Use of Caveolin-1, Thyroid Transcription Factor-1, and Cytokeratins 7 and 20 in Discriminating Between Primary and Secondary Pulmonary Adenocarcinoma from Breast or Colonic Origin

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The objectives of this study were firstly to compare the immunostaining patterns of antibodies against caveolin-1, thyroid transcription factor-1 (TTF-1), cytokeratin 7 (CK7) and cytokeratin 20 (CK20) in primary and secondary pulmonary adenocarcinomas of breast or colonic origin, and secondly, to investigate their use alone and in combination, in distinguishing between primary and secondary lung adenocarcinomas from breast or colonic origin. Of the 49 lung adenocarcinoma specimens that were enrolled in this study, 30 were primary pulmonary adenocarcinomas, and 19 (9, breast origin; 10, colonic origin) were metastatic pulmonary carcinomas. Immunohistochemical staining was used to detect the expression of caveolin-1, TTF-1, CK7, and CK20. Primary pulmonary adenocarcinoma most often had the CK7-positive/CK20-negative immunohistochemical phenotype and was either TTF-1 positive or caveolin-1 negative. Secondary pulmonary adenocarcinoma of breast origin most often had the CK7-positive/CK20-negative immunohistochemical phenotype and was either TTF-1 negative or caveolin-1 positive, while secondary pulmonary adenocarcinoma of colonic origin most often had the CK20-positive/CK7-negative immunohistochemical phenotype and was either TTF-1 negative or caveolin-1 positive. The results suggest that caveolin-1, TTF-1, or CK7/CK20 alone did not distinguish reliably between primary and secondary pulmonary adenocarcinomas originating from breast or colon. The use of a panel of antibodies that includes TTF-1, caveolin-1, and CK7/CK20 may have higher sensitivity in discriminating between primary adenocarcinomas and metastatic lung adenocarcinomas from breast or colonic origin.

Key Words: caveolin-1, cytokeratin 7, cytokeratin 20, pulmonary adenocarcinoma, thyroid transcription factor-1
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The lung is a very common site for metastatic disease. It is clinically important to differentiate between primary and secondary lung adenocarcinomas, because treatment and prognosis differ for patients with these malignancies. Pathologists are often asked to identify

the primary site. It has been reported that immunohistochemistry is a useful method for ascertaining the site of origin in such cases. However, until recently, the use of immunohistochemistry was hampered by the lack of commercially available antibodies that are specific for lung tumor-associated antigens.

Caveolin-1, a metastasis-promoting molecule, was first discovered as a tyrosine-phosphorylated target in Rous sarcoma virus (RSV)-transformed avian fibroblasts, suggesting a possible role for this protein in cellular transformation [1,2]. It is also the principal component of caveolae, which has been recognized as a key player in the regulation of several signal transduction molecules [3–5]. Recently, elevated expression of caveolin-1 was found to be associated with metastases and poor prognosis of prostate, esophageal and pancreatic carcinomas [6–8].

Thyroid transcription factor-1 (TTF-1), a 38-kDa protein, is located primarily in the nuclei of type II pneumocytes and Clara's cells in the lung, thyroid tissues, and the diencephalons of the brain [9,10]. Markers have recently been described to recognize carcinomas of pulmonary or thyroid origin. In lung cancer a high frequency of TTF-1 expression has been observed in small cell carcinomas (85–90%) and in adenocarcinoma (75–80%) [11].

Cytokeratin 7 (CK7), a 54-kDa basic cytokeratin protein, is expressed in a wide variety of epithelia, such as lung, breast, endometrium, urothelium, stomach, and skin adnexal glands [12]. Cytokeratin 20 (CK20) is a 46-kDa acidic protein and is distributed predominantly in carcinomas of the gastrointestinal, pancreatobiliary tracts, and mucinous ovarian tumors [13]. The coordinated expression of CK7 and CK20 has been used to determine the site of origin of carcinomas [14,15]. Each immunophenotype is associated with a group of epithelial neoplasms. For example, the CK7-positive/CK20-negative phenotype is seen in a wide variety of carcinomas, including those of the lung, breast and female genital tract, whereas the CK7-negative/CK20-positive phenotype is often associated with carcinomas of colorectal origin [15].

Limited information is available in the literature regarding the comparison of the diagnostic utility of these markers in distinguishing between primary lung carcinomas and nonpulmonary neoplasms. In the present study, we compared the immunostaining patterns of antibodies against caveolin-1, TTF-1, CK7, and CK20 in primary and secondary pulmonary adenocarcinomas, in addition to investigating their use alone and in combination for distinguishing between primary and secondary lung carcinomas from the breast or colon.

Materials and Methods

Tissue specimens were obtained from 49 cases of lung adenocarcinoma diagnosed at the Department of Pathology, Kaohsiung Medical University Chung-Ho Memorial Hospital, between 1998 and 2004. All tumors were surgical resection specimens. There were 30 primary pulmonary adenocarcinomas, nine metastatic adenocarcinomas from the breast, and 10 metastatic adenocarcinomas from the colon.

Hematoxylin and eosin staining was performed and the slides were examined under light microscope. Tissue microarray was prepared according to the authors' published method [16]. For each donor tissue block, two tissue cores were punched with a bone marrow aspiration needle (2 mm diameter). The cylinder was carefully transferred with forceps to a recipient metal paraffin block box. After all the cylinders were aligned in the box, the box was covered with a plastic cassette and then liquid wax was gently poured into the box until full. The box was then cooled to room temperature slowly. Before sectioning, the tissue array paraffin blocks were chilled to –20°C and removed from the box. Three-micrometer sections were cut and mounted on silane-coated slides.

For immunostaining, deparaffinized and rehydrated sections were heated in an oven at 121°C for 30 minutes in citrate buffer to retrieve antigenic activity and then they were cooled at room temperature. Endogenous peroxidase activity was inhibited by incubation with 0.3% hydrogen peroxide in methanol for 20 minutes. After nonspecific reactions had been blocked with 10% normal bovine serum, the sections were incubated with polyclonal antibodies to caveolin-1 alpha-isoform (1:50; Chemicon, USA) for 3 hours, and monoclonal antibodies to TTF-1 (1:50; M3575; DAKO), to CK7 (1:50; M7018; DAKO), and to CK20 (1:50; M7019; DAKO) for 40 minutes. The sections were first incubated with biotinylated goat anti-mouse/rabbit immunoglobulin (Ig) for 20 minutes and then with streptavidin–peroxidase complex for 20 minutes. Careful rinses were performed with several changes

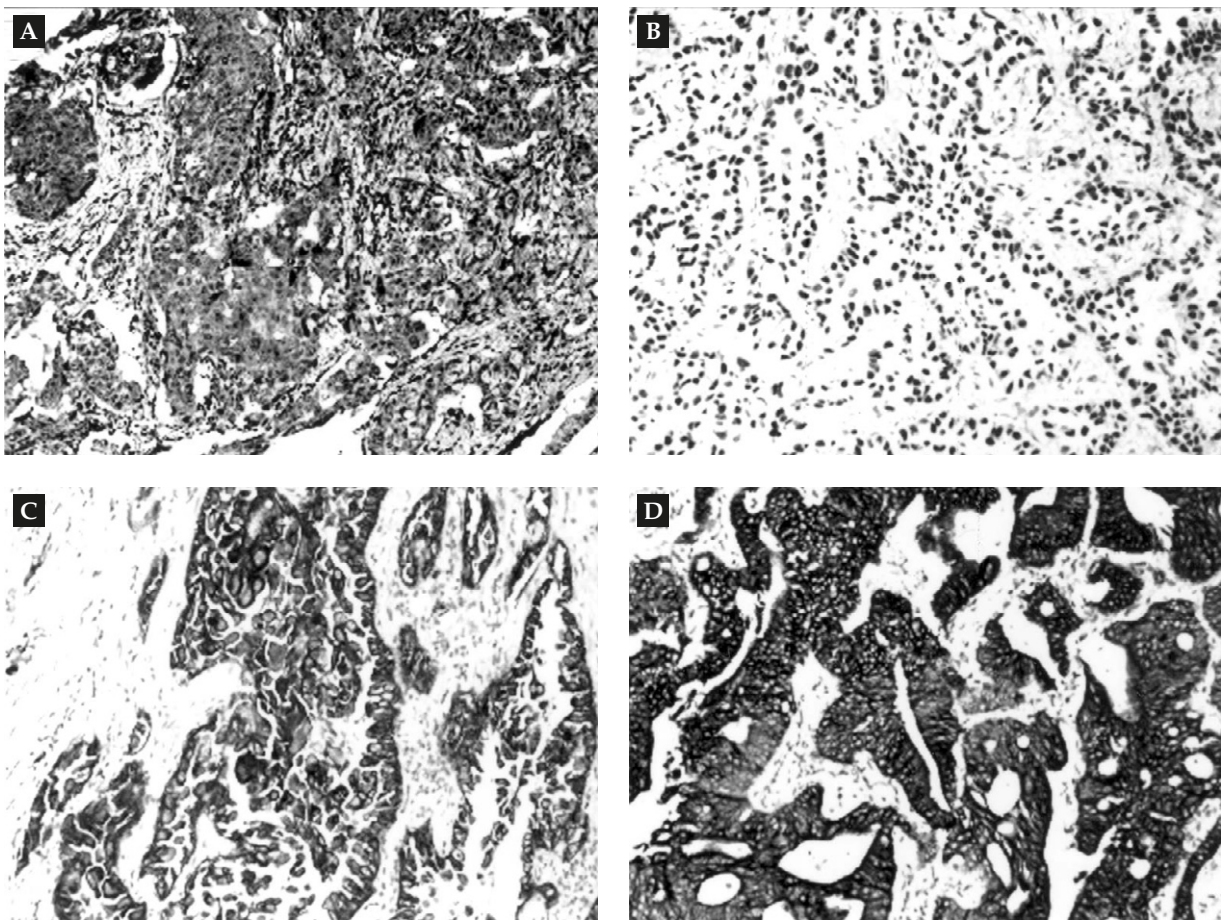

Figure. *(A) Breast origin lung adenocarcinoma shows strong staining for caveolin-1. (B) Primary lung adenocarcinoma shows strong staining for thyroid transcription factor-1. (C) Breast origin lung adenocarcinoma shows strong staining for cytokeratin 7. (D) Colonic origin lung adenocarcinoma shows strong staining for cytokeratin 20 (original magnification, 200×).*

of phosphate-buffered saline between each stage of the procedure. Finally, the sections were incubated in 3,3′-diaminobenzidine for 5 minutes. The slides were then rinsed gently with distilled water, counterstained in hematoxylin, dehydrated and mounted. Negative controls were performed by replacing the primary antibody with nonimmune rabbit IgG.

The staining slides were evaluated by two pathologists (Wang and Chai) who were blinded to knowledge of the clinicopathologic data. The intensity of the caveolin-1 immunostaining was evaluated by light microscopy as described elsewhere [17]. Caveolin-1 intensity was graded as follows: 1, no staining; 2, weak staining; 3, moderate staining; and 4, strong staining. For the purpose of further analysis, using a cut-off point to define two groups of negative and positive caveolin-1 expression, grades 1 or 2 were regarded as negative, and grades 3 or 4 were regarded as positive (Figure A). TTF-1 expression was based on the presence of nuclear staining whereas CK7 and CK20 expressions were based on the presence of cytoplasmic staining. Tumors were considered to show negative immunostaining if staining was found in fewer than 10% of neoplastic cells and positive if staining was present in more than 10% (Figure B–D).

Results

Patient ages ranged from 28 to 78 years (mean, 46.7 years) and the male-to-female ratio was 0.75 (21 males; 28 females).

The immunohistochemical findings are summarized in Table 1. TTF-1 and caveolin-1 positivity was shown in 70% (21/30) and 27% (8/30) of primary pulmonary adenocarcinomas. The CK7-positive/CK20-negative and CK7-negative/CK20-positive immunostaining patterns were demonstrated in 77%

Table 1. Results of immunohistochemical staining of primary and secondary pulmonary adenocarcinomas for caveolin-1, thyroid transcription factor-1 (TTF-1), cytokeratin 7 (CK7), and cytokeratin 20 (CK20)

Immunomarker	Lung origin ($n=30$)	Breast origin ($n=9$)	Colonic origin ($n=10$)
TTF-1			
Positive	21 (70%)	0 (0%)	0 (0%)
Caveolin-1			
Positive	8 (27%)	7 (78%)	6 (60%)
CK7/CK20			
CK7-positive/CK20-positive	0 (0%)	0 (0%)	0 (0%)
CK7-positive/CK20-negative	23 (77%)	5 (56%)	0 (0%)
CK7-negative/CK20-positive	7 (23%)	0 (0%)	9 (90%)
CK7-negative/CK20-negative	0 (0%)	4 (44%)	1 (10%)

Table 2. Caveolin-1 expression in cytokeratin 7 (CK7)-positive/cytokeratin 20 (CK20)-negative primary and metastatic pulmonary adenocarcinomas of breast origin

	Lung origin ($n=23$)	Breast origin ($n=5$)
Caveolin-1		
Positive	7 (30%)	5 (100%)
Negative	16 (70%)	0 (0%)

Table 3. Caveolin-1 expression in cytokeratin 7 (CK7)-negative/cytokeratin 20 (CK20)-positive metastatic pulmonary adenocarcinoma of colonic origin

	Colonic origin ($n=9$)
Caveolin-1	
Positive	5 (56%)
Negative	4 (44%)

(23/30) and 23% (7/30) of primary pulmonary adenocarcinomas. TTF-1 was not shown in metastatic pulmonary adenocarcinomas while caveolin-1 positivity was evident in 78% (7/9) and 60% (6/10) of metastatic pulmonary adenocarcinomas. A CK7-positive/CK20-negative immunostaining pattern was demonstrated in 56% (5/9) of pulmonary adenocarcinomas of breast origin, while a CK7-negative/CK20-positive immunostaining pattern was demonstrated in 90% (9/10) of pulmonary adenocarcinomas of colonic origin. Forty-four percent (4/9) of pulmonary adenocarcinomas of breast origin and 10% (1/9) of colonic origin showed a CK7-negative/CK20-negative immunostaining pattern.

Of the 23 CK7-positive/CK20-negative primary lung adenocarcinomas, only 30% (7/23) were caveolin-1 positive. Caveolin-1 was expressed in 100% (5/5) of CK7-positive/CK20-negative pulmonary adenocarcinomas of breast origin (Table 2). Caveolin-1 positivity was demonstrated in 56% (5/9) of CK7-negative/CK20-positive metastatic pulmonary adenocarcinomas of colonic origin (Table 3).

According to the above findings, the primary pulmonary adenocarcinoma most often has the CK7-positive/CK20-negative immunohistochemical phenotype and can be either TTF-1 positive or caveolin-1 negative. The secondary pulmonary adenocarcinoma of breast origin most often has the CK7-positive/CK20-negative immunohistochemical phenotype and can be either TTF-1 negative or caveolin-1 positive. The secondary pulmonary adenocarcinoma of colonic origin most often has the CK20-positive/CK7-negative immunohistochemical phenotype and can be either TTF-1 negative or caveolin-1 positive.

Discussion

It is important to determine whether a lung tumor is primary or secondary lung adenocarcinoma, because the treatment protocol and prognosis differ considerably for patients with these malignancies. A reliable immunohistochemical marker is required in differentiation between these malignancies. In this study, we compared four antibodies—caveolin-1, TTF-1, CK7 and CK20—as markers of primary and secondary lung adenocarcinomas of breast or colonic origin.

Caveolin-1 plays a key role in membrane traffic, normal vesicular transport, cholesterol homeostasis, and signal transduction [18]. Animal studies have found that caveolin-1 is most abundant in adipocytes,

endothelial cells, type I pneumocytes, fibroblasts and smooth muscle cells [18]. The application of caveolin-1 immunoexpression in predicting prognosis has been reported in some malignancies, such as squamous cell carcinoma of lung, pleomorphic carcinoma of lung, and clear-cell renal cell carcinoma [17,19,20]. Other studies have reported that caveolin-1 expression was upregulated in human cancers, including esophageal squamous cell carcinoma, lung adenocarcinoma and prostate cancer, and also that this upregulation was associated with metastasis [6,7,21]. The present study similarly reported that the intensity of caveolin-1 increased in secondary adenocarcinoma, be it either of breast or colonic origin. This research indicated that caveolin-1 plays a role in cancer cell metastasis.

Several previous studies have found that TTF-1 was useful as a marker of pulmonary carcinoma in histologic specimens [11,22–24]. Immunoreactivity for TTF-1 was present in 70–90% of primary pulmonary carcinomas. All metastatic breast or gastrointestinal adenocarcinomas were nonreactive for TTF-1. Similar results were obtained in our study. Although TTF-1 was fairly specific for pulmonary carcinomas, it showed a lower sensitivity for pulmonary carcinoma and, thus, was not useful as an independent marker in differentiating between primary pulmonary adenocarcinoma and metastatic adenocarcinoma.

Since the early 1990s, the clinical utility of monoclonal antibodies directed against CK20 paired with anti-CK7 antibodies in the differential diagnosis of primary and secondary lung cancers has been reported [25]. Adenocarcinoma from the colon is usually CK7 positive/CK20 negative, whereas adenocarcinoma from the lung or breast is usually CK7 negative/CK20 positive [26]. Although our present study obtained the same result, the combined panel of CK7/CK20 certainly has relatively low sensitivity and low specificity.

There is no report on the use of an antibody panel combining CK7, CK20, TTF-1, and caveolin-1 in differentiating between primary and secondary pulmonary adenocarcinomas of breast or colonic origin. In the current study, we confirmed that CK7, CK20, and TTF-1 expression are useful as immunohistochemical markers for the diagnosis of lung tumors and for the differential diagnosis of primary pulmonary adenocarcinomas from extrapulmonary adenocarcinomas metastatic to the lung. On the other hand, the accuracy of diagnosis seemed to increase when caveolin-1 was added to the panel of TTF-1 combined with CK7/CK20. In conclusion, we suggest that a panel comprising caveolin-1, TTF-1 and CK7/CK20 immunostains would be beneficial in the differential diagnosis of primary and secondary lung cancers of breast or colonic origin.

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利用 Caveolin-1，TTF-1，CK7 和 CK20 來區分原發性肺腺癌和來自乳房或大腸的轉移性肺腺癌

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本篇研究的目的首先是要比較 caveolin-1、TTF-1、CK7 和 CK20 個別在原發性肺腺癌和來自乳房或大腸的轉移性肺腺癌的表現並且調查這些因子單獨使用或是聯合這些因子的表現比較能區分原發性肺腺癌和來自乳房或大腸的轉移性肺腺癌。此次研究所選定的 49 個肺腺癌樣本，分別為 30 個原發性肺腺癌和 19 個轉移性肺腺癌(包括有 9 個來自乳房轉移和 10 個來自大腸轉移之肺腺癌樣本)。我們利用免疫組織化學染色的方法來評估 caveolin-1、TTF-1、CK7 和 CK20 的表現。當染色結果呈現 CK7 陽性/CK20 陰性並且 TTF-1 為陽性或 caveolin-1 陰性時最有可能的是原發性肺腺癌。當呈現 CK7 陽性/CK20 陰性及 TTF-1 陰性或 caveolin-1 陽性最可能是來自乳房轉移的肺腺癌。若染色結果為 CK20 陽性/CK7 陰性和 TTF-1 陰性或 caveolin-1 陽性則最可能是來自大腸轉移的肺腺癌。本篇研究結果顯示，單獨使用 caveolin-1、TTF-1、或 CK7/CK20 並不能有效的區別原發性肺腺癌和來自乳房或大腸的轉移性肺腺癌。聯合這些因子時敏感性較高比較能區分肺腺癌是原發性的或是從乳房、大腸轉移來的。

關鍵詞：caveolin-1，CK7，CK20，肺腺癌，TTF-1

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