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Thyroid Transcription Factor-1, Cytokeratin 7and Cytokeratin 8 for Differentiating Primary and Metastatic Lung Adenocarcinoma

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Abstract: Adenocarcinomas are the most common cause of malignancy in pleura fluids. Usual primary sites include the lung, breast, gastrointestinal tract and genitourinary tracts. Predicting the site of origin of an adenocarcinoma is difficult due to overlapping morphologic characteristics. Thyroid transcription factor-1 (TTF-1), Cytokeratin7 (CK7) and Cytokeratin8 (CK8) were investigated to differentiate the primary and metastatic lung adenocarcinoma in 14 pleura fluid samples. There was a significant difference between TTF-1, CK 7 and CK 8 expressions in primary lung adenocarcinomas (P=0.028; Chi-squared test) and in metastatic lung adenocarcinomas (P=0.004; Chi-squared test). The sensitivity of TTF-1, CK7 and CK8 as a marker for primary lung adenocarcinomas were 0, 87 and 57%, respectively. Moreover, the sensitivity of TTF-1, CK7 and CK8 as a marker for metastatic lung adenocarcinomas were 16, 86 and 75%, respectively. Overall results for primary lung adenocarcinomas demonstrated CK8 reactivity in 4 (57%) of 7 cases. Nine metastatic lung adenocarcinoma samples (75%) were positive for CK8. TTF-1 was expressed in 16% of metastatic lung adenocarcinomas, but negative TTF-1 staining was observed in all primary lung adenocarcinoma cases. It was concluded that these results confirmed that the method of TTF-1, CK7 and CK8 immunohistochemistry is not sensitive enough for the differential diagnosis of primary and metastatic lung adenocarcinomas.

Key words: Lung adenocarcinoma · Transcription factor-1 · Cytokeratine 7 · Cytokeratine 8 · Pleura fluids

INTRODUCTION

The incidence of lung cancer in the United States has been steadily increasingduring recent years; lung cancer now ranks as the primary cause of death from cancer in American men [1]. Non-small cell carcinomas account for about 85% of all lung cancers. About 60% of lung cancers are adenocarcinomas and about 26% are squamous cell carcinomas [2]. The proportion of adenocarcinomas (ADC) has been increasing recently and the percentage of women with adenocarcinoma has also been rising [3].

Adenocarcinomas are the most common epithelial malignancies found in body cavity fluids. Common primary sites include the lung, breast, gastrointestinal tract and genitourinary tract. Identification of the specific site of origin may have important prognostic as well as therapeutic implications. However, it is challenging,, to determine the primary site of adenocarcinoma based on cytomorphology alone. Tissue specific immunohistochemical markers offer an attractive means for confirming metastatic disease in patients with known primary tumors and they may assist in identifying the primary sites in patients with adenocarcinomas of unknown origin.

TTF-1 (thyroid transcription factor-1) is a tissue-specific homeodomain-containing transcription factor that plays an important role in the early differentiation and morphogenesis of the developing lung and thyroid gland. The expression of TTF-1 has been found in malignant tumors highly selectively in lung and thyroid cancers. In lung cancer, high frequency of TTF-1 expression has been observed in small cell carcinomas (85–90%) and in adenocarcinoma (75–80%), whereas squamous cell cancers and large cell carcinomas showed no expression at all, or expression at very low frequency [4].

Blobel *et al.* [5] showed that alveolar cells of human lung contained cytokeratin (CK) polypeptides typical of simple epithelia (CK7, 8, 18 and 19). Basal cells of

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the bronchial epithelium, on the other hand, contained CK5 and small amounts of CK6. Consequently, it has been also found "simple-epithelium-type" cytokeratins in all adenocarcinomas and later also in squamous cell carcinomas [6]. CK8 is the most widely expressed cytokeratin in various epithelial cells and cancer cells among at least 21 related cytokeratins [7]. One study has demonstrated that CK8 is found in the serum of a subgroup of patients with non-small cell lung carcinomas [8]. CK7, a neutral-basic type II cytokeratin found in adenocarcinomas of breast and lung, among others [9 - 11].

The distinction between primary and metastatic lung adenocarcinoma could be difficult by routine histology. This study was undertaken to evaluate the clinical utility of TTF-1, CK7 and CK8 in the diagnosis of primary and metastatic lung adenocarcinoma in pleura fluids specimens.

MATERIALS AND METHODS

Cases: The cytological files of the Hannover Cytopathology Institute were reviewed during August 2009. The 22 pleural fluids were collected from 22 patients who were diagnosed with adenocarcinoma. Medical records were reviewed to verify that the cases were of primary lung origin and did not represent metastatic disease from primary tumors at other sites. The selection of patients was restricted to include those for whom the primary site of origin for the carcinoma was confirmed based on clinical findings (n=22). Slides that were stained previously by routine Giemsa methods were retrieved for each patient. The diagnosis of adenocarcinoma based on cytomorphology was confirmed. Classic cytologic features of malignancy included three-dimensional aggregates comprised of cells with increased nuclear-to-cytoplasmic ratios, irregular nuclear membranes, course chromatin, large irregular nucleoli and finely vacuolated cytoplasm. The sensitivity of CK7, CK8 and TTF-1 in pleura fluid samples for primary lung adenocarcinomas was calculated.

Immunocytochemical Staining: Cytological smears were prepared by the standard cytologic method. The slides were hydrated in decreasing ethanol solutions. Endogenous peroxidase was blocked with hydrogen peroxidase for 3 minutes. The slides were rinsed in water and then incubated with the biotin blocking system (Dako) prior to the application of the primary antibody. The TTF-1, CK7 and CK8 monoclonal antibodies were used at a dilution of 1:100 and incubated with the samples for one hour. The slides were then rinsed in buffer and incubated for 25 minutes with the linking solution (LSAB+ kit; Dako; biotinylated antimouse, antirabbit and antigoat). This was followed by a rinse in buffer and incubation with streptavidin peroksidase for 25 minutes. After rinsing in buffer, the slides were submerged in AEC for 5 minutes. The slides were counterstained with Mayer's hematoxilen. Only cytoplasmic staining was regarded as a positive result. The intensity of staining was graded on the following scale: 0 (negative), 1+ (weak), 2+ (moderate) and 3+ (strong). All material was evaluated blindly by two observers.

Statistical analysis was performed using the chi-squared test and the orthogonal test.

RESULTS

The results of TTF-1, CK7 and CK8 immunostaining of primary lung adenocarcinomas are shown in Table 1. CK7 was expressed in 87% of primary lung adenocarcinomas. Positive immunoreactivity for CK7 was characterized by a red, diffusely cytoplasmic staining in tumor cells that occurred singly or in groups (Fig. 1). The 9 (100%) pleura fluids from adenocarcinoma of lung origin were negative for TTF-1. The 4 (57%) primary lung carcinoma samples were positive for CK8 (Fig. 2) (Table 1). There was a significant difference between TTF-1, CK7 and CK8 expressions in primary lung adenocarcinomas (P=0.028; Chi-squared test). The statistical values are given in Table 2.

Table 3 shows the staining patterns of metastatic lung adenocarcinomas from pleural effusions for TTF-1, CK7 and CK8. For all metastatic lung adenocarcinomas studied, 2(16%) of the cases exhibited TTF-1, 6 (86%) exhibited CK7, 9 (75%) exhibited CK8 immunostaining.

There was a significant differenceamong TTF-1, CK7 and CK8 expressions in metastatic lung adenocarcinomas (P=0.004; Chi-squared test). The statistical values were given in Table 4. The sensitivity of TTF-1, CK7 and CK8 as markers for metastatic lung adenocarcinoma were 16 86 and 75%, respectively. CK7 had the highest sensitivity of the metastatic lung adenocarcinoma studied, with a sensitivity of 86%. Overall results for metastaic lung adenocarcinomas demonstrated TTF-1 reactivity in 2 (16%) of 13 cases (Fig. 3).

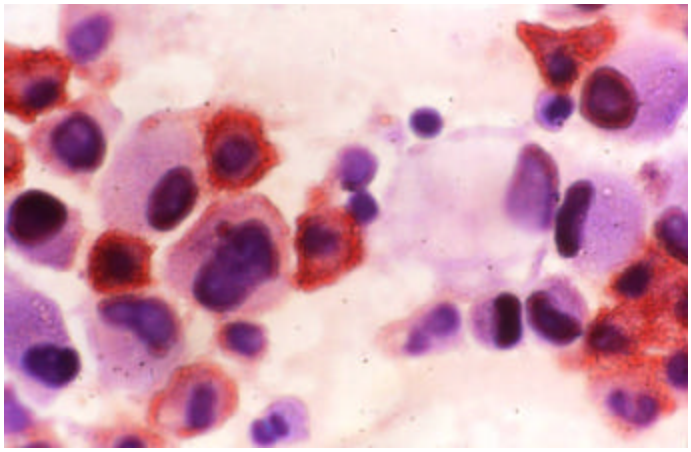

Fig. 1: CK7 positive staining in a case of primary lung adenocarcinoma (pleura, original magnification x 600)

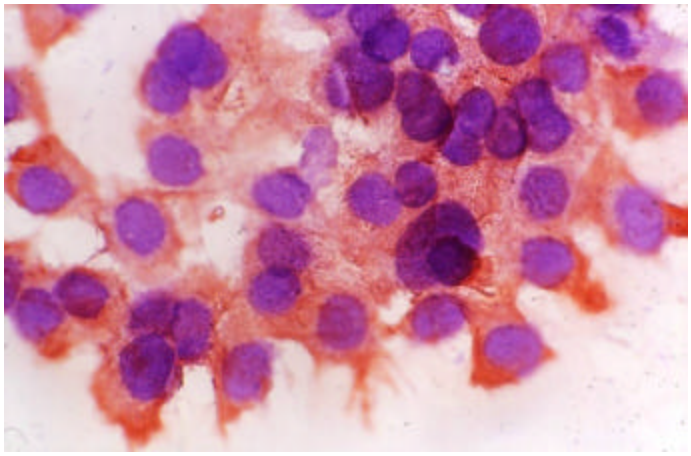

Fig. 2: Strong cytoplasmic staining for CK8 in a case of primary lung adenocarcinoma (pleura, original magnification x 600)

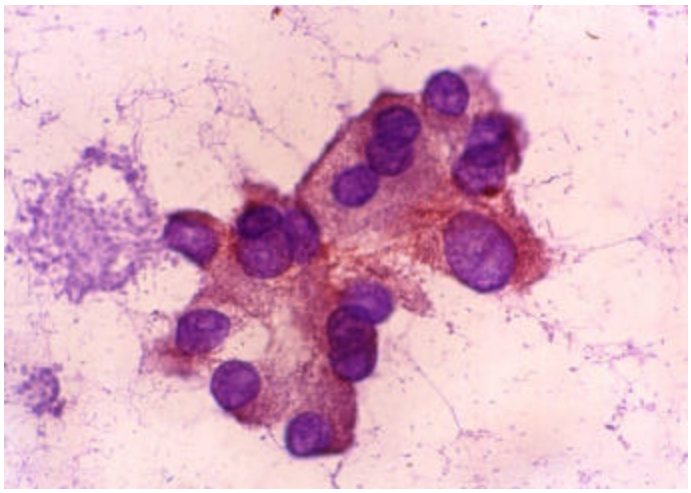

Fig. 3: TTF-1 positive staining in a case of metastatic lung adenocarcinoma (pleura, original magnification x 00)

Table 1: Staining intensity of TTF-1, CK7 and CK8 in primary lung adenocarcinoma

Tumor Markers	n	Negative n (%)	Positive n (%)	1+ (%)	2+ (%)	3+%)
Thyroid transcription factor-1	9	9(100)	0(0)	0(0)	0(0)	0(0)
Cytokeratin 7	8	1(13)	7(87)	3(37)	3(37)	1(13)
Cytokeratin 8	7	3(43)	4(57)	1(14)	2(29)	1(14)

Table 2: Sensitivity values of TTF-1, CK7 and CK8 in primary lung adenocarcinoma

Tumor Markers	Sensitivity =TP/(TP+FN)*100
TTF-1	0.00
CK7	87.50
CK8	57.14

Sensitivities were calculated from the formula (Sensitivity=True positive/(True positive +False negative)*100).

Table 3: Staining intensity of TTF-1, CK7 and CK8 in metastatic lung adenocarcinoma

Tumor Markers	n	Negative n (%)	Positive n (%)	1+ (%)	2+ (%)	3+ (%)
Thyroid transcription factor-1	13	11(84)	2(16)	1(8)	1(8)	0(0)
Cytokeratin 7	7	1(14)	6(86)	0(0)	4(57)	2(29)
Cytokeratin 8	12	3(25)	9(75)	4(33)	4(33)	1(9)

Table 4: Sensitivity values of TTF-1, CK7 and CK8 in metastatic lung adenocarcinoma

Tumor Markers	Sensitivity =TP/(TP+FN)*100
TTF-1	15.38
CK7	85.71
CK8	75.00

Sensitivities were calculated from the formula (Sensitivity=True positive/(True positive +False negative)*100).

DISCUSSION

Adenocarcinoma is the most common cause of malignancy in body cavity fluids. Usual primary sites include the lung, breast, gastrointestinal tract and genitourinary tracts. Predicting the site of origin of an adenocarcinoma can be difficult due to overlapping morphologic characteristics. Lung is a common primary site for adenocarcinomas in body cavity fluids [12-14]. the use of TTF-1, CK7 and CK8 to distinguish primary and metastatic adenocarcinomas of lung in 22 pleura fluids was investigated herein.

Organ specific markers using immunocytochemistry offer an attractive means for subclassifying adenocarcinomas according to primary site. CK7 and CK8 are tissue specific proteins expressed selectively in the epithelial cells of the lung [15]. Cytokeratins, belonging to the intermediate filament (IF) protein family, are particularly useful tools in oncology diagnostics. At present, more than 20 different cytokeratins have been identified, of which cytokeratins 8, 18 and 19 are the most abundant in simple epithelial cells. Upon release from proliferating or apoptotic cells, cytokeratins provide useful markers for epithelial malignancies. Previous examination has demonstrated CK 7 and CK 8 positivity in the vast majority of lung adenocarcinomas studied [8, 16]. Using commercially available monoclonal antibody for CK8, it demonstrated a sensitivity of 57.14% for primary lung adenocarcinomas. 7 primary lung carcinoma samples (87%) were positive for CK7. The sensitivity of CK7 as a marker for primary lung adenocarcinomas was 87.5% [16]. Oğuztüzün *et al.* [17] reported that CK8 and CK 7 reactivity in 72 and 65% of primary lung carcinoma samples.

In primary lung adenocarcinomas, most authors describe TTF-1 immunopositivity higher than 75%, however, within this group important differences could be observed [18]. Goldstein *et al.* has studied 40 bronchiolo-alveolar carcinomas and observed frequent (92%) and strong TTF-1 expression in nonmucinous tumors, whereas it was weak and was found only in 21% of the mucinous counterpart [19-20]. TTF-1 immunohistochemistry was also found to be very specific in distinguishing primary signet-ring cell carcinoma of the lung and of extrapulmonary origin, such as breast, stomach and colon [21, 22]. TTF-1 expression was observed as a highly

sensitive and specific immunomarker for distinguishing pulmonary and extrapulmonary adenocarcinomas also in malignant pleural effusion fluid [23, 24]. TTF-1 immunopositivity is characteristic for lung adenocarcinomas and thyroid cancers, but as the latter rarely metastasize to the serosal surfaces, therefore TTF1 is an important marker for malignant pleural fluid of pulmonary origin.

In conclusion, the present work domesnstrated the expression of TTF-1, CK 7 and CK 8 in 13 primary and 9 metastatic lung adenocarcinomas. It was found that TTF-1, CK 7 and CK 8 expressions are not valuable in distinguishing primary lung adenocarcinomas from adenocarcinomas that metastases to the lung.

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