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Utility of Thyroid Transcription Factor-1 and Cytokeratin 20 in Identifying the Origin of Metastatic Carcinomas of Cervical Lymph Nodes

The identification of primary location of a metastatic tumor is a difficult diagnostic problem and sometimes can be facilitated by the use of immunohistochemical markers. Thyroid transcription factor-1 (TTF-1) is a 38-kDa nuclear homeodomain transcription factor that is expressed specifically in lung or thyroid neoplasms. Cytokeratin 20 (CK20) is a 46-kDa low-molecular-weight cytokeratin that shows restricted expression in adenocarcinomas of the gastrointestinal tract (GIT) and transitional cell carcinomas of the urinary tract. We studied the immunohistochemical expression of TTF-1 and CK20 in 68 metastatic carcinomas in cervical lymph nodes. The primary sites were the lung in 29 cases, stomach in 13, colorectum in 3, and other sites in 23. TTF-1 expression was detected in 69.0% of metastatic lung carcinomas and none in metastatic GIT carcinomas, whereas CK20 expression was detected in 68.8% of metastatic GIT carcinomas and none of metastatic lung carcinomas. TTF-1 had a specificity of 0.95 and a sensitivity of 0.69 for metastatic lung carcinoma, whereas CK20 had a specificity of 1.00 and a sensitivity of 0.69 for metastatic GIT carcinoma. These results indicate that TTF-1 and CK20 should be the first choice as a component of antibody panel to prove or to exclude the lung and GIT origin, respectively, especially in patients presenting with metastatic carcinomas of unknown primary site.

Key Words : *Thyroid Transcription Factors; Cytokeratin 20; Neoplasms Metastasis; Lymph Nodes, Cervical*

Mee Sook Roh, Sook Hee Hong

Department of Pathology and Institute of Medical Science, Dong-A University College of Medicine, Busan, Korea

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Address for correspondence
Sook Hee Hong, M.D.
Department of Pathology, Dong-A University College of Medicine 1, 3-ga, Dongdaeshin-dong, Seo-gu, Busan 602-713, Korea
Tel : +82.51-240-5351, Fax : +82.51-243-7396
E-mail : shhong@daunet.donga.ac.kr

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INTRODUCTION

Lymph nodes are the most common site of metastatic tumor and sometimes constitute the first clinical manifestation of the disease (1). One of the pathologist's task is to provide information about possible primary source for clinicians. The metastatic tumors in the lymph node sometimes show nearly identical histopathologic features irrespective of the site of origin, and identification of the primary tumor location may be impossible on purely morphologic ground. The anatomic location of a lymph node involved by metastatic tumor provides an important indication of the primary site of origin. Especially in cervical lymph node, high cervical lymph nodes may harbor metastatic carcinomas originating in head and neck, whereas low cervical lymph nodes are commonly the site of metastases from intrathoracic carcinomas, particularly in the lungs, and intraabdominal tumors, and the supraclavicular lymph nodes are most often the site of metastases for abdominal (gastric) cancers (2). Immunohistochemical approaches using tissue-specific antibodies, especially against lung and gastrointestinal tract (GIT), are helpful to locate the primary tumor sites in work-up for metastatic tumors of cervical lymph nodes.

Thyroid transcription factor-1 (TTF-1) is a nuclear transcription protein identified by Civitareale et al. (3) in 1989. TTF-1 is a 38-kDa homeodomain-containing nuclear protein that is a member of the *Nkx2* gene family and plays a role in transcriptional activation during embryogenesis in the thyroid, diencephalon, and respiratory epithelium (4-6). TTF-1 is encoded by a single locus gene on chromosome 14q13 and regulates the expression of several genes including thyroglobulin, thyroperoxidase, sodium-iodide transport protein, calcitonin, and major histocompatability complex class I gene in the thyroid (7-9) and surfactant proteins A, B, and C, and Clara cell secretory protein gene in the lung (10-13). TTF-1 expression has been demonstrated in neoplasms of the lung and thyroid (14, 15).

Cytokeratin 20 (CK20) is a low-molecular-weight cytokeratin that was originally identified by Moll et al. (16-18) as protein IT in two-dimensional gel electrophoresis of cytoskeletal extracts of intestinal epithelium. In normal tissues, it is expressed only in the gastrointestinal epithelium, urothelium, and Merkel cell (17, 18).

Therefore, we speculated that TTF-1 and CK20 immunohistochemial expressions might be useful in distinguishing metastatic lung and GIT carcinomas in the cervical lymph node, respectively.

MATERIALS AND METHODS

Materials

The study material was obtained from the files of the Department of Pathology at Dong-A University Hospital. It consisted of 68 metastatic carcinomas in the cervical lymph nodes. The primary sites of the study specimens were the lung in 29 cases (11 adenocarcinomas, 8 squamous cell carcinomas, 7 small cell carcinomas, and 3 undifferentiated carcinomas), adenocarcinoma of the stomach in 13, undifferentiated or squamous cell carcinomas of the nasopharynx in 5, infiltrating ductal carcinomas of the breast in 4, adenocarcinomas of the colorectum in 3, squamous cell carcinomas of the esophagus in 3, transitional cell carcinomas of the ureter in 2, squamous cell carcinomas of the uterine cervix in 2, and in one each for papillary and medullary carcinoma of the thyroid, carcinoma ex pleomorphic adenoma of the submandibular gland, adenocarcinoma of the prostate, mucinous cystadenocarcinoma of the ovary, hepatocellular carcinoma of the liver, and squamous cell carcinoma of the tongue. The primary sites of each case were verified by comparing the histologic features between metastases and primary tumors that had been resected or biopsied simultaneously, previously, or afterward.

Immunohistochemical Staining for TTF-1 and CK20

Immunohistochemical studies for TTF-1 and CK20 were performed on formalin-fixed, paraffin-embedded, 4 μm-thick tissue sections using the avidin-biotin-peroxidase complex method. The primary antibodies used were the 8G7G3/1 anti-TTF-1 monoclonal antibody (1:50, Neomarker, CA, U.S.A.) and the KS20.8 anti-CK20 monoclonal antibody (1:100, Dako, Copenhagen, Denmark). Deparaffinization of all sections was performed through a series of xylene baths and rehydration was performed through graded alcohols. To enhance the immunoreactivity, microwave antigen retrieval at 750W for 30 min in citrate buffer (pH 6.0) was performed for TTF-1, and the sections for CK20 immunostaining were digested with pepsin at room temperature for 15 min. After blocking the endogenous peroxidase activity with 5% hydrogen peroxidase for 10 min, the primary antibody incubation for TTF-1 and CK20 was performed at 4°C overnight. Detection of the immunoreactive staining was carried out by the avidin-biotin-peroxidase complex method using the Histostain-plus kit (Zymed, CA, U.S.A.). The antigen-antibody reaction was visualized using 3-amino-9-ethylcarbazole as a chromogen. The Mayer's hematoxylin counterstain was performed.

To exclude equivocal reactions from data, staining was considered positive if >10% of the nuclei reactivity with any intensity for TTF-1 and cytoplasmic or membranous reactivity for CK20 were seen.

Statistical Analysis

The specificity and sensitivity of immunohistochemical stainings were calculated by using standard statistical methods as follows:

Specificity=true negatives/true negatives+false positives

Sensitivity=true positives/true positives+false negatives

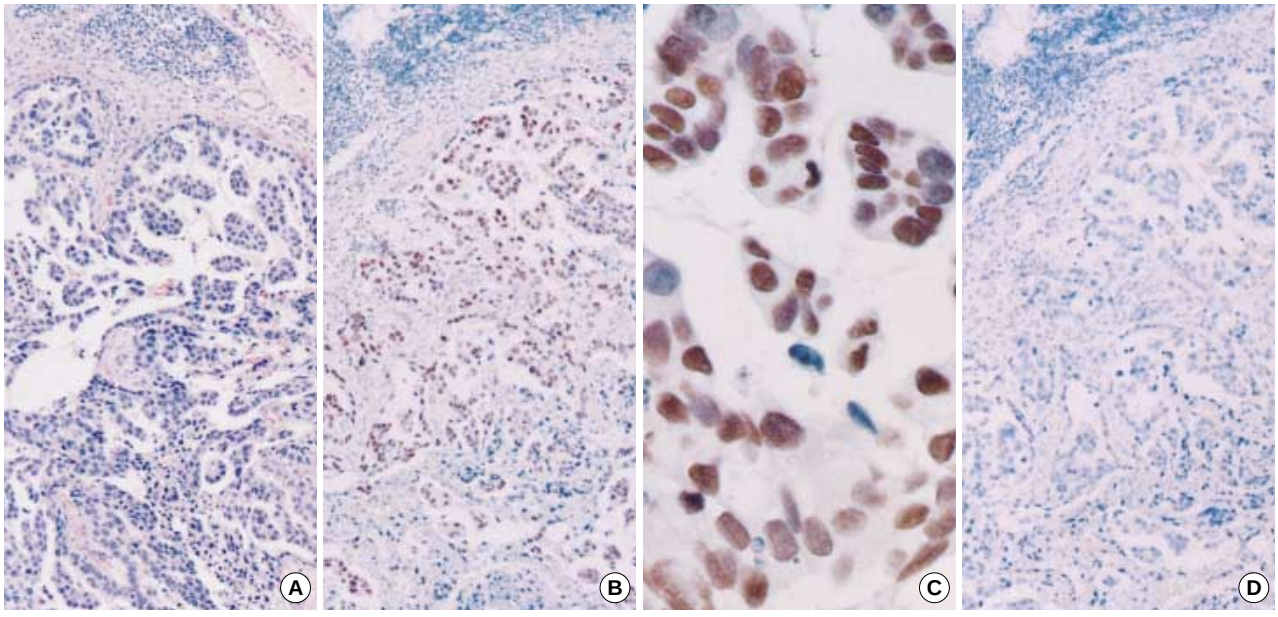

Fig. 1. Metastatic adenocarcinoma from the lung. (A) Photomicrograph showing papillary adenocarcinoma (H&E, ×40). (B) Immunohistochemical stain for TTF-1 shows nuclear positivity (×40). (C) High magnification of TTF-1 immunoreactivity (×400). (D) Immunohistochemical stain for CK20 shows negative reactivity (×40).

For TTF-1 immunohistochemical study, false positives were defined as extrapulmonary tumors with positive staining for TTF-1 and false negatives as tumors of pulmonary origin lacking detectable staining for TTF-1. For CK20 immunohistochemical study, false positives were defined as extra-GIT tumors with positive staining for CK20 and false negatives as tumors of GIT origin lacking detectable staining for CK20.

RESULTS

TTF-1 immunoreactivity was highlighted by a fine granular to diffuse pattern of staining confined to the nuclei independent of labeling intensity. Cytoplasmic immunostaining was sometimes encountered as a faint and diffuse labeling, but it was not counted as TTF-1 immunoreactivity (Fig. 1).

CK20 immunostaining showed cytoplasmic or membranous reactivity. In many cases, however, the staining showed a mosaic-like pattern of alternating positive and negative cells (Fig. 2).

TTF-1 expression was detected in 69.0% (20/29) in metastatic lung carcinomas and none (0/16) in metastatic GIT adenocarcinomas, whereas CK20 expression was detected in 68.8% (11/16) in metastatic GIT adenocarcinomas and none (0/29) in metastatic lung carcinomas. Metastatic tumors from other sites showed TTF-1 expression in each case of 2 thyroids and 2 uterine cervical cancers and did not express CK20 in all other tumors (Table 1).

The metastatic tumor from the lung showed TTF-1 immunoreactivity in 90.9% (10/11) of adenocarcinomas and in 85.7% (6/7) of small cell carcinomas. The metastatic tumor from the GIT showed CK20 immunoreactivity in 100% (3/3) of colorectal adenocarcinomas and in 61.5% (8/13) of gastric adenocarcinomas. TTF-1 was expressed in 2 of 39 extrapulmonary tumors and CK20 was not expressed in any of the 52 extra-GIT tumors.

Table 1. Results of TTF-1 and CK20 immunohistochemical staining in metastatic carcinomas of cervical lymph node

Primary site	No. of cases	Positive (%)	
		TTF-1	CK20
Lung	29	20 (69.0)	0 (0)
Adenocarcinoma	11	10 (90.9)	0 (0)
Small cell carcinoma	7	6 (85.7)	0 (0)
Squamous cell carcinoma	8	3 (37.5)	0 (0)
Undifferentiated carcinoma	3	1 (33.3)	0 (0)
Gastrointestinal tract	16	0 (0)	11 (68.8)
Stomach	13	0 (0)	8 (61.5)
Colorectum	3	0 (0)	3 (100)
Nasopharynx	5	0 (0)	0 (0)
Breast	4	0 (0)	0 (0)
Esophagus	3	0 (0)	0 (0)
Thyroid	2	1 (50)	0 (0)
Ureter	2	0 (0)	0 (0)
Uterine cervix	2	1 (50)	0 (0)
Submandibular gland	1	0 (0)	0 (0)
Prostate	1	0 (0)	0 (0)
Ovary	1	0 (0)	0 (0)
Liver	1	0 (0)	0 (0)
Tongue	1	0 (0)	0 (0)
Total	68	22 (32.4)	11 (16.2)

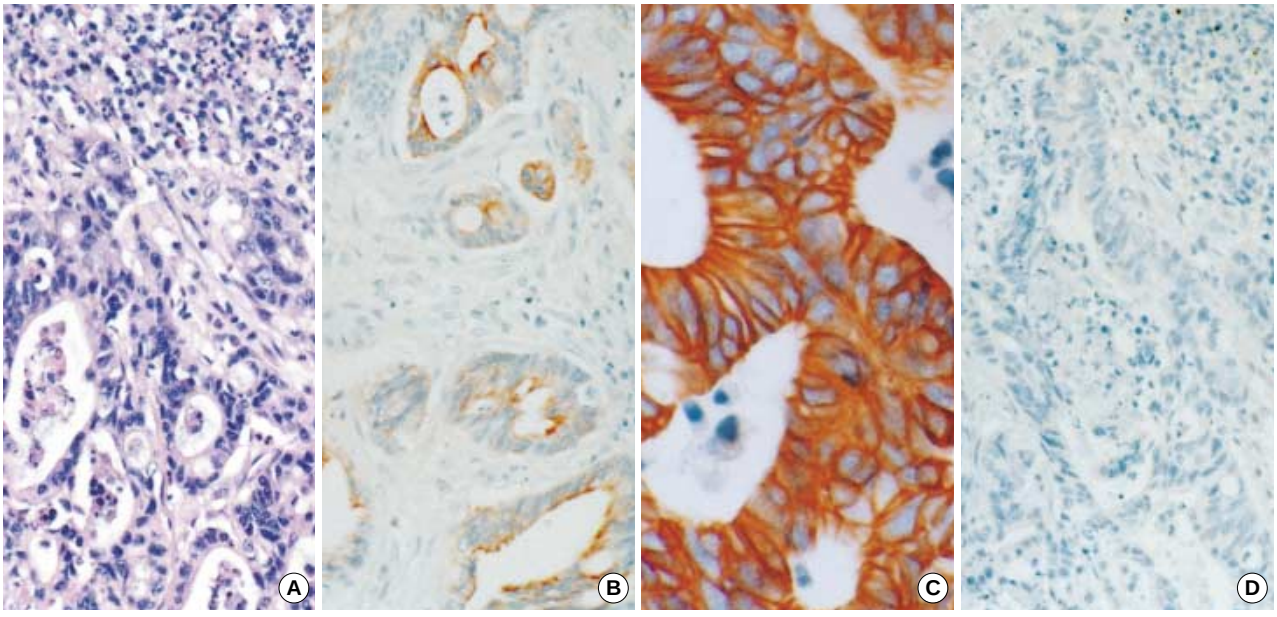

Fig. 2. Metastatic adenocarcinoma from the colon. (A) Photomicrograph showing moderately differentiated tubular adenocarcinoma (H&E, ×100). (B) Immunohistochemical stain for CK20 shows membranous positivity (×100). (C) High magnification for CK20 immunoreactivity (×400). (D) Immunohistochemical stain for TTF-1 shows negative reactivity (×100).

Thus TTF-1 had a specificity of 0.95 (39/39+2) and a sensitivity of 0.69 (20/20+9) for diagnosis of metastatic lung carcinomas, whereas CK20 had a specificity of 1.00 (52/52+0) and a sensitivity of 0.69 (11/11+5) for diagnosis of metastatic GIT carcinomas.

DISCUSSION

In the surgical pathology practice, a relatively common problem is to identify the primary site in the presence of a lymph node metastasis. We frequently encounter patients presenting initially with cervical lymphadenopathy. According to our experience, these patients with unknown primary tumors were subjected to extensive, uncomfortable, and very costly diagnostic procedures, including bronchoscopy, enteric endoscopy, upper and lower gastrointestinal series, intravenous pyelograms, angiography, mammography, and thyroid scanning in an attempt to locate the primary tumor site.

Sometimes the anatomic location of a lymph node involved by metastatic tumor provides an important indication of the primary site of its origin. Notwithstanding the anatomic connections between the location of neoplasms and their draining lymph nodes, metastatic tumors can be occasionally found in unexpected and remote areas. Because of blockage of lymphatics by tumor cells with subsequent retrograde flow and circulation shunts, paradoxical metastases, contralateral metastases, and metastases of deep-seated tumors to peripheral lymph nodes may be encountered (2). Therefore although the most common sites of origin for each regional group of lymph nodes are considered first, any other possible locations of primary tumors must be systemically reviewed.

Moreover, altered histopathological appearance of tumors in metastatic sites can often make the diagnosis more difficult. Identifying the primary tumor is usually difficult, often frustrating, yet indispensable to the treatment. All available methods of histopathology, electron microscopy, histochemistry, and immunohistochemistry must be employed for this important diagnostic process.

Various monoclonal antibodies have been developed with high degrees of specificity, by which the detection and identification of numerous classes of molecules are feasible. The monoclonal antibodies against specific markers for particular tissue or cell type can be used to identify their neoplastic counterparts.

TTF-1, also known as thyroid-specific enhance-binding protein or N*kx*-2.1, is a member of the N*kx*-2 gene family of the homeodomain-containing transcription nuclear factors that plays a pivotal role in tissue morphogenesis and cytodifferentiation (5, 19, 20). TTF-1 gene expression is regulated in respiratory epithelial cells at the transcriptional and posttranscriptional levels by means of cross-regulatory mechanism, involving hepatocyte nuclear factor 3 (21), Oct-1 protein (22), GATA-6 (23), and calreticulin (24). TTF-1 is consistently expressed in all pulmonary epithelial cells during early embryogenesis. As the development proceeds, it is progressively downregulated in tracheal and bronchial columnar epithelium, while remaining predominantly expressed in type II pneumocytes and Clara cells (13). Current information indicates that TTF-1 is almost exclusively expressed in carcinomas of the thyroid and lung. Furthermore, the TTF-1 expression in thyroid carcinomas is of only minor diagnostic importance since thyroid carcinomas can be specifically and sensitively identified by the immunohistochemical detection of thyroglobulin and thus discriminated from pulmonary carcinomas.

TTF-1 expression has been demonstrated in all types of lung carcinomas. TTF-1 has been localized in 26% to 76% of adenocarcinomas, in 0% to 38% of squamous cell carcinomas, in 40% of large cell carcinomas, in 0% to 50% of typical carcinoids, in 40% to 75% of large cell neuroendocrine carcinomas, and in 81% to 100% of small cell carcinomas (14, 25, 26). In our study on metastatic lung tumors, TTF-1 immunoreactivity was noted in 90.9% (10/11) of adenocarcinomas, in 37.5% (3/8) of squamous cell carcinomas, and in 85.7% (6/7) of small cell carcinomas. The results of our study performed on metastatic tumors of cervical lymph nodes were similar to those from the previous reports of primary lung tumors (14, 25, 26). Interestingly, we found a rather higher percentage of TTF-1 expression in metastatic adenocarcinomas than in primary lung tumors. Puglisi et al. (28) reported that non-small cell lung carcinomas with a higher percentage of immunoreactive cells for TTF-1 showed a poor prognosis. Pelosi et al. (27) reported that in adenocarcinomas, a stronger TTF-1 immunoreativity was generally seen in tumors with increased microvessel diameter, size less than 3 cm, and low proliferative fraction. They suggested that TTF-1 may affect the growth of small adenocarcinomas, which usually show low proliferative activity, via a mechanism involving an increased number of vascular channels. Our findings showed some discrepancy with the results of Pelosi et al. (27). The cause for these differences may be explained by subtle technical differences among the studies and diagnostic criteria for positive immunostaining. Further investigations are needed to determine the expression pattern of differentiation markers of their cells of origin, in view of the heterogeneous and variable nature of tumors during progression of metastasis. And it remains to be explained whether the expression of TTF-1 protein is switched off or on during tumor progression or represents local maturation in metastasis.

It is known that cytokeratin intermediate filaments have twenty subtypes (17, 18). They are of different molecular weights and show differential expression in various cell types and tumors. Monoclonal antibodies to specific cytokeratin subtypes have been used in an attempt to classify tumors according to site of origin. Cytokeratin immunohistochemistry has important applications in surgical pathology (17, 29).

CK20 is a major protein in mature enterocytes and goblet cells of the intestinal villi. Although the biological function of CK20 is unknown, the predominance of CK20-rich cells in the luminal-exposed to regions of intestinal mucosa has been proposed to confer a special mechanical stability (17, 18). Gastrointestinal adenocarcinomas are frequently CK20-positive, whereas adenocarcinomas of the lung or female genital tract (with the exception of mucinous adenocarcinoma of the ovary) are almost always CK20-negative (17, 29, 30).

According to studies of CK20 immunohistochemistry in lung cancer, CK20 immunoreactivity was low and observed in 16.6% of metastatic non-small cell carcinomas of the lung in the pleural fluid (31) and in 15% (5 of 33) of pulmonary adenocarcinomas (18). In colon cancers, CK20 immunoreactivity was high and noted in 90 of 93 (97%) of adenocarcinomas of the colon (18) and shown to have a 100% sensitivity in cytologic specimens of metastatic carcinomas (32). In stomach cancers, CK20 immunoreactivity was noted in 50% of adenocarcinomas of the stomach (17) and in 68.7% in cytologic specimen of metastatic carcinomas (31). The positive rates of CK20 immunoreactivity for metastatic gastric cancer of our study and Ascoli et al. (31) were slightly higher than that of primary lesion in study of Moll et al. (17). It also remained to be studied whether these differences are produced by the technical or interpretation differences or CK20 involves in tumor progression and metastasis.

In summary, the results of the present study indicate that TTF-1 and CK20 should be the first choice as a component of antibody panel to prove or to exclude the lung and GIT origin, respectively, especially in patients presenting with metastatic carcinomas of unknown primary site. Although this method may not identify the primary tumor site in all cases, it compares favorably with other special techniques in this difficult diagnostic setting.

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