Review Article

Immunohistochemistry for Assessment of Pulmonary and Pleural Neoplasms: A Review and Update

Dongfeng Tan[1] and Dani S. Zander[2]

[1]*Department of Pathology, MD Anderson Cancer Center, Houston, TX, USA and* [2]*Department of Pathology, Pennsylvania State University Hershey Medical Center, Hershey, PA, USA*

Abstract: Diagnosis and classification of lung and pleural neoplasms are complex due to diverse histopathology and tumor heterogeneity. A large number of immunohistochemical markers have recently become available to facilitate accurate diagnosis and classification of pulmonary and pleural neoplasms. The purpose of this article is to review the current data available on these markers and to provide a practical approach to evaluate pulmonary and pleural neoplasms immunohistochemically. Current literature of immunohistochemical markers related to pulmonary and pleural neoplasms was collected and reviewed. Literature emphasizing differential diagnosis was selected. Data useful in the diagnosis and classification of pulmonary and pleural neoplasms was collated. This review provides an updated overview and general guideline for the practicing surgical pathologists to resolve some of the common differential diagnostic situations in their daily immunohistochemical assessment of pulmonary and pleural neoplasms.

Key Words: Immunohistochemistry, lung, small cell carcinoma, adenocarcinoma, non-small cell carcinoma, pleura, mesothelioma, neuroendocrine neoplasm, spindle cell neoplasm

Evaluation of pulmonary and pleural neoplasms requires determination of histopathologic type and differentiation, as well as assessment of probable site of origin. Although this process is currently based primarily on histopathologic features, immunohistochemistry (IHC) can provide valuable information in several settings. First, IHC can assist in diagnosis and classification of a neoplasm as a non-small cell lung cancer (NSCLC) or small cell lung cancer (SCLC), a distinction critically important for determining therapy. Second, IHC can provide evidence to support the interpretation of a neoplasm with neuroendocrine differentiation. And finally, IHC can help the differential diagnosis between lung carcinomas and malignant mesotheliomas, and between lung carcinomas and metastatic extrapulmonary malignancies. In the last decade, a broad spectrum of antibodies or immunohistochemical markers has been developed and used to resolve these differential diagnostic questions [1-8]. Strategies employing these antibodies are the focus of this review.

Primary Pulmonary or Extrapulmonary Neoplasm?

The lung is the most frequent site of involvement by metastatic neoplasms [9]. In fact, metastatic neoplasms to the lungs are more common than primary lung tumors [10-13]. Based on clinicopathologic data, the lungs are involved by metastatic disease in one third to half of all malignant lesions [11]. Therefore, metastatic tumors must be considered in the differential diagnosis of primary lung cancer. Any newly detected lung mass should be evaluated with a goal of determining whether it represents a primary or secondary neoplasm. Detailed clinical evaluation and physical findings are very useful in the separation of primary and secondary pulmonary neoplasms [14]. Unfortunately, such information is often not conveyed to pathologists despite the fact that it has been demonstrated to enhance diagnostic accuracy [15]. Therefore, active and open communication between patholo-gists and oncologists can never be over-emphasized.

Although tumor morphology on hematoxylin and eosin (H&E) sections oftentimes is sufficient to answer the question of whether the tumor is primary or metastatic, there is considerable overlap between pulmonary neoplasms and neoplasms originating from other anatomic sites. For instance, it can be difficult to differentiate primary lung adeno-carcinoma from metastatic gastro-intestinal and pancreatic adenocarcinoma or from meta-static breast carcinoma. Although in some cases identification of an in situ component (bronchioloalveolar carcinoma) may point towards a lung primary, IHC is extremely useful to confirm the histological diagnosis. Construction of an IHC panel with antibodies supportive and against each potential diagnostic entity can often result in a higher level of confidence in recommending the site of origin. Practically, there is also value to include at least one marker (such as pan-cytokeratin) that should be positive in each of the differential diagnostic possibilities in order to establish the antigenic integrity of the tissue [16].

Among the "specific" markers studied for pulmonary epithelium, thyroid transcription factor-1 (TTF-1) has received the most attention. TTF-1, a 38-kilodalton nuclear protein and a member of the *Nkx*2 homeo-domain transcription factor family, was originally characterized as a promoter of thyroid-specific transcription of the thyro-globulin and thyroperoxidase genes [17, 18]. TTF-1 was subsequently detected in the fetal lung as well as within certain areas of the diencephalon [19, 20]. In normal lung tissues, TTF-1 has been observed primarily in the nuclei of alveolar cells, particularly type II pneumocytes, non-ciliated bronchiolar cells (Clara cells), and basal cells [21]. Among NSCLCs, up to 94% of adenocarcinomas have been reported to express TTF-1 [22-25].

By virtue of its tissue-specific expression in tumors of lung and thyroid, TTF-1 has recently been used as a marker for the diagnosis of primary and metastatic lung cancer, especially for identification of the lung as the primary site of metastatic adenocarcinomas [22-24]. For example, Bejarano *et al* studied 170 metastatic adenocarcinomas from various sites [26]. They found that TTF-1 was positive in 63% of lung adenocarcinomas, whereas adenocarcinomas of the colon (n=18), prostate (n=9), kidney (n=8), and breast

(n=51) were entirely negative for TTF-1. Although they reported that one of 66 gastric and one of eight endometrial adeno-carcinomas showed focal positivity, details were not provided about the characteristics of these focal reactions. Other studies have also found extrapulmonary adenocarcinomas to be immunonegative for TTF-1 [22, 23]. Tan and colleagues examined the prevalence of TTF-1 expression in human NSCLCs using the high-throughput tissue microarray (TMA) technique [27] and found similar frequencies to those obtained from conventional whole-block sections [28, 29]. TTF-1 has also been proven useful in pulmonary fine needle aspiration (FNA) specimens [30]. In summary, the high specificity and relatively good sensitivity of TTF-1 make it a valuable antibody for differentiating primary pulmonary adeno-carcinoma from metastatic adenocarcinoma. However, TTF-1 has limited value in differentiating pulmonary from extrapulmonary squamous cell carcinomas, since most pulmonary squamous cell carcinomas are immunonegative [27, 31-33]. TTF-1 is also expressed in more than 80% of small cell carcinomas originating in the lung, 39% of small cell carcinomas of the bladder, and a small percentage of gastrointestinal and cervical small cell carcinomas, limiting its utility in determining the primary site of small cell carcinoma [34-36].

Antibodies against surfactants have been evaluated in the differential diagnosis of pulmonary adenocarcinomas. While the sensitivity of surfactants is generally com-parable with TTF-1, the specificity is inferior [34]. Yatabe and coworkers evaluated the expression of surfactant apoprotein in pulmonary adenocarcinomas (n=64), and found a high correlation with TTF-1 (p <0.001) [37]. However, surfactant antibodies appear to be less specific than TTF-1 for pulmonary adenocarcinoma. Bejarano and associates evaluated antibodies to the pulmonary epithelial cell-specific proteins surfactant proteins A and B (SP-A and SP-B) and TTF-1 for their utility in differentiating primary pulmonary non-small cell carcinomas (n = 57) from carcinomas of the breast (n = 51) [26]. Expression of SP-A, SP-B and TTF-1 was detected in 49%, 53%, and 63% of NSCLCs, and 54%, 63% and 76% of pulmonary adenocarcinomas, respectively. No immuno-reactivity to SP-B and TTF-1 was observed in any of the 51 breast carcinomas, whereas four

Table 1 Immunohistochemical profiles of primary and metastatic lung adenocarcinomas

	TTF-1[a]	CK7	CK20	ER	PSA	GCDFP	INHB	HEP	CD10	RCC Ma
Lung	+[b]	+	---	---	---	---	---	---	---	---
Colorectal	---	---	+	---	---	---	---	---	---	---
Breast	---	+	---	+	---	+	---	---	---	---
Prostate	---	+/-	+/-	---	++	---	---	---	---	---
Kidney	---	+/-	---	---	---	---	---	---	+	++
Adrenocortical	---	---	---	---	---	---	++	---	---	---
Ovary	---	+	---	+	---	---	+	---	---	---
Hepatocellular	---	---	---	---	---	---	---	---	---	---

[a]TTF-1: thyroid transcription factor-1; ER: estrogen receptor; PSA: prostate specific antigen; GCDFP: gross cystic disease fluid protein; INHB: inhibin; HEP: hepatocyte-related antigen; RCC Ma: renal cell carcinoma monoclonal antigen.
[b]++, almost always diffuse, strong positive; +, mostly positive with variable staining; +/-, mostly negative with variable staining; ---, almost always negative.

of these tumors stained positive for SP-A. Thirteen breast carcinomas metastatic to the lung were non-reactive for TTF-1, while six (46%) showed reactivity for both SP-A and SP-B. Surfactant staining, however, can be helpful in the distinction between TTF-1+ lung adenocarcinoma and thyroid neoplasm, and thyroglobulin staining can also play a role in distinguishing these tumors.

Cytokeratin (CK) 7 is present in many simple and pseudostratified epithelia, including bronchogenic epithelium. The specific diagnostic utility of CK7 lies in the fact that primary lung cancer is characteristically strongly and diffusely positive, whereas carcinomas of colorectal, hepatocellular, renal, and adrenal cortical origin are virtually always negative or very rarely focally and weakly positive. CK7 is particularly useful, when used in combination with CK20, in identifying colon cancer metastases to the lung (CK20+/CK7-), and distinguishing pulmonary small cell carcinoma (CK7+or-/CK20-) from Merkel cell carcinoma (CK7-/CK20+) [38, 39].

Unfortunately, the CK7+/CK20- profile is not restricted to lung cancers; breast carcinomas, endometrial adenocarcinomas and non-mucinous ovarian carcinomas are also classically CK7+/CK20-. Furthermore, a CK7+/CK20+ profile can be seen in some mucinous and mixed bronchioloalveolar carcinomas, and has been associated with features of "enteric differentiation" by some authors [40, 41]. The differential diagnosis for CK7+/CK20+ adenocarcinomas also includes neoplasms with origins in the pancreas, ampulla, bladder, and ovary.

In practice, we find it is helpful to combine CK7/CK20 with other markers, particularly TTF-1, to differentiate between primary and metastatic carcinomas. If one is generally confident that the neoplasm is an adeno-carcinoma, a basic panel consisting of pan-keratin, CK7, CK20 and TTF-1 can be supplemented with other antibodies geared to be specific for other potential primary sites. If lymphoma, melanoma, sarcoma, or germ cell tumor is a consideration, then appropriate markers for these neoplasms can be added to the panel. A practical panel of IHC markers to distinguish primary lung adenocarcinoma from metastatic carcinoma is summarized in **Table 1**.

Small Cell Carcinoma or Non-Small Cell Carcinoma?

Accurate distinction between SCLC and poorly differentiated NSCLC is a key decision point in clinical management. However, separating the two groups of tumors morphologically can sometimes be challenging due to biopsy crush artifact, tumor necrosis, limited tumor representation, and overlapping morphologic features. Although chromatin characteristics in well-preserved tumor cells can help to direct one to the correct diagnosis, chromatin detail is often impaired by crush artifact, necrosis and suboptimal preservation. Moreover, some SCLCs and NSCLCs fall into a morphologically indeterminate gray zone in cellular characteristics including cell and nuclear sizes, amount of cytoplasm, and presence or absence of neuroendocrine differentiation. Particular challenges may occur in dis-tinguishing small cell squamous carcinomas of

the lung from SCLCs.

Many investigators have applied immuno-histochemical techniques to the evaluation of SCLCs and NSCLCs [42-44]. Recent studies suggest the potential utility of several newer antibodies, including p63 and high molecular weight keratin, to help differentiate SCLCs from NSCLCs. P63, a recently discovered member of the p53 family of nuclear transcription factors, is a dominant-negative regulator that is characterized by its ability to transactivate different reporter genes and induce apoptosis. P63 has been shown to play a critical role in maintaining stem cell populations in squamous and other stratified epithelia [45, 46]. Abnormal expression of p63 was originally documented in squamous cell carcinomas of the oral cavity and esophagus, and in premalignant and invasive squamous lesions of the cervix [47, 48]. The relationship of p63 to squamous cell differentiation and stem cell commitment prompted an examination of p63 expression in pulmonary epithelium, squamous metaplasia, and neoplasms [49, 50].

Wang and associates examined p63 expression in benign lung and in neoplasms of pulmonary origin [50]. In normal lung, p63 intensely stained the nuclei of bronchial reserve cells but not the ciliated cells, alveolar epithelial cells, or non-epithelial cells. The lower strata of the metaplastic squamous bronchial epithelium stained positively, as did all squamous cell carcinomas (n=30). In some well-differentiated carcinomas, staining was found at the periphery of tumor nests but not in central zones with squamous maturation. In poorly differentiated carcinomas, a very high proportion (80% to 100%) of the nuclei were positive for p63. All SCLCs (n=9) were p63-negative. Staining of bronchioloalveolar carcinomas (n=7) and adenocarcinomas (n=23) was variable with some tumors showing undetectable staining and others showing heterogeneous positive staining. Adeno-squamous cell carcinomas (n=5) displayed a unique basalar staining pattern. The authors concluded that p63 is expressed in benign bronchial stem cells, in neoplastic cells with either squamous differentiation or squamous differentiating potential, and in a sub-population of adenocarcinomas, but not in SCLCs. Subsequently, Pelosi and co-workers provided additional data indicating a high prevalence of p63 expression in NSCLCs [49].

They evaluated 278 patients with NSCLC. P63 immunoreactivity was seen in 109/118 squamous cell carcinomas, 15/95 adeno-carcinomas, 2/2 adenosquamous carcinomas, 4/6 large cell carcinomas, and 10/57 poorly differentiated neuroendocrine tumors (NET).

Most recently, Zhang et al evaluated a battery of antibodies including p63 for their utility in distinguishing SCLC from poorly differentiated squamous cell carcinoma [36]. All but one poorly differentiated squamous cell carcin-omas (27/28, 96%) showed diffuse moderate or strong staining for p63 and high molecular weight keratin, and no or minimal staining for TTF-1. In contrast, most SCLCs (26/28, 93%) manifested opposite immunoreactivities, showing diffuse moderate or strong staining for TTF-1 with no staining for p63 or high molecular weight keratin. This study demon-strates that a panel of antibodies against p63, high molecular weight keratin and TTF-1 is highly effective for distinguishing between SCLC and poorly differentiated squamous cell carcinoma. This panel also facilitates diagnosis of combined small cell and non-small cell carcinomas. Wu and colleagues obtained similar results. In their study, negative or rarely equivocal immunohisto-chemical reactivity for p63 was observed in all 23 SCLCs, while diffuse strong staining was seen in all 13 poorly differentiated squamous cell carcinomas [35]. Differential immuno-histochemical profiles of SCLC and poorly differentiated squamous cell carcinoma are summarized in **Table 2**.

Neuroendocrine Neoplasm or Non-Neuroendocrine Neoplasm?

Malignant pulmonary NET is a distinct subset of lung neoplasms [51-55]. In the recently revised World Health Organization (WHO) classification of lung tumors, this category embraces typical carcinoid, atypical carcinoid, large cell neuroendocrine carcinoma (LCNEC), and SCLC [9, 51]. In this spectrum of neoplasms, typical carcinoid represents the lowest grade neoplasm, and SCLC and LCNEC represent the highest grade neoplasms. All of these tumors share, to varying degrees, certain histological, ultrastructural, immuno-histochemical and molecular characteristics. Microscopically, neuroendocrine architectural characteristics include organoid nesting, palisading, a trabecular pattern and rosette-like structures. Mitoses, necrosis, and

Table 2 Immunohistochemical profiles of small cell carcinoma of lung (SCCL) and poorly differentiated squamous carcinoma of lung (PDSCL)

	TTF-1[a]	p63	HMWK	Synaptophysin	Chromogranin A
SCCL	++[b]	---	---	+	+/-
PDSCL	---	++	++	---	---

[a]TTF-1: thyroid transcription factor-1; HMWK: high molecular weight keratin.
[b]++, almost always diffuse, strong positive; +, mostly positive with variable staining; +/-, mostly negative with variable staining; ---, almost always negative.

pleomorphism are present in varying degrees in this spectrum of neoplasms, as outlined in the WHO classification scheme. Cytomorphologic characteristics such as cell and nuclear size and shape, chromatin pattern, nucleoli, and amount of cytoplasm also vary among these neoplasms.

Neuroendocrine differentiation can be demonstrated by electron microscopy or IHC in virtually all typical and atypical carcinoid tumors, and in a smaller percentage of the higher grade neuroendocrine neoplasms. For this reason, IHC is helpful for confirming neuroendocrine differentiation, but has limited value for separating individual neuroendocrine tumors from each other. A wide range of immunohistochemical markers to detect neuroendocrine differentiation has been studied [52-60]. Chromogranin, synaptophysin and neural cell adhesion molecule (NCAM)-CD56 are the most reliable and widely used, offering confident results with high sensitivity and specificity [44, 54, 61, 62]. Ultrastructurally, synaptophysin is present in microvesicles, whereas chromogranin is present in secretory granules [63]. These differences suggest that chromogranin and synaptophysin may be complementary generic neuroendocrine markers. CD57 (Leu-7) is also a frequently used neuroendocrine marker. However, CD57 is not restricted in its distribution to neuroendocrine tumors, since its reactivity is also present in non-neuroendocrine tumors including prostate carcinomas, thymomas, and a variety of small round blue cell tumors [64]. Therefore, the use of CD57 antibody alone is unreliable for specific identification of neuroendocrine tumors. Neuron-specific enolase catalyzes the interconversion of 2-phosphoglycerate and phosphoenolpyruvate in the glycolytic pathway, and was widely used in earlier times to identify neuroendocrine differentiation. Because of its broad reactivity in non-neuroendocrine neoplasms, this antibody has been replaced by

more specific neuroendocrine markers that are now available. Using TMA analysis [7], Nitadori and coauthers systematically studied 48 antibodies and the phenotypic differences between LCNEC and SCLC. They found four proteins were significantly over-expressed in LCNEC as compared to SCLC: CK7, 113 vs. 49 ($p<0.03$); CK18, 171 vs. 120 ($p<0.001$); E-cadherin, 77 vs. 9 ($p<0.001$); and beta catenin, 191 vs. 120 ($p<0.03$). These antibodies may be useful in the routine differential diagnosis between LCNEC and SCLC, but further studies are needed.

Immunohistochemical staining for neuroendocrine substances is also not restricted to neuroendocrine neoplasms. Lung neoplasms that are not classified by histological criteria as neuroendocrine neoplasms may express neuroendocrine markers. Up to 20% of NSCLCs that do not show neuroendocrine morphology by light microscopy demonstrate immunohistochemical and/or ultrastructural evidence of neuroendocrine differentiation [65, 66]. These tumors are collectively referred to as "NSCLC with neuroendocrine differentiation" (NSCLC-ND). If histological features of a more specific histological type of NSCLC are seen (i.e., squamous differentiation), then the neoplasm should be classified according to its specific histological features (i.e., squamous cell carcinoma) and a comment made regarding neuroendocrine differentiation.

Currently, the diagnosis of LCNEC requires confirmation of neuroendocrine differentiation by either IHC or electron microscopy, in a large cell carcinoma with neuroendocrine architectural characteristics. If the tumor is a large cell carcinoma with neuroendocrine architectural characteristics but no neuroendocrine staining, the term "large cell carcinoma with neuroendocrine architecture" has been used. If the tumor is a large cell carcinoma with neuroendocrine staining but without

neuroendocrine architectural features, then it can be classified as a "large cell carcinoma with neuroendocrine differentiation".

Basaloid carcinoma can occasionally be difficult to distinguish from SCLC and LCNEC, and IHC can offer assistance [48, 67]. Basaloid carcinoma typically displays a solid, nested or trabecular growth pattern, nuclear palisading at the periphery of the neoplastic lobules, rosette-like structures in one-third of the cases, and commonly comedo-type necrosis. However, in contrast to LCNEC, neoplastic cells are characterized by hyper-chromatic nuclei, increased nuclear to cyto-plasmic ratio and inconspicuous nucleoli. Basaloid carcinomas usually express low and high molecular weight keratins and only about 10% of basaloid carcinomas focally express a neuroendocrine marker, with only 5% to 20% of tumor cells immunopositive in these cases [49, 67]. TTF-1 is negative.

Sturm et al have recently shown the power of a specific cytokeratin antibody CK34βE12 to discriminate basaloid carcinoma from LCNEC [54]. Monoclonal antibody 34βE12 (CK34βE12) recognizes a subset of high molecular weight cytokeratins identified as 1, 5, 10, and 14 in Moll's catalog [68]. It is expressed in basaloid carcinomas, but not in LCNECs [54]. In tumor pathology, Morice and Ferreiro first retrospectively investigated the expression of CK34βE12 in upper aerodigestive tract tumors. 22 of 23 basaloid squamous cell carcinomas showed strong positivity for CK34βE12, whereas none of the 10 small cell carcinomas showed staining [69]. Similar results were subsequently reported in lung cancers. Lyda and Weiss showed that CK34βE12 was immunoreactive in 97% of squamous cell carcinomas, but only in 1 of 37 (3%) SCLCs and focally positive in 1 of 6 (17%) LCNECs [44]. Viberti et al investigated the expression of CK34βE12 in cytological specimens and transbronchial biopsies [70]. In their study, CK34βE12 was negative in 93% (40/43) of SCLCs and in all six other neuroendocrine tumors (2 typical carcinoids, 2 atypical carcinoids and 2 LCNECs), while strong positivity was observed in virtually all non-neuroendocrine tumors: squamous cell carcinomas (15 of 16), adenocarcinomas (10 of 10) and basaloid carcinoma (4 of 4). All these findings suggest that expression of CK34βE12 is largely restricted to non-neuroendocrine pulmonary carcinomas.

Sturm and colleagues' recent study also confirmed the lack of staining for CK34βE12 in most neuroendocrine lesions [54]. In their large cohort of neuroendocrine lesions, all cases of neuroendocrine cell hyperplasia (n=15), tumorlet (n=23), typical (n=27) and atypical carcinoid (n=23), as well as some LCNECs were completely negative for CK34βE12. While the majority of high grade NETs lacked CK34βE12 immunoreactivity, combined carcinomas and a proportion of histologically pure cases demonstrated focal immunostaining. The lack of CK34βE12 staining in SCLCs reported by Zhang et al provided further evidence of association between neuroendocrine differentiation and negative IHC staining for CK34βE12 [36].

Therefore, CK34βE12 should be included in the routine diagnostic panel of antibodies in the differential diagnosis of lung cancers, particularly when the differential diagnosis involves both neuroendocrine and non-neuroendocrine neoplasms. This antibody may also be useful in resolving questions of basaloid carcinoma versus SCLC, SCLC versus poorly differentiated squamous cell carcinoma, LCNEC versus other poorly differentiated NSCLCs, and carcinoid versus NSCLC excluding LCNEC.

Mesothelioma or Non-Mesothelioma?

The differential diagnosis of a malignant epithelial neoplasm in the peripheral lung or pleura focuses first and foremost on the distinction between malignant mesothelioma and adenocarcinoma (primary and metastatic). IHC has essentially replaced histochemistry and electron microscopy as the ancillary technique today to diagnose mesotheliomas and to differentiate mesotheliomas from primary lung cancers with direct invasion of the pleura (most commonly adenocarcinomas) and primary neoplasms outside the chest cavity with metastasis to the pleura. IHC is usually not helpful in differentiating atypical reactive mesothelial hyperplasia from malignant mesothelioma.

Traditionally, a panel of antibodies has been used to distinguish adenocarcinoma from mesothelioma. Adenocarcinomas are usually

positive for one or more of the following antibodies: carcinoembryonic antigen (CEA), Leu-M1, B72.3, BER-EP4 and MOC-31 [42]. These antibodies generally show low frequencies of reactivity in mesotheliomas. Most pathologists will choose two or three of these antibodies in their panel that also includes two or three antibodies reactive to mesothelial cells. Until recent years, however, few antibodies were available to provide positive evidence for mesothelioma. Antibodies against epithelial membrane antigen (EMA) and human milk fat globule protein-2 (HMFG-2) were used for diagnosis of epithelial mesotheliomas since the majority of epithelial mesotheliomas show a distinctive membranous staining pattern because of the extensive microvillus surface of mesothelium [71].

Recently, a few new antibodies have shown value for diagnosis of mesotheliomas. They comprise part of the "mesothelioma versus adenocarcinoma" panel of stains used in many centers [13, 68, 72-75]. CK5/CK6 was among the first "positive" markers for mesothelioma. Moll and associates evaluated CK5 expression in pulmonary and pleural malignancies. Using polyclonal antibodies, they demonstrated that 12 of 13 epithelial mesotheliomas expressed CK5, whereas none of the 21 lung adenocarcinomas did, indicating the value of CK5 assessment in distinguishing mesotheliomas from pulmonary adenocarcinomas [68]. The utility of CK5 in the diagnosis of mesothelioma was further confirmed with monoclonal antibody (D5/16B4) that reacted with CK5/CK6 [72]. Epithelial mesotheliomas were strongly positive in most cases (385/450), and pulmonary adenocarcinomas were largely negative or only focally positive. While CK5/CK6 may stain some lung carcinomas and non-pulmonary carcinomas focally [42], strong and diffuse CK5/CK6 staining in the neoplastic cells, along with concordant immunoreactivities of other markers in the panel, supports an interpretation of mesothelial differentiation.

Antibody against calretinin is another specific and reproducible positive marker of epithelial mesothelioma, and decorates cells in a cytoplasmic and nuclear distribution. Calretinin is a 29-kd intracellular calcium-binding protein that has been described in renal convoluted tubules, eccrine glands,

steroid-producing cells and neurons with variable staining patterns, and is almost always focal or weak. In contrast, calretinin is frequently expressed in mesothelial cells, usually in a diffuse and strong manner [72]. However, similar immunoreactivity was also reported in rare adenocarcinoma cases, supporting the use of a panel of antibodies for differential diagnosis of mesothelioma. This notion was further illustrated by Ordonez in a recent study [73], in which a comprehensive IHC with a number of markers was performed to distinguish epithelioid mesotheliomas from lung adenocarcinomas. These markers included calretinin, CK5/CK6, WT1, thrombo-modulin, mesothelin, CD44S, HBME-1, N-cadherin, E-cadherin, MOC-31, TTF-1, BG-8 (Lewisy), CEA, Ber-EP4, B72.3 (TAG-72), Leu-M1, CA19-9, EMA and vimentin [72]. 100% of mesotheliomas were immunoreactive for calretinin, CK5/CK6 and mesothelin; 93% for WT1, 93% for EMA, 85% for HBME-1, 77% for thrombomodulin, 73% for CD44S, 73% for N-cadherin, 55% for vimentin, 40% for E-cadherin, 18% for Ber-EP4, 8% for MOC-31, 7% for BG-8, and none for CEA, B72.3, Leu-M1, TTF-1 or CA19-9. Among adeno-carcinomas, 100% were positive for MOC-31, Ber-EP4, and EMA, 96% for BG-8, 88% for CEA, 88% for E-cadherin, 84% for B72.3, 74% for TTF-1, 72% for Leu-M1, 68% for HBME-1, 48% for CD44S, 48% for CA19-9, 38% for mesothelin, 38% for vimentin, 30% for N-cadherin, 14% for thrombomodulin, 8% for calretinin, 2% for CK5/CK6 and none for WT1.

These results suggest that calretinin, CK5/CK6 and WT1 are the best positive predictive markers while CEA, MOC-31, Ber-EP4, BG-8 and B72.3 are the best negative predictive markers for epithelioid meso-thelioma. A panel of four to six markers (2-3 with relative specificity for mesothelioma and 2-3 with relative specificity for adeno-carcinoma), selected based upon availability and the quality of staining in an individual laboratory, is recommended. Addition of TTF-1, which has a high sensitivity and very high specificity for lung adenocarcinoma, may also be useful for confirming pulmonary origin. To our knowledge, no convincing TTF-1-positive mesothelioma case has been reported in the literature [5, 13, 74, 75].

A recent review of immunohistochemical diagnosis of epithelioid mesothelioma highlighted a few newer markers that may

Table 3 Immunohistochemical differential diagnosis between primary lung adenocarcinoma and epithelioid mesothelioma

	CALR[a]	CK5/CK6	TTF-1	CEA	CD15	Ber-EP4	B72.3
Adenocarcinoma	+/-[b]	+/-	+	+	+	+	+
Mesothelioma	++	++	---	+/-	+/-	+/-	+/-

[a]CALR: calretinin; CK5/CK6: cytokeratin 5/6; TTF-1: thyroid transcription factor-1; CEA: carcinoembryonic antigen.

[b]++, almost always positive; +, mostly positive with variable staining; +/-, mostly negative with variable staining; ---, almost always negative.

offer additional help in the differential diagnosis between epithelioid mesothelioma and metastatic carcinoma [6]. D2-40 and podoplanin are particularly promising since both markers appear to be highly sensitive and specific for epithelioid mesotheliomas [13]. However, it should be kept in mind that their utility has not been fully determined in routine diagnostic work. A suggested IHC panel for epithelioid mesothelioma and its differential diagnostic entities is presented in **Table 3**. The differential diagnosis of sarcomatous mesothelioma and its histological mimics sarcomatoid carcinoma and sarcomas will be discussed later.

In short, although IHC has proven to be valuable in the differentiation of epithelioid mesothelioma from pulmonary or metastatic adenocarcinoma, no single antibody has demonstrated absolute sensitivity or specificity in making this distinction. A battery of immunohistochemical markers is required. The specific antibodies selected may vary depending on individuals' preference, but we advocate inclusion of several markers of adenocarcinoma and several markers of mesothelioma in the panel, to enhance its sensitivity and specificity. While use of more stains may mean increasing the likelihood of one aberrant result, the other results should help to put it into perspective.

Spindle Cell Neoplasms of the Lung

Primary pulmonary spindle cell neoplasms are uncommon and can occasionally be a source of diagnostic difficulty. Sarcomatoid carcinoma, also referred to as spindle cell carcinoma or carcinosarcoma, is a neoplasm of epithelial derivation that shows variable types of differentiation. Several studies have evaluated sarcomatoid carcinoma by IHC using keratin antibodies or electron microscopy and concluded that the spindle cell component of the neoplasm was of epithelial derivation [76,

77]. In most instances, the spindle cells co-express keratin and vimentin, similar to sarcomatoid mesotheliomas. The differential diagnosis of sarcomatoid carcinomas of the airways should include all true primary pulmonary sarcomas and metastatic sarcomas, particularly synovial sarcoma, fibrosarcoma, leiomyosarcoma and malignant fibrous histiocytoma.

A selected panel of antibodies can facilitate diagnosis of some specific but relatively rare pulmonary spindle cell entities. Intra-pulmonary localized fibrous tumors occur as neoplasms in the subpleural regions of the lung. Histologically, these neoplasms are composed of spindle cells with varying degrees of cellularity and varying amounts of extracellular collagenous or myxoid matter. They are usually diffusely positive for CD34, and lack keratin expression [78]. Epithelioid hemangioendothelioma consists of round or polygonal cells with an epithelioid appearance. The neoplastic cells characteristically express endothelial markers CD31, CD34 and factor VIII antigen [79, 80]. Kaposi's sarcoma, another endothelial neoplasm, is frequently associated with human immunodeficiency virus and human herpes virus 8 infections. It consists of more spindle-shaped cells compared to those seen in epithelioid hemangioendothelioma. Typically, Kaposi's sarcoma is immunopositive for endothelial markers as well as vimentin [81]. HMB-45 is a helpful marker, when combined with epithelial and mesenchymal cell markers, to confirm spindle cell melanoma as well as define the presence of lymph-angioleiomyomatosis [82]. Other malignant spindle cell neoplasms, including leiomyo-sarcoma, malignant fibrous histiocytomoa, synovial sarcoma and melanoma, can rarely present as primary tumors, although a metastatic tumor should first be ruled out before classifying a neoplasm in this group as a lung primary. Immuno-profiles of malignant spindle cell neoplasms in

Table 4 Immunohistochemical profiles of malignant spindle cell neoplasms of the lung

	PK[a]	VIM	Actin	CD31	CD34	CD68	CD99	S100	HMB45	EMA	CALR
Spindle cell carcinoma	+[b]	++	--	--	--	--	--	--	--	+	--
Melanoma	--	+	--	--	--	--	--	++	+	--	--
Leiomyosarcoma	--	++	++	--	--	--	--	--	--	--	--
Kaposi's sarcoma	--	++	+	+	++	--	--	--	--	--	--
MFH	--	++	+	--	+	++	--	--	--	--	--
Synovial sarcoma	+	+	+	--	--	--	+	--	--	+	--

[a]PK: pan-cytokeratin; VIM: vimentin; EMA: epithelial membrane antigen; CALR: calretinin.
[b]++, almost always positive; +, mostly positive with variable staining; --, almost always negative.

the lung are summarized in **Table 4**.

Differentiating sarcomatoid mesothelioma from other pleural-based spindle cell tumors by light microscopy can be challenging. The contribution of IHC to resolution of this differential diagnosis appears to be lower than it is for distinguishing epithelioid meso-thelioma from carcinoma. Significant immuno-phenotypic overlap exists among sarcomatoid mesotheliomas and sarcomas and sarco-matoid carcinomas, which are the main entities in the differential diagnosis. Nevertheless, studies have been performed to investigate the utility of diagnostic IHC in this setting. For example, Lucas and coauthors recently stained 20 mesotheliomas with sarcomatoid components (10 biphasic and 10 sarcomatoid mesotheliomas) with a panel of antibodies including pan-cytokeratin, CK5/CD6, calretinin, WT-1, thrombomodulin, and smooth muscle actin, and compared the immunophenotypic profiles of these tumors with 24 high-grade sarcomas and 10 pulmonary sarcomatoid carcinomas [83]. Pan-cytokeratin stained 70% of sarcomatoid mesotheliomas, 17% of sarcomas, and 90% of sarcomatoid carcinomas. CK5/CK6 and WT-1 rarely stained sarcomas, sarcomatoid carcinomas, or the sarcomatoid components of mesothelioma. Calretinin and thrombo-modulin each stained 70% of sarcomatoid mesotheliomas. However, calretinin was also positive in 17% of sarcomas and in 60% of sarcomatoid carcinomas, while thrombo-modulin was positive in 38% of sarcomas and in 40% of sarcomatoid carcinomas. Smooth muscle actin was expressed in 60% of sarcomatoid mesotheliomas and in 58% of sarcomas, but in only 10% of sarcomatoid carcinomas. These results suggest that cytokeratin and calretinin have the most value in differentiating sarcomatoid mesothelioma from sarcoma, but that none of these markers is useful for distinguishing sarcomatoid mesothelioma and sarcomatoid carcinoma. For sarcomatoid tumors involving the pleural lining, clinicopathological data, especially information about the pattern of involvement of the tumor (i.e. localized versus diffuse, pleural versus parenchymal with pleural extension), should be noted and carefully correlated with microscopic and immuno-histochemical findings. Comparison of the immunohistochemical features of sarcomatoid mesothelioma, localized fibrous tumors of the pleura, and sarcomas is summarized in **Table 5**.

Table 5 Immunohistochemical features of sarcomatoid mesothelioma, spindle cell carcinoma and sarcomas

	PK[a]	VIM	Actin	CD31	CD34	Desmin	S100	CALR
Sarcomatoid mesothelioma	+	++	+	--	--	--	--	+
Spindle cell carcinoma	+[b]	++	--	--	--	--	--	--
Sarcoma	--	++	+[c]	+[c]	+[c]	+[c]	+[c]	--

[a]PK; pan-cytokeratin; VIM: vimentin; CALR: calretinin.
[b]++, almost always positive, + mostly positive with variable staining; +[c], dependent on histological type of sarcoma; --, almost always negative.

In conclusion, applications of IHC to the diagnosis of pulmonary and pleural neoplasms have grown considerably as an increasing number of antibodies have been developed in the last decade. These immunohistochemical markers are invaluable for complementing morphologic evaluation and maximizing the information that can be derived from human tissue samples. As IHC becomes integrated into more clinical decision-making algorithms, its scope will likely expand further.

Address all correspondences to Dongfeng Tan, MD, Department of Pathology, Unit 85, MD Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA. Tel: 713-745-4977; Fax: 713-745-1105; Email: dtan@mdanderson.org

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