), and epiregulin (autocrine loop activation), account for approximately 50 % of cases of TKI-resistance [50, 60–62].

20.8.2 Genotype-Phenotype Correlations

In patients with lung adenocarcinoma treated with erlotinib and gefitinib, favorable responses were associated with adenocarcinoma with lepidic patterns [18]. This finding led to trials of gefitinib and erlotinib in patients that showed that 17–22 % of patients had a response to gefitinib [63, 64]. The weak correlation between EGFR mutation status and adenocarcinoma subtypes [65, 66] led to the adoption of reflex genetic

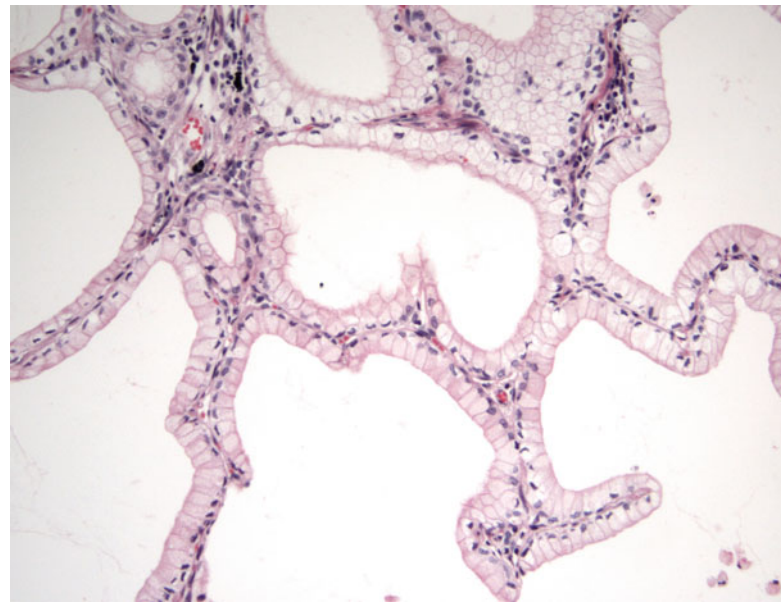

Fig. 20.13 Mucinous adenocarcinoma is exclusively TTF1 negative, EGFR mutation negative but may have Ras mutation (H&E, 100× magnification)

testing of all lung cancers and investigation for treatable molecular targets [67]. Genetic abnormalities can be seen in different histology types although with various frequency. One characteristic correlation is that mucinous adenocarcinoma (Fig. 20.13) may be exclusively TTF1 negative, EGFR mutation negative but may have Ras mutation, and expresses CDX2 possibly because of their presumed derivation from bronchiolar mucinous goblet cells [15, 68]. However, more recently, molecular genetic analyses of lung adenocarcinoma have recently become the standard of care for treatment selection [57].

20.8.3 Targeted Therapies with Angiogenesis Inhibitors in Nonsquamous NSCLC

Recent studies show that NSCLC with histology types other than SCC appear to be more associated with response to treatment with bevacizumab. Bevacizumab (Avastin®) is a monoclonal antibody with high affinity for VEGF. Despite the potential benefit of bevacizumab for some patients with previously untreated advanced NSCLC [69, 70], the appropriate clinical setting for the use of this antiangiogenic agent is stringent, due to safety issues raised in patients with SCC, which requires an accurate diagnosis on the pretreatment biopsy specimens. The clinical activity of bevacizumab in inoperable locally advanced, metastatic, or recurrent NSCLC was first shown in chemotherapy-naive patients [71]. Patients with nonsquamous NSCLC histology are the only patients who benefit from treatment with bevacizumab in combination with chemotherapy [69].

Bevacizumab is currently contraindicated in patients with SCC on the basis of the results of a recently published phase II trial [71] that 31 % of patients with SCC histology developed a life-threatening or fatal hemoptysis associated with bevacizumab, although it is still not clear whether histology alone is the reason for increased bleeding risk. Excluding patients with SCC appeared to markedly limit the risk of life-threatening bleeding complications associated with bevacizumab.

20.8.4 Targeted Therapies in Lung Cancers with Anaplastic Lymphoma Kinase (ALK) Abnormalities

On August 26, 2011, the Food and Drug Administration (FDA) approved crizotinib for the treatment of patients with locally advanced or metastatic NSCLC that is ALK-positive by FISH (Fig. 20.14). Anaplastic large-cell lymphoma kinase gene (ALK) was originally identified through cloning of the t [2, 5] (p23;35) translocation found in a subset of anaplastic large-cell lymphomas (ALCLs), a tumor of T-cell lineage [72, 73]. ALK encodes a tyrosine kinase receptor that is normally expressed only in select neuronal cell types. In ALK-rearranged ALCLs, the intracytoplasmic portion of ALK is fused to the N-terminal portion of nucleophosmin (NPM) resulting in a chimeric protein with constitutive kinase activity. Several other balanced translocations involving ALK have been discovered in ALCLs; however, the various resulting chimeric proteins all retain the ALK kinase domain [74]. The importance of the kinase activity is exemplified by ALK-rearranged ALCL cell lines which are dependent upon ALK enzymatic activity for growth and survival.

Recently, ALK rearrangements were identified in rare NSCLC cell lines and in isolated primary adenocarcinomas from Japanese and Chinese populations [75, 76]. The majority of the ALK rearrangements within NSCLCs result from an interstitial deletion and inversion in chromosome 2p and result in the EML4-ALK fusion gene product [75, 76]. Murine tumors, human cell lines, and a recent published clinical trial have shown that lung cancers expressing EML-ALK are sensitive to inhibitors of ALK kinase activity [77–79]. Thus, it is critical to efficiently and accurately identify those lung adenocarcinomas that harbor ALK rearrangements in routine practice in order to guide the appropriate clinical therapy.

None of the ALK-rearranged adenocarcinomas showed coexistent mutations in EGFR. Published studies show that ALK-rearranged adenocarcinomas are more likely to present in younger patients with a history of never-smoking, and at higher stage relative to those without ALK rearrangements (ALK germline) [80]. The majority of ALK-rearranged adenocarcinomas had a distinct histology represented by solid tumor growth and frequent signet-ring cells with abundant intracellular mucin (Fig. 20.15) [80].

The developing evidence-based guideline recommendations of the College of American

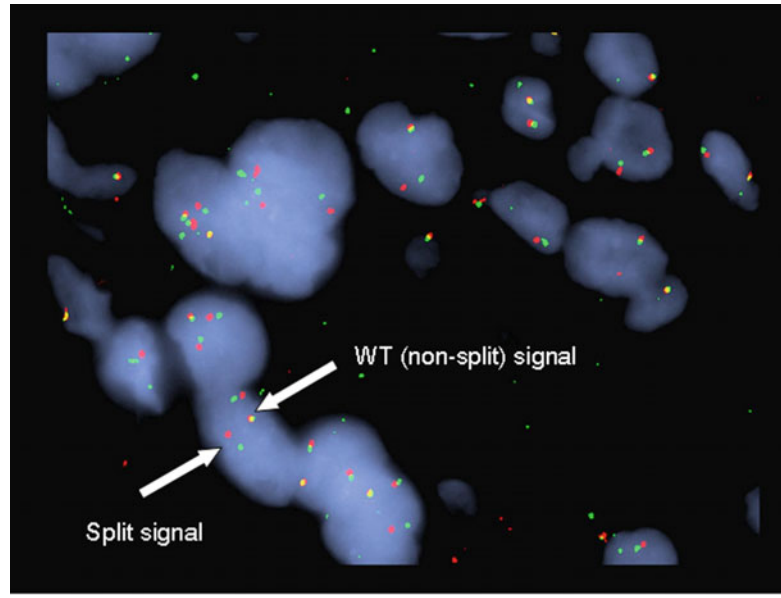

Fig. 20.14 Identification of lung cancers with chromosomal translocations involving ALK requires fluorescence in situ hybridization on formalin-fixed, paraffin-embedded tumor tissues using a break-apart probe to the ALK gene (FISH, 1000× magnification)

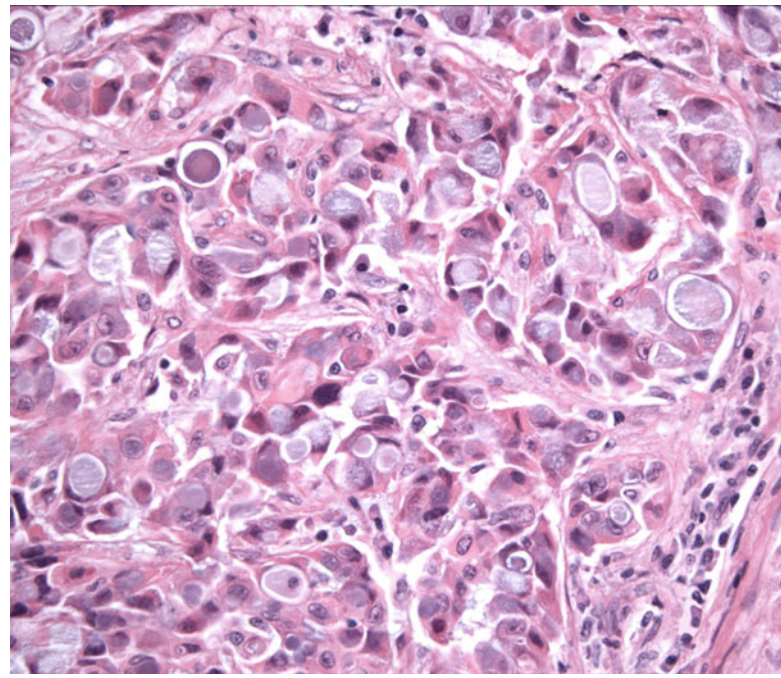

Fig. 20.15 The majority of ALK-rearranged adenocarcinomas had a distinct histology represented by solid tumor growth and frequent signet-ring cells with abundant intracellular mucin (H&E, 100× magnification)

Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology (AMP) for the molecular testing of lung cancers will likely conclude that ALK rearrangements be typically assessed by molecular cytogenetic techniques such as FISH and the currently commercially available ALK monoclonal antibodies help in screening lung cancers for ALK rearrangements. Therefore, IHC is useful in identifying cases with ALK rearrangements at the present time [80–83].

20.8.5 Other Molecular Abnormalities that Show Promise for Targeted Therapies in Lung Cancer

20.8.5.1 ROS1

Due to multiplex genomic analysis, such as that conducted by the Lung Cancer Mutation Consortium, and the emergence of clinically available Next Generation sequencing, understanding of the molecular underpinnings and vulnerabilities of lung cancer has evolved well beyond EGFR and ALK [84]. ROS1 rearrangements occur in 1–2 % of NSCLC [85]. ROS1 has a high degree of homology with ALK (approximately 50 % within the kinase domain and 75 % within the ATP-binding site), and the majority of cases respond to the first-generation ALK inhibitor crizotinib; however, certain other ALK inhibitors such as alectinib do not appear to have activity against ROS1-positive cases. ROS1 is a distinct receptor with a kinase domain that is phylogenetically related to the anaplastic lymphoma kinase/lymphocyte-specific protein tyrosine kinase (ALK/LTK) and insulin receptor (INSR) RTK families, suggesting that tyrosine kinase inhibitors for these receptors could have cross-activity against ROS1 [86].

20.8.5.2 Her-2

Unlike the other members of the HER family, HER-2 is not strictly a receptor tyrosine kinase because no high-affinity endogenous ligand has been identified. HER-2 acts as a signaling network coordinator and amplifier when it heterodimerizes with other HER family members. HER-2 mutations (in contrast to amplification, which is the

pathogenic event in breast and gastroesophageal cancer) occur in 2–4 % of NSCLC. Dual EGFR/HER2 inhibitors such as afatinib and lapatinib, as well as the anti-HER2 antibody trastuzumab, have activity against these cases [87, 88]. They are in-frame insertions in exon 20 and have targeted the corresponding TK domain region, as in EGFR-insertion mutations. These mutations occur in the same subpopulation as those with EGFR mutations (adenocarcinoma, never-smoker, East Asian, and women) [66]. Although HER-2 mutations occur in only 2 % of patients, HER-2 is frequently overexpressed to some degree in NSCLC and appears to be associated with drug resistance, increased metastatic potential, increased production of vascular endothelial growth factor (VEGF), and poor prognosis [89]. HER-2–mediated resistance to DNA-damaging agents requires the activation of Akt, which phosphorylates murine double minute 2 (MDM2) and therefore enhances MDM2-mediated ubiquitination and degradation of p53. Blocking the Akt pathway mediated by HER-2 increases the cytotoxic effect of DNA-damaging drugs in tumor cells with wild-type p53. Furthermore, recent studies have shown that the G/G genotype of the MDM2 polymorphism is associated with worse overall survival among early-stage NSCLC patients, particularly those with squamous cell histology [90].

Trastuzumab (Herceptin) is a chimerized monoclonal antibody against HER-2. Combinations of trastuzumab and chemotherapy are well tolerated, with response rates of 21–40 % [91]. One trial showed that patients whose tumors highly overexpressed HER-2 (3+) by IHC or evidence of amplification by FISH showed a good response. It appears that highly overexpressing HER-2 cases of NSCLC (3+ by IHC), although relatively infrequent (3–9 %), may show benefit with treatment with trastuzumab.

20.8.5.3 MET Proto-oncogene

MET can be activated by mutations, autocrine/paracrine growth, overexpression by gene amplification, or decreased degradation [92]. Germline and somatic MET gene mutations have been reported in hereditary and sporadic papillary renal cell cancers [93]. MET gene mutations and amplifications have been reported in other cancers to be predictors of response to therapy [94]. Expression of MET and phospho-MET has been studied in lung cancer, and recently it was shown that 40 % of lung cancer tissues overexpressed MET [95–97]. Recent studies have shown that survival in NSCLC patients with ≥5 copies/cell is worse than those with less than 5 copies/cell and that MET gene amplification leads to EGFR tyrosine kinase resistance in EGFR-mutant patients [62]. Anti-HGF antibodies, anti-MET antibodies, and small-molecule MET TKI inhibitors are all in various stages of development, and predictive biomarkers for MET inhibitors will be important to elucidate for future trials and treatment decisions [92].

20.8.5.4 Other Targeted Molecular Therapies

There has been tremendous research and investment in the development of small molecules that target key proteins in cell signaling pathways that are aberrantly altered in disease, particularly in carcinogenesis. For instance, receptor tyrosine kinases (RTKs) serve as potential therapeutic targets in several solid tumors, including lung cancer.

Gene fusions involving the rearranged during transfection (RET) gene occur in approximately 1 % of NSCLC. Several commercially available multitargeted kinase inhibitors have RET activity (e.g., vandetanib, sorafenib, sunitinib, cabozantinib); cabozantinib has led to radiographic responses.

BRAF mutations occur in 1–3 % of NSCLC. Of these, approximately 50 % are V600 and respond to BRAF inhibitors such as vemurafenib and dabrafenib, both currently approved for V600 BRAF-mutant melanoma.

The RTK c-kit is highly expressed in SCLC (although it is not mutated), and this has led to clinical trials with the specific c-KIT inhibitor (STI-571, Glivec, Novartis), alone and in combination therapy. However, these trials have failed to show a meaningful benefit from the imatinib treatment [98]. Antibodies against the angiogenic factor VEGF and small molecules

against VEGF receptors, such as SU5416, which is an inhibitor of Flk-1 receptor, are being tested in NSCLC and other tumor types. More recently, modification of gene expression using siRNAs has the promise of being the most powerful tool yet.

20.9 Conclusion

Surgical excision remains the only therapeutic modality that can cure selected lung cancer patients. Pathologists play an important role in the surgical management of patients with lung cancer, from preoperative diagnosis and staging, to intraoperative evaluation and postoperative assessment of tumor genetic alterations. With the design of new targeted therapies, pathologists are required to identify the "targeted" population or the subset of patients that benefits the most from these novel therapies.

The clinical application of molecular diagnostic techniques has allowed a more precise and rapid assessment of lung cancer and will triage the patient to "personalized" therapies that will have the highest rates of eradicating the tumor. The convergence of genetics, informatics, and imaging, along with other novel technologies, like circulating free DNA (cfDNA) is rapidly expanding the area of "precision medicine" by refining the classification of lung cancer, often with important prognostic and treatment implications [99–101]. Among these new technologies, genetics and next-generation DNA sequencing methods are having the greatest effect. The prospect of sequencing whole genomes at lesser costs reshapes our approaches to genetic testing [102]. The clinical implications will be greatest when the results of genetic testing are actionable to inform about prognosis and prediction to therapeutic modalities [103].

Our knowledge about lung cancer changed radically in the past decade, and progress mainly depends on identifying new predictive biomarkers. We need to better understand both the tumor and the host biology that underlies tumor sensitivity and resistance in order to provide a rationale for specific targeted therapy.

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