

The value of immunohistochemical expression of TTF-1, CK7 and CK20 in the diagnosis of primary and secondary lung carcinomas

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ABSTRACT

الأهداف: دراسة مدى فائدة معامل الغدة الدرقية-1 (TTF-1) ومجموعة من المضادات المناعية للستوكيراتين-7 في التفريق بين أورام الرئة الأولية والثانوية الخبيثة.

الطريقة: شملت الدراسة 43 حالة من الأورام الخبيثة للرئة، و14 حالة ورم خبيث حرشفي، و12 حالة ورم خبيث طلائي، و8 حالات من أورام الخلايا الصغيرة، و3 حالات أورام خبيثة من الغشاء البلوري، و6 حالات أورام ثانوية. تم جمع المعلومات من ملفات المرضى في قسم علوم الأمراض في مستشفى الملك عبد العزيز الجامعي - جدة - المملكة العربية السعودية، في الفترة ما بين 2004م وحتى 2006م. صبغة جميع الحالات بمضادات معامل الغدة الدرقية-1 (TTF-1)، الستوكيراتين-20 (CK20)، سيتوكيراتين-7 (CK7) باستخدام الصبغة المناعية الهستوكيميائية.

النتائج: أظهر معامل الغدة الدرقية-1 (TTF-1)، صبغة موجبة في جميع نوايا خلايا الأورام الأولية للرئة مثل: الأورام الطلائية، أورام الخلايا الصغيرة، والأورام الثانوية الناشئة من الغدة الدرقية، في حين أظهرت صبغة سالبة في الأورام الحرشفية والأورام الثانوية وأورام الغشاء البلوري. أظهر سيتوكيراتين-7 (CK7) صبغة ايجابية في الأورام الأولية للرئة لمختلف أنواعها، كما أظهرت صبغة موجبة في حالات أورام الثدي، الغدة الدرقية الثانوية، وفي الرئة. أظهر الستوكيراتين-20 (CK20) صبغة إيجابية في حالات الأورام الثانوية الناشئة من القولون، في حين كان سالبا في باقي الأورام.

خاتمة: يتبين من الدراسة أن معامل الغدة الدرقية-1 (TTF-1) حساسة للأورام الأولية الناشئة من الرئة إذا استُبعدت الأورام الثانوية الناشئة من الغدة الدرقية، كما يمكن استخدامه للتفرقة بين الأنواع المختلفة من أورام الرئة في حالة ما إذا كانت الأورام عالية الدرجة سيتوكيراتين-7 (CK7). لا يمكن التفريق بين الأورام الأولية والثانوية للرئة لظهورها في عدة أورام أخرى غير الرئة مثل: أورام الثدي وأورام الغدة الدرقية. يعتبر الستوكيراتين-20 (CK20) من دلائل الأورام المميزة لأورام القولون الخبيثة الثانوية في الرئة.

Objective: To study the value of immunohistochemical staining of thyroid transcription factor-1 (TTF-1), cytokeratin 7 (CK7), and cytokeratin 20 (CK20) in the differentiation between primary and secondary pulmonary carcinomas.

Methods: Forty-three cases of lung carcinoma, 14 squamous cell carcinoma, 12 adenocarcinoma, 8 small cell carcinoma, 3 mesothelioma, and 6 metastatic tumors, were collected from the files of the Pathology Department, King Abdul-Aziz University Hospital, Jeddah, Saudi Arabia between 2004 and 2006. All cases were stained immunohistochemically following Avidin biotin method using monoclonal antibodies to TTF-1, CK7, and CK20.

Results: Immunohistochemical staining of 43 cases of lung carcinoma revealed nuclear immunoreactivity for TTF-1 in all primary adenocarcinoma, and small cell carcinoma, while cases of squamous cell carcinoma were negative. Mesotheliomas were negative to TTF-1, CK7, and CK20. Metastatic tumors (except for one case metastatic from the thyroid gland) were negative to TTF-1. Cytokeratin 7 was positively expressed in primary tumors of lung, as well as metastatic tumors from the thyroid and breast. Cytokeratin 20 was negative in all primary lung tumors, while positive in metastatic carcinomas from the colon.

Conclusion: Thyroid transcription factor-1 is a sensitive marker for diagnosis of primary pulmonary adenocarcinoma, and differentiation between poorly differentiated squamous cell carcinoma and small cell carcinoma and adenocarcinoma. Cytokeratin 20 could be a marker for metastatic tumors from the colon to the lung since it was negative in all primary lung tumors.

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Lung cancer is the leading cause of cancer death worldwide.¹ The lung is one of the most common visceral sites of metastasis from internal malignancies. The classic presentation of metastatic tumors to the lung is in the form of multiple bilateral peripheral nodules. Approximately 9% of metastatic tumors (particularly from colorectum, breast, kidney, and pancreas), present by an unusual growth pattern, whether as isolated growth (coin lesion) or it may present as endobronchial mass, which may be difficult to interpret as secondary tumor. Distinguishing a primary lesion from a solitary metastasis to the lung may be difficult based on morphology alone. A multidisciplinary approach that involves careful clinical history, thorough clinical and radiographic examination, and the application of special pathologic techniques as immunochemical studies are usually indicated.¹⁻³ Thyroid transcription factor-1 (TTF-1) is a tissue specific homeodomain containing DNA-binding protein of the Nkx-2 gene family. It plays an important role in the early differentiation and morphogenesis of the developing lung and thyroid. In adults, TTF-1 activates transcription of thyroglobulin and thyroperoxidase genes in the follicular thyroid cells. In lungs, it activates the promoters for Clara cell secretory protein, and surfactant apoproteins. It is selectively expressed in alveolar cells and in a subset of bronchiolar epithelial cells. It has also been found in benign lung tumors as sclerosing hemangioma, and alveolar adenoma.^{4,6} In cases of lung carcinomas the level of TTF-1 immunoreactivity varies extensively according to the tumor type, the difference in TTF-1 expression might be of clinical importance in distinguishing different histological types of lung carcinoma.^{7,8} Cytokeratins (CK) are soft epithelial intermediate filaments that comprise approximately 20 different keratin polypeptides. This family of intermediate filaments is found to be crucial in the diagnostic immunohistochemistry for the identification of carcinomatous differentiation and for identification of specific carcinoma subtypes.⁹ Cytokeratin 7 (CK7) is a simple keratin that has restricted distribution in many simple, stratified and ductal epithelium, such as breast, ovary, lung, uterus, transitional epithelium, pancreaticobiliary epithelium, and neuroendocrine cells. Cytokeratin (CK20) is a low molecular weight cytokeratin that is limited to the gastrointestinal epithelium and tumors, transitional cell carcinoma, and Merkel cell neoplasms.^{9,10} Many studies showed that combined use of CK7 and CK20 (with their specific tissue distribution) is of great value to identify colon cancer metastatic to the lung, to differentiate transitional cell carcinoma from squamous cell carcinoma and poorly differentiated carcinomas.^{9,10} The aim of the present

work is to investigate the potential use of TTF-1, CK7, and CK20 immunoreactivity to improve the diagnosis of different subtypes of lung carcinomas, and in the differentiation between primary and metastatic lung cancers particularly metastatic adenocarcinoma.

Methods. The material of the present study constituted 43 cases of lung biopsies collected from the files of the Pathology Department, King Abdul-Aziz University Hospital, Jeddah, Saudi Arabia between 2004 and 2006. Approval was obtained for the local ethics committee prior to the commencement of the study, and that informed patient consent was received from all study participants. Thirty-four cases were diagnosed as primary lung tumors based on clinical data, endoscopic features, and thorough body examination by the clinicians to exclude primary tumor elsewhere in the body, 12 cases were diagnosed as adenocarcinoma, 14 cases were diagnosed as squamous cell carcinoma, and 8 cases were diagnosed as small cell carcinoma. Three cases were diagnosed as mesothelioma. Six cases were metastatic tumors to the lung with previously known primary in breast (3 cases), colon (one case), thyroid (one case), and soft tissue sarcoma (one case). Primary lung tumors were classified histologically according to the World Health Organization criteria.¹¹ All sections were stained for Periodic Acid Schiff stain (PAS) and Periodic Acid Schiff-Diastase stain (PASD) in order to demonstrate intracytoplasmic mucin in cases of adenocarcinoma. Five-micrometer sections from selected tumor blocks were mounted on 3-aminopropyltriethoxysilane coated (Sigma, St. Louis, MO) slides and deparaffinized in xylene, rehydrated in graded alcohols, and rinsed in 0.05 M Tris-buffered saline. Endogenous peroxidase was inhibited in H₂O₂ for 30 minutes at 37°C. Heat-induced antigen retrieval was performed by boiling sections in 10 mM citrate buffer at pH 6.0. The sections were then incubated for one hour at room temperature with monoclonal antibodies to TTF-1 (Dako, Carpentina, USA) (1:100), cytokeratin-7 (Dako, Carpentina, USA) (1:50), and CK20 (Dako, Carpentina, USA) (1:50). Antibodies to synaptophysin and neuron specific enolase were used when needed to document a diagnosis of small cell lung carcinoma. The antigen-antibody complexes were visualized using 3, 3'-diaminobenzidine tetrachloride (DAB) as chromogen. All sections were then counterstained with hematoxylin, dehydrated, and cover-slipped. The TTF-1 was localized in the nucleus of tumor cells. Both CK 7, and CK 20 appeared as positive brown staining in the cytoplasm of tumor cells. The scoring system for tumor staining could not be applied to the studied cases due to small size of the specimens.

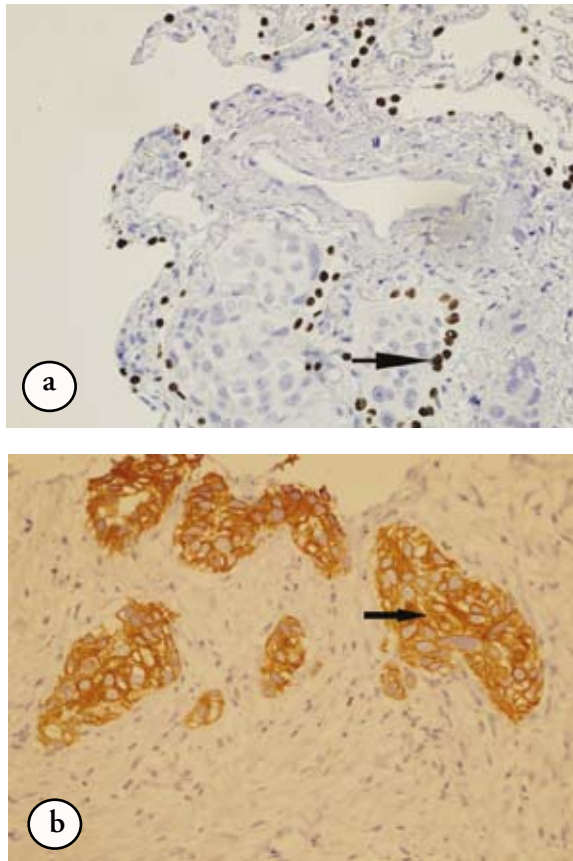


Figure 1 - Squamous cell carcinoma of the lung showing, a) negative staining of tumor cells to TTF-1, while adjacent normal pulmonary cells are immunopositive (ABC x 200), b) Squamous cell carcinoma case showing strong positive cytoplasmic staining for CK7 (ABC x 200).

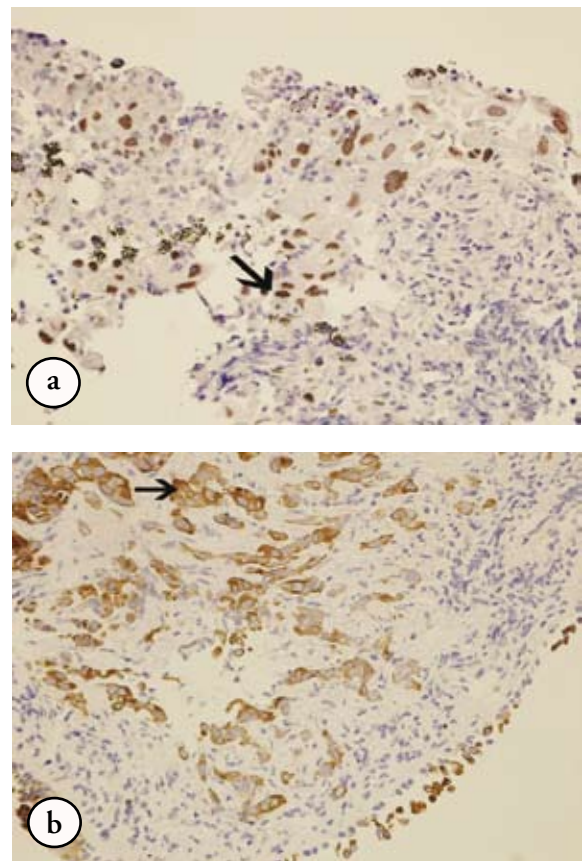


Figure 2 - Primary adenocarcinoma of the lung showing, a) strong positive nuclear staining of all tumor cells to TTF-1, b) diffuse cytoplasmic staining of tumor cells to CK7 (ABC x 200).

Results. Fourteen cases of primary squamous cell carcinoma were histologically diagnosed depending on the presence of evidence of keratinization in tumor cells and the absence of PAS, and PASD cytoplasmic staining. All cases showed negative immunoreactivity of tumor cells to TTF-1, and CK 20, while adjacent normal alveolar cells were strongly immunoreactive to TTF-1. Tumor cells showed strong cytoplasmic brown staining to CK 7, which appeared in more than 50% of tumor cells (Figure 1). Twelve cases were diagnosed as primary adenocarcinoma, tumor cells showed evidence of glandular formation and intracytoplasmic mucin secretion evidenced by PAS and PASD positive cytoplasmic staining. All cases showed positive immunoreactivity to TTF-1 in more than 50% of neoplastic cells as intense nuclear brown staining in tumor cells, regardless of the tumor grade, positive strong diffuse cytoplasmic staining to CK7, while all cases were negative to CK20 (Figure 2). Eight cases

were diagnosed as small cell lung carcinoma based on the presence of small cells arranged in sheets, having hyperchromatic nuclei, inconspicuous nucleoli, and scanty cytoplasm. Diagnosis was supported by positivity of these tumor cells to synaptophysin and neurospecific enolase. Small cell carcinoma cells showed strong diffuse immunoreactivity of tumor cells to TTF-1 and CK7, while they were absolutely negative to CK20 (Figure 3). Mesothelioma cases showed negative immunoreactivity to TTF-1, CK7, and CK20. As regards metastatic carcinomas, all the adenocarcinoma cases (except for metastatic thyroid carcinoma) showed negative immunoreactivity to TTF-1, while metastatic thyroid carcinoma, showed intense nuclear immunostaining. Metastatic carcinoma from breast showed positive CK7 and negative CK20, while metastasis from colon showed positive immunoreactivity to CK20 and negative immunoreactivity to CK7. One case of metastatic sarcoma showed negative immunoreactivity to TTF-1, CK7, and CK20. (Figure 4).

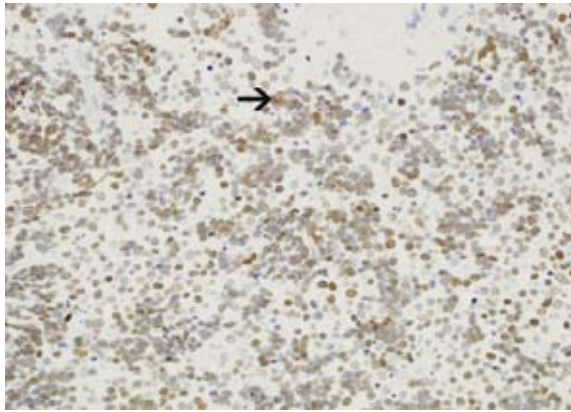


Figure 3 - A case of small cell carcinoma showing positive immunoreactivity to TTF-1 in the form of nuclear staining of more than 50% of tumor cells. (ABC x 200).

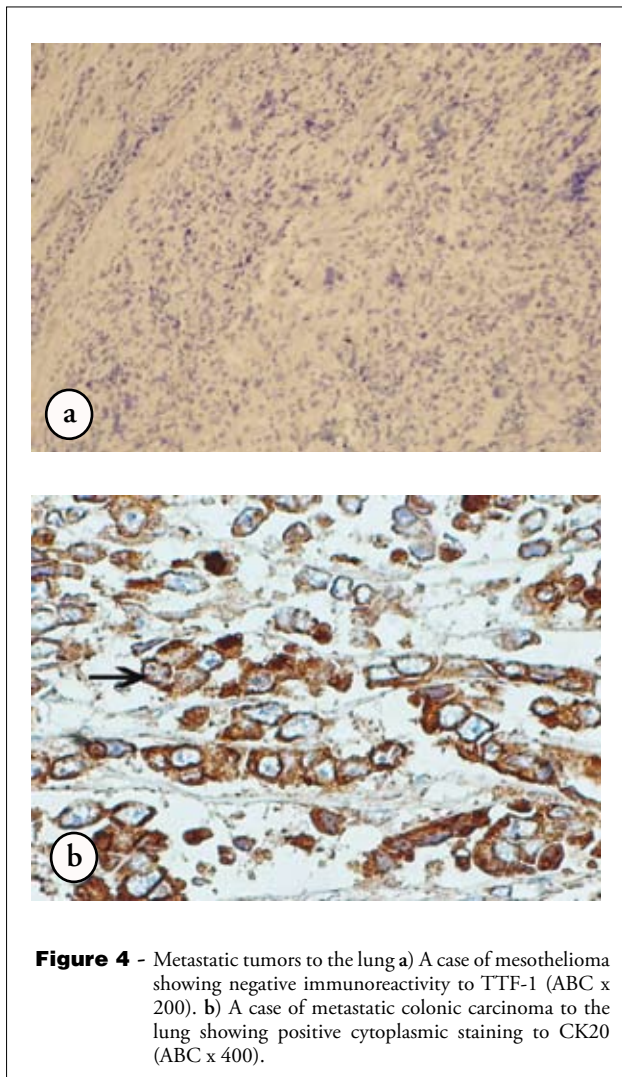


Figure 4 - Metastatic tumors to the lung a) A case of mesothelioma showing negative immunoreactivity to TTF-1 (ABC x 200). b) A case of metastatic colonic carcinoma to the lung showing positive cytoplasmic staining to CK20 (ABC x 400).

Discussion. Lung carcinoma is the leading cause of cancer death worldwide. Adenocarcinoma accounts for approximately 30% of lung carcinoma, and shows an steadily increasing incidence, especially among women. Distinguishing primary adenocarcinoma of the lung from metastatic lesion in the lung is often a challenging task, especially when the tumor presents as solitary nodule. In the present study, 43 biopsies of lung carcinomas obtained by Fibro-optic bronchoscope or by guided needle biopsy were studied for immunoreactivity to TTF-1, CK7, and CK20. All cases of primary adenocarcinoma showed strong positive immunoreactivity to TTF-1, and CK7, while they were absolutely negative to CK20. These results are comparable to those of many other studies that showed 54-97% of immunoreactive adenocarcinoma cells to TTF-1, and showed that negative primary adenocarcinoma cases were classified as mucinous adenocarcinoma or goblet cell-type bronchioalveolar carcinoma.¹²⁻¹⁴ Squamous cell carcinoma cases showed negative immunoreactivity to TTF-1 and CK 20 in all cases, while all cases were positive to CK7.¹⁵⁻¹⁷ Small cell carcinomas showed 100% strong positivity to TTF-1. These results agreed with other studies that showed 81-100% positivity to TTF-1.^{12,14} In a recent study, comparison between immunoreactivity of pulmonary and non-pulmonary small cell carcinoma to TTF-1 was conducted, it showed that both groups expressed TTF-1 by the same percentage and that TTF-1 is of no use to differentiate between primary and secondary small cell carcinoma.¹⁶ Also, TTF-1 could not be used to distinguish between adenocarcinoma and small cell carcinoma of the lung, while it is of great help in distinguishing poorly differentiating squamous cell carcinoma from poorly differentiating adenocarcinoma and small cell carcinoma. In contrast, cases of metastatic carcinomas from breast, and colon were totally negative to TTF-1. Only metastatic thyroid carcinoma to the lung showed nuclear positivity to TTF-1, which agreed with previous studies.^{16,18} The combination of TTF-1 with CK7 in the diagnosis of primary lung adenocarcinoma from metastatic cases did not increase the sensitivity of the test over the use of TTF-1 alone since metastatic carcinoma from the breast and thyroid showed positive immunoreactivity to CK7 (while they were negative to CK20). In our study, all cases of mesothelioma were negative to TTF-1, CK7, and CK20, these results are in keeping with those of other results that showed the specificity of TTF-1 for pulmonary adenocarcinoma versus mesothelioma was found to be 100%.¹⁹

From the above results, we can conclude that the combined use of TTF-1, CK7, and CK20 immunoreactivity could be helpful in distinguishing primary pulmonary adenocarcinoma from cases

of mesothelioma and metastatic adenocarcinoma to the lung (excluding cases of metastatic thyroid carcinoma), especially when the tumor appears as a solitary pulmonary nodule. Our results confirm that TTF-1 immunohistochemistry is a very sensitive and a highly specific method and could be used in every day practice.

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