Expression of Cytokeratin 5/6 in Epithelial Neoplasms: An Immunohistochemical Study of 509 Cases

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Cytokeratin 5/6 (CK 5/6) immunoreactivity has been observed in the vast majority of cases of malignant mesothelioma but only rarely in pulmonary adenocarcinomas. Thus, CK 5/6 has been used to distinguish malignant mesothelioma from pulmonary adenocarcinoma. However, the utility of CK 5/6 in distinguishing pleural malignant mesothelioma from pleural metastases from nonpulmonary adenocarcinoma, as well as peritoneal malignant mesothelioma from peritoneal metastatic adenocarcinoma, has not yet been adequately addressed because the tissue expression of CK 5/6 in nonpulmonary neoplasms has not been well defined. We have studied the CK 5/6 expression in 509 cases of various epithelial tumors by immunohistochemistry. We found that the vast majority of cases of squamous cell carcinoma, basal cell carcinoma, thymoma, salivary gland tumor, and biphasic malignant mesothelioma were positive for CK 5/6. In addition, CK 5/6 immunoreactivity was detected in 15 of 24 cases (62%) of transitional cell carcinoma, in 5 of 10 cases (50%) of endometrial adenocarcinoma, in about one third of cases of pancreatic adenocarcinoma (38%) and breast adenocarcinoma (31%), and in one quarter of cases of ovarian adenocarcinomas (25%). Our study confirms the diagnostic utility of CK 5/6 immunohistochemistry in distinguishing biphasic mesothelioma from pulmonary adenocarcinoma but raises caution about its use for the differential diagnosis of pleural or peritoneal malignant mesothelioma *versus* pleural or peritoneal metastatic nonpulmonary adenocarcinoma, because many types of nonpulmonary adenocarcinomas may be positive for CK 5/6.

KEY WORDS: Cytokeratin 5/6, Immunohistochemistry, Mesothelioma, Nonpulmonary adenocarcinoma, Pulmonary adenocarcinoma.

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Malignant mesothelioma (MM) arises from the serous membranes of various body cavities. About three quarters of MM derive from the pleural cavity; one quarter are from the peritoneal cavity; approximately 2% of MMs arise in the pericardium; and a smaller number originate in the tunica vaginalis testis (1, 2). Unlike the case with other adenocarcinomas, the diagnosis of MM is rarely made based on routine histology alone and must be confirmed by immunohistochemistry because many mimics can present a similar morphologic appearance at the same anatomic sites (*e.g.*, the pleural and peritoneal cavities) as those typically involved by MM. Distinguishing MM and adenocarcinoma from various organs has been problematic for years because of the lack of completely sensitive and specific mesothelial markers. In recent years, several monoclonal antibodies have been found to be almost exclusively expressed in MM and only rarely in pulmonary adenocarcinomas, or to be almost exclusively expressed in pulmonary adenocarcinomas and only rarely in MM. The former antibodies include cytokeratin 5/6 (CK 5/6) (3), *N*-cadherin (4), calretinin (5), thrombomodulin (6), MoAb 44–3A6 (7), and CD44H (8); and the latter antibodies include MOC 31 (9), E-cadherin (10), carcinoembryonic antigen (CEA), BER-ER4, B72.3, BG8, and CD15 (11).

In recent years, CK 5/6 has emerged as one of most useful positive markers for confirming a diagnosis of MM when used in the proper way and on the appropriate setting (12). However, approximately 14% of adenocarcinomas have been reported also to be positive for CK 5/6 (3, 8). Thus, it is important to know which types of adenocarcinomas are positive for CK 5/6 when one is considering use of CK 5/6 antibody to distinguish MM from nonpulmonary adenocarcinoma.

The purpose of the current study is to perform comprehensive testing of CK 5/6 to determine which types of adenocarcinoma may be CK 5/6 positive and to determine the frequency of positivity.

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MATERIALS AND METHODS

Cases

509 cases of carcinomas from different organs were selected from our surgical pathology files. The diagnoses were reconfirmed. All tissues had been fixed in 10% neutral formalin and embedded in paraffin. The organ origins of carcinomas are listed in Table 1. Of 26 cases of breast carcinoma, 20 cases were ductal and six were lobular carcinoma. Of 28 cases of salivary gland tumors, 11 cases were mixed tumor, nine were adenoid cystic carcinoma, four were Warthin's tumor, three were ductal adenocarcinoma, and one was a mucoepidermoid tumor. Of 54 cases of thyroid tumor, 24 cases were follicular adenoma, 16 were medullary carcinoma, five were follicular carcinoma, and nine were papillary carcinoma. Squamous cell carcinomas were derived from head and neck (11 cases), lung (four cases), and esophagus (10 cases). Of 15 cases of small cell carcinoma, nine were from skin (Merkel cell carcinoma), five from lung, and one from parotid gland.

Immunohistochemistry

Commercially available CK 5/6 antibody was purchased from Chemicon International, Inc (dilution 1:200, Temecula, CA). Serial 5-μm sections were cut from each case. The sections were deparaffinized and rehydrated in graded alcohol. For heat-induced epitope retrieval, the sections were subjected into 100 mM EDTA buffer (pH 8.0) in a steamer (Black & Decker, Shelton, CT) at 100° C for 20 minutes. The sections were then brought to an automated stainer (DAKO Corporation, Carpinteria, CA) according to the vendor's protocol. EnVision Plus and peroxidase detection methods were used.

Cytoplasmic immunoreactivity was assessed. Only those cases showing >5% tumor cell positivity were regarded as positive.

TABLE 1. CK 5/6 in Epithelial Neoplasms

Organs	Tumor Types	Total Cases (*n*)	Positive Cases (*n*)	%
Skin & mucosa	Squamous cell carcinoma	25	25	100
Skin	Basal cell carcinoma	20	20	100
Thymus	Thymoma	8	8	100
Salivary gland	All tumors	28	26	93
Mesothelioma	Biphasic	17	13	76
	Sarcomatoid	12	3	25
Bladder	Transitional cell carcinoma	24	15	62
Uterus	Endometrioid carcinoma	10	5	50
Pancreas	Adenocarcinoma	13	5	38
Breast	Adenocarcinoma	26	8	31
Ovary	Adenocarcinoma	24	6	25
Liver	Cholangiocarcinoma	14	2	14
Lung and GI	Carcinoid tumor	10	1	10
Multiple[a]	Undifferentiated carcinoma	27	2	7
Lung	Adenocarcinoma	21	1	5
Liver	Hepatocellular carcinoma	28	1	4
Adrenal	Cortical tumor	20	0	0
Colon	Adenocarcinoma	20	0	0
Germ cell	Germ cell tumor	14	0	0
Kidney	Carcinoma	19	0	0
Lung, liver, & GI	Neuroendocrine carcinoma	9	0	0
Lung & skin	Small cell carcinoma	15	0	0
Prostate	Adenocarcinoma	18	0	0
Soft tissue	Epithelioid sarcoma	12	0	0
Soft tissue	Synovial sarcoma	6	0	0
Stomach	Adenocarcinoma	15	0	0
Thyroid	All tumors	54	0	0
Total		509	141	27

GI, gastrointestinal tract.

[a] Undifferentiated carcinoma are from lung, stomach, breast, thyroid, nasopharynx, ovary, and parotid gland.

RESULTS

CK 5/6 positivity was cytoplasmic. The results of staining with CK 5/6 are summarized in Table 1.

CK 5/6 in Malignant Mesothelioma

Thirteen of 17 cases of biphasic MM (76%) and 3 of 12 cases of sarcomatoid MM (25%) were positive for CK 5/6. The CK 5/6 immunoreactivity was mainly identified in the epithelioid component in biphasic MM, whereas rare spindle cells in biphasic MM and in sarcomatoid MM were positive.

CK 5/6 in Carcinomas from Stratified Epithelia

All 25 cases of squamous cell carcinoma and all 20 cases of basal cell carcinoma showed strong cytoplasmic positivity for CK 5/6 (Fig. 1A-B). There was no difference in staining intensity in carcinomas showing different grades of differentiation or of different origin. Fifteen of 24 cases of transitional cell carcinoma were positive for CK 5/6 (63%). The CK 5/6 staining pattern and intensity varied between well differentiated and poorly differentiated transitional cell carcinoma. In low-grade papillary

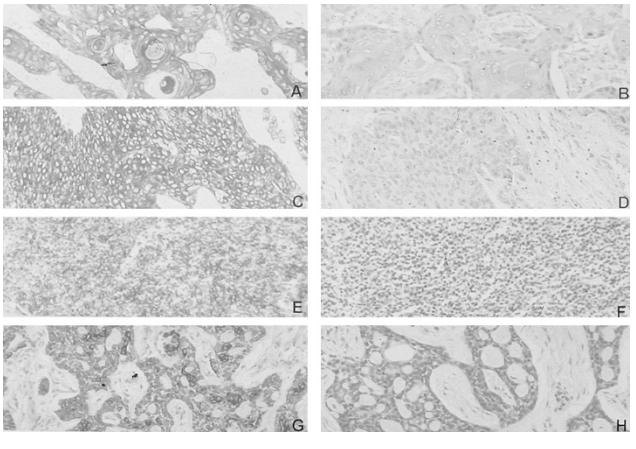

FIGURE 1. CK 5/6 expression in carcinomas from stratified epithelia. The CK 5/6 immunoreactivity is diffuse in squamous cell carcinoma (**A**), transitional cell carcinoma (**C**), and thymoma (**E**), and in adenoid cystic carcinoma of the salivary gland (**G**). The corresponding hematoxylin and eosin stained sections are shown at the right as **B**, **D**, **F**, and **H**, respectively.

transitional cell carcinoma (TCC), the CK 5/6-positive cells were observed at the basal layers of the papillae; whereas in high-grade TCC, tumor cells were diffusely positive for CK 5/6 in some cases (Fig. 1C-D). All eight cases of thymoma were positive for CK 5/6. The positive tumor cells usually formed a network between small lymphocytes (Fig. 1E-F). Twenty-six of 28 cases of salivary gland tumor were positive for CK 5/6. CK 5/6–positive cells were seen at the basal layers of epithelial projections in Warthin's tumor, spindled (myoepithelial) and glandular cells in mixed tumor, and in all tumor cells in adenoid cystic carcinoma (Fig. 1G-H) and salivary gland ductal adenocarcinoma.

CK 5/6 in Adenocarcinomas from Simple Epithelia

In contrast to carcinomas of stratified epithelia in which the tumor cells were diffusely positive for CK 5/6, adenocarcinomas were frequently focally positive for CK 5/6. Five of 10 cases of endometrial adenocarcinoma (50%) and 6 of 24 cases of ovarian adenocarcinoma (25%) were positive for CK 5/6. Serous papillary (Fig. 2A-B), endometrioid (Fig. 2C-D), and clear-cell adenocarcinomas were positive in a significant subset of cases. Eight of 20 cases of invasive breast ductal carcinoma were positive for CK 5/6 (40%; Fig. 2E-F), whereas none of six cases of lobular carcinoma were positive. Five of 13 cases (38%) of pancreatic adenocarcinoma were positive for CK 5/6. In addition, occasional cases of undifferentiated carcinoma (2/27), cholangiocarcinoma (2/14), hepatocellular carcinoma (1/28), carcinoid tumor (1/10), and pulmonary adenocarcinoma (1/21) were focally positive for CK 5/6. In undifferentiated carcinomas, CK 5/6 positivity was found in areas with squamous differentiation.

In prostate, all 18 cases of adenocarcinoma were negative for CK 5/6, whereas CK 5/6 highlighted basal cells of normal prostatic glands, resembling the staining pattern of high molecular weight keratin as identified by antibody 34βE12 in normal prostate tissue. As in prostate, CK 5/6 also stained myoepithelial cells of breast lobules and ducts, as well as intraductal carcinoma.

There was no CK 5/6 immunoreactivity detected in adrenal cortical tumor (0/20), colon adenocarcinoma (0/20), gastric adenocarcinoma (0/15), thyroid tumors (0/54), small cell carcinoma (from skin, lung, and parotid gland) (0/15), large cell neuroendocrine carcinoma (0/9), renal cell carcinoma (0/19), germ cell tumor (0/14), and soft-tissue sarcoma with an epithelioid component (epithelioid sarcoma and biphasic synovial sarcoma; 0/21).

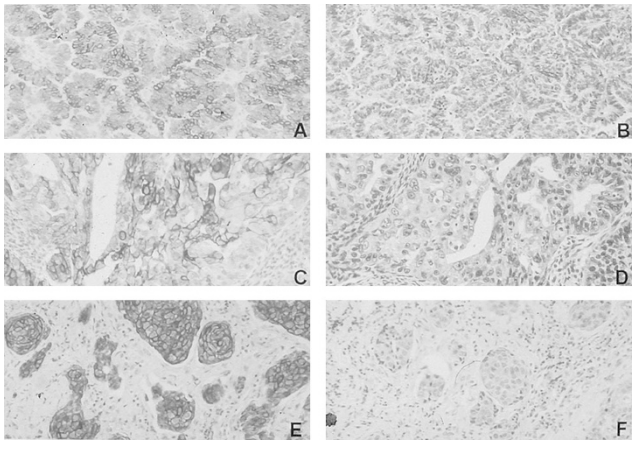

FIGURE 2. CK 5/6 expression in adenocarcinomas from simple epithelia. The CK 5/6 immunoreactivity in a serous papillary adenocarcinoma of the ovary (**A**) and is focally positive in an endometrioid adenocarcinoma of the uterus (**C**) and is diffuse in an infiltrating ductal carcinoma of the breast (**E**). The corresponding hematoxylin and eosin stained sections are shown at the right as **B**, **D**, and **F**, respectively.

DISCUSSION

CK5/6 are intermediate-sized basic keratins. In normal tissue, CK5/6 are mainly expressed in keratinizing (epidermis) and nonkeratinizing (mucosa) squamous epithelium, as well as in basal–myoepithelial cell layer of the prostate, breast, and salivary glands. CK5/6 are also seen in benign and malignant tumors of epidermal, squamous mucosal, and myoepithelial origins (13, 14). In 1989, Moll *et al.* (15) demonstrated CK 5 protein in 12 of 13 cases of biphasic malignant mesothelioma (MM) but in none of 21 cases of pulmonary adenocarcinomas. This work did not draw most pathologists' attention until approximately 10 years later, when Ordóñez (3) found that all of 40 cases of mesothelioma and none of 30 cases of pulmonary carcinomas were positive for CK 5/6. Additional study by Cury et al. (8) confirmed these observations. CK 5/6 is currently used by many pathologists as one of several mesothelial markers in the immunohistochemical differential diagnosis of MM *versus* adenocarcinoma.

Two issues should be considered when using CK 5/6 as an aid to the differential diagnosis of MM *versus* pleural metastatic tumors. First, the positive rate of CK 5/6 in sarcomatoid MM is much lower (30%) than that found in biphasic MM (76%). Although the current study and the study by Attanoos *et al.* (16) showed that only one third of cases of sarcomatoid mesothelioma expressed CK 5/6, they can usually be distinguished from metastatic adenocarcinoma by routine histologic examination. In fact, sarcomatoid MM is more often confused with primary or metastatic pleural or peritoneal-based sarcomas. In this case, other mesothelial markers, such as thrombomodulin and calretinin, should be

TABLE 2. Summary of Reported CK 5/6–Positive Tumors from Simple Epithelia

Organs	Tumor Types	Current	Ordóñez (3)	Baschinsky et al. (18)	Cury et al. (8)	%
Pancreas	Adenocarcinoma	5/13	ND	5/10	0/1	42
Uterus	Endometrioid carcinoma	5/10	2/10	ND	1/1	38
Ovary	Adenocarcinoma	6/24	10/30	ND	1/6	28
Breast	Adenocarcinoma	8/26	1/18	ND	2/21	17
Liver	Cholangiocarcinoma	2/14	ND	2/10	ND	17
Lung and GI	Carcinoid tumor	1/10	ND	ND	ND	10
Lung etc.	Undifferentiated carcinoma	2/27	ND	ND	ND	7
Colon	Adenocarcinoma	0/20	0/10	ND	3/10	7
Liver	Hepatocellular carcinoma	1/28	ND	0/10	ND	4
Kidney	Carcinoma	0/19	0/10	ND	1/4	3
Lung	Adenocarcinoma	1/21	0/30	ND	1/19	3
Thyroid	All tumors	0/54	1 /7	ND	ND	2
Adrenal	Cortical tumor	0/20	ND	ND	ND	0
Germ cell	Germ cell tumor	0/14	ND	ND	ND	0
Lung & GI	Neuroendocrine carcinoma	0/9	ND	0/13	ND	0
Lung & skin	Small cell carcinoma	0/15	ND	ND	ND	0
Prostate	Adenocarcinoma	0/18	0 /8	ND	ND	0
Stomach	Adenocarcinoma	0/15	ND	ND	ND	0

ND, not done. GI, gastrointestinal tract.

used in conjunction with CK 5/6. Attanoos *et al.* (16) have shown that approximately one third of cases of sarcomatoid mesothelioma expressed at least one of those three markers, whereas other spindle cell sarcomas are virtually always negative.

The second consideration in the use of CK 5/6 as an aid to the diagnosis of MM is that a variety of adenocarcinomas may also be positive for CK 5/6 (Table 2). Ordóñez (3) and Cury *et al.* (8) both studied CK 5/6 expression in adenocarcinoma; they showed that approximately 15% of adenocarcinomas of various tissue origins are positive for CK 5/6. The current study obtained a slightly lower CK 5/6 positive rate of 9% (Table 1), probably because of variation in the types of adenocarcinomas studied. Interestingly, all three studies demonstrated that CK 5/6–positive adenocarcinoma was mainly derived from the uterus, ovary, breast, pancreas, and biliary tract (Table 3). Breast adenocarcinoma may be easily confused with pleural MM when it metastasizes to the pleura. Additional immunohistochemical markers may be helpful, such as estrogen receptor (ER), GCDFP-15, CEA, BerEP4, MOC31, CD15, and BG8 for breast cancer and thrombomodulin and calretinin for MM. It may also be problematic when CK 5/6 is used to help differentiate a peritoneal MM from peritoneal metastatic adenocarcinomas, as metastatic pancreatic, endometrial, or ovarian adenocarcinoma have among the highest rates of CK 5/6 positivity (30–40%) among adenocarcinomas arising from simple epithelia. These adenocarcinomas frequently exhibit papillary or glandular features that are also frequently seen in MM. In addition, adenocarcinomas from the pancreas, uterus, and ovary frequently metastasize to the peritoneal cavity and may even express other mesothelial markers, such as thrombomodulin, CD44H, and calretinin (8). Therefore, it may be extremely difficult to distinguish a peritoneal mesothelioma from metastatic adenocarcinoma by histologic and immunohistochemical studies alone. Additional clinical history (asbestos exposure), radiologic findings, and other immunomarkers (ER, CEA, MOC-31, BerEp4, and CD15) may be crucial to establish the correct diagnosis.

TABLE 3. Expression of CK (Cytokeratin) 5/6, CK 7, and CK 14 in Malignant Mesothelioma, Adenocarcinoma, and Squamous Cell Carcinoma

Tumor Types	Cytokeratin 5/6	Cytokeratin 7	Cytokeratin 14
MM	+++/−	+++/−	—
Adenocarcinoma	—/+	++++	—
SCC	++++	—	++++

MM, malignant mesothelioma; SCC, squamous cell carcinoma.

Assessment of CK 5/6 immunoreactivity may be useful in several other diagnostic situations. Like other high-molecular weight cytokeratins such as CK 14 (17), CK 5/6 protein is expressed mainly in stratified epithelia and cognate neoplasms (Table 1). Therefore, similar to what has been described with CK 14, CK 5/6 may be used as an aid to the identification of squamous differentiation. CK 5/6 may also be useful in the differential diagnosis of hepatocellular carcinoma (rarely CK 5/6 positive) *versus* metastatic pancreatic carcinoma (CK 5/6 positive in one third to one half of cases), as first described by Baschinsky *et al.* (18). Third, CK 5/6 may be useful in the immunodiagnosis of bladder *versus* prostate carcinoma. The current study demonstrated that 63% of transitional cell carcinoma were positive for CK 5/6, whereas all cases of prostate adenocarcinomas were CK 5/6 negative. In addition, because CK 5/6 stains basal cells of benign prostatic glands but not malignant glands, CK 5/6 antibodies may be useful in the distinction of benign *versus* malignant prostate proliferation, similar

to 34βE12 antibody. In fact, Abrahams *et al.* (19) found that CK 5/6 had superior sensitivity (97%) as compared with 34βE12 (33%) in reliably staining atrophic prostatic glands.

In summary, the current study has confirmed the utility of CK 5/6 in distinguishing mesothelioma from pulmonary adenocarcinoma and from nonpulmonary adenocarcinoma, if used in conjunction with histology, other immunomarkers, clinical history, and radiological findings. CK 5/6 stains carcinomas from stratified epithelia and myoepithelial cells of various tissue origins; thus, it can be used as a marker for squamous cell carcinoma, basal cell carcinoma, transitional cell carcinoma, salivary gland tumors, and thymoma. CK 5/6 may also be useful in separating benign from malignant prostatic glands. Whether CK 5/6 is a superior marker to 34βE12 for the diagnosis of prostate carcinoma requires additional studies.

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