Use of cytology and small biopsy specimens in diagnosing, treating lung cancer

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Lung cancer remains the major cancer killer of men and women. Because of stage at presentation or medical morbidities, most lung cancer patients will never come to surgery. Therefore, cytology and small biopsy specimens are currently being used for diagnosis and clinical management. These specimens vary in type and amount of material, and in the era of targeted therapy and the use of histological subtyping as a tool for selecting therapy, much information is needed from these often tiny specimens. Therefore, the pathologist, and especially the cytopathologist, plays a pivotal role in the appropriate triage of these specimens at the time of biopsy. It is still necessary to separate small cell from non-small cell lung cancer (NSCLC), but that is only the initial step in managing patients with primary lung cancer. The pathologist must distinguish adenocarcinoma from squamous cell carcinoma. Although this may be done by morphology and ancillary special stains, we also have a variety of immunohistochemical markers to resolve this differential diagnosis. These steps should be done before molecular testing because only adenocarcinomas are tested routinely for the presence of activating mutations of the epidermal growth factor receptor (EGFR) or the mutually exclusive mutation of KRAS. To ensure adequate material for the necessary studies, it is recommended that on-site cytology assessment be available in the interventional radiology and operating room suites. Activating mutations in the tyrosine kinase domain of the epidermal growth factor receptor were discovered in 2004. The need to distinguish adenocarcinoma from squamous cell carcinoma stems not only from the recognition that EGFR mutations are found only in adenocarcinoma and to a lesser degree in adenosquamous carcinoma but also from the indications (and contraindications) for the use of certain anti-neoplastic agents that are dependent on the histologic subtype of cancer.

Pemetrexed is a chemotherapeutic agent that in combination with cisplatin therapy is indicated for the initial treatment of patients with locally advanced or metastatic nonsquamous non-small cell lung cancer. It is not as effective in patients with squamous cell carcinoma and hence clinicians increased use of the term “nonsquamous histology.”

Bevacizumab is a humanized monoclonal antibody that recognizes and blocks vascular endothelial growth factor A (VEGF-A) that stimulates angiogenesis in cancer. The phase two trial reported by Johnson, et al showed an association between fatal hemoptysis in patients with squamous cell carcinoma of the lung treated with bevacizumab. Therefore, bevacizumab is contraindicated for tumors with squamous cell histology.

Thus, in order for the oncologist to administer the safest and most effective therapy to the patient, pathologists need to subtype primary lung cancers. A number of excellent immunohistochemical stains that work well in surgical and cytological material are available to aid in this differential diagnosis.

The most commonly used stains to support glandular differentiation in NSCLC are TTF-1 and napsin A. TTF-1, thyroid transcription factor-1, is a protein that regulates transcription of genes specific for the thyroid, lung, and dienecephalon. It is also known as thyroid-specific enhancer binding protein. It is used to determine if a tumor arises from the lung or thyroid. If metastatic thyroid cancer to the lung is ruled out by morphology or thyroglobulin positivity, a positive TTF-1 IHC stain supports a diagnosis of adenocarcinoma originating from the lung, p63 is my preferred IHC stain to support squamous differentiation, but 34BE12 can also be used to confirm the squamous subtype of the tumor. While there can be some overlap in the staining between adenocarcinoma and squamous cell carcinoma, it is exceedingly rare for squamous cell carcinomas to lack p63 expression. TTF-1 and p63 are nuclear stains, while napsin A and 34BE12 are cytoplasmatic (Figs. 1 and 2). Napsin A can stain pulmonary macrophages, but it is useful in identifying pulmonary metastases, which will lack these histiocytes. Napsin A has a characteristic granular staining pattern (Fig. 1). Any amount of p63 positivity along with a negative TTF-1 stain would indicate squamous differentiation, and therefore bevacizumab would be contraindicated in this setting.

TTF-1 is a more sensitive marker than p63. So, if a tumor stains positive for both of these immunohistochemical stains, it is more likely to be an adenocarcinoma rather than a squamous cell carcinoma. An additional stain that is helpful in this setting is CK 5/6. A negative CK 5/6 is useful in ruling out squamous carcinoma. Mucin is also a useful stain that is somewhat underused. Mucin positivity in a tumor with TTF-1 and p63 expression supports a diagnosis of adenocarcinoma. These IHC stains are particularly valuable in small biopsy and cytology specimens, which have limited amounts of material to determine histologic subtype. They work well in cytology cell blocks. It is important to remember that cytology cell blocks often show a “squamoid” appearance artificially even when the tumor is adenocarcinoma. Correct application of TTF-1, and p63 in this setting can avoid histologic misclassification of the tumor (Fig. 2).

Histology has important clinical implications: selection of the most efficacious drug (possibly pemetrexed), avoidance of a potentially dangerous drug (bevacizumab), and staging of NSCLC for molecular mutation analysis. All adenocarcinomas should be tested routinely for the presence of EGFR and KRAS mutations. ALK testing is appropriate if EGFR and KRAS are negative because of the availability of crizotinib, an ALK (anaplastic lymphoma kinase) inhibitor in clinical trials for patients with EGFR/KRAS-negative, ALK-positive adenocarcinomas.

IHC stains work well in small biopsy and cytology material and allow the separation of adenocarcinoma from squamous cell carcinoma with an accuracy of up to 100 percent when compared with definitive histologic subtyping done on corresponding surgical resection material, as demonstrated in a recent publication by Rekhtman, et al. We do not agree with Langer, et al that only histologic material is appropriate for the subtyping of