SHORT REPORT

Positive immunostaining for thyroid transcription factor-1 in primary and metastatic colonic adenocarcinoma: a note of caution

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Immunohistochemistry for thyroid transcription factor-1 (TTF-1) is considered an important ancillary technique for distinguishing primary pulmonary adenocarcinomas from metastases. A case of metastatic colonic adenocarcinoma in a bronchial biopsy specimen that showed focal positive nuclear staining for TTF-1 is reported here. The primary colonic adenocarcinoma was retrieved from the file and immunostaining also showed focal nuclear positivity for TTF-1. This confirmed the morphological and clinical impression of colonic metastases in the bronchial biopsy specimen. To investigate this apparent aberrant TTF-1 expression, further sections from primary colonic adenocarcinoma were immunostained for TTF-1 using the SPT24 antibody clone. Three of the six cases studied showed focal nuclear positivity with the SPT24 clone. All six cases were subsequently shown to be negative with the 8G7G1 clone. It is concluded that the SPT24 antibody to TTF-1 may show focal nuclear positivity in adenocarcinomas of colorectal origin such that focal staining in small biopsy specimens should be interpreted with caution.

Thyroid transcription factor-1 (TTF-1) is a homeodomain transcription factor specific to the thyroid and lung. Antibodies to TTF-1 are widely used in clinical pathology, and many studies have found the specificity for thyroid and lung to be almost 100%.^{1–4} A few studies, however, have highlighted occasional positivity for TTF-1 in adenocarcinomas from other sites. For example, Bejarano *et al*⁵ found focal positivity for TTF-1 in 1 of 66 gastric adenocarcinomas and in 1 of 8 endometrial adenocarcinomas by using a polyclonal antibody. Medina-Flores *et al*⁶ reported expression in 1 of 20 colon adenocarcinomas and 1 of 23 gastric adenocarcinomas by using a monoclonal antibody. We report a case of focal TTF-1 staining in metastatic colonic adenocarcinoma and the findings of a small study of TTF-1 staining in primary colonic adenocarcinoma.

CASE REPORT

A 70-year-old man with a history of colonic adenocarcinoma, Dukes's stage B, presented with bilateral endobronchial tumours. The bronchial biopsy specimen showed an adenocarcinoma with histological features suggesting metastases from the colonic primary (fig 1A). Appropriate immunohistochemistry was carried out to confirm the primary site. The tumour displayed focal positive immunostaining for cytokeratin 7 (CK7) and TTF-1 (fig 1B). Staining for cytokeratin 20 (CK20) was negative, whereas that for the homeobox gene product (CDX-2) was positive. This immunoprofile is more in keeping with a primary origin in the lung. This, however, seemed unlikely both on clinical and morphological grounds.

In view of the discrepancy between the immunoprofile and the histological appearances, we reviewed sections from the

previous sigmoid colectomy and immunostained them for CK7, CK20, CDX-2 and TTF-1. The sigmoid tumour was a moderately differentiated colonic adenocarcinoma and seemed similar to that in the bronchial biopsy specimens (fig 1C). This tumour also showed focal nuclear positivity for TTF-1 (fig 1D) and focal staining for CK7. In addition, CK20 was positive in around 60% of the tumour cells. These findings supported the clinical and morphological opinions that the endobronchial foci of the adenocarcinoma were indeed metastases from the previous primary sigmoid tumour. Furthermore, this case highlighted a potential source of diagnostic error and prompted a further evaluation of TTF-1 immunostaining in colonic adenocarcinoma.

MATERIALS AND METHODS

Tissue samples

Six cases of primary colonic adenocarcinoma were retrieved from the pathology files of the Western Infirmary, Scotland. These included four resections and two biopsy specimens, which were reviewed histologically to confirm the diagnosis.

TTF-1 immunohistochemistry

Paraffin wax block sections of 3 µm thickness were immunostained for TTF-1 with an anti-TTF-1 monoclonal antibody (SPT24; 1:50 dilution; Vector, Peterborough, UK). Immunohistochemical staining was carried out in a Dako Autostainer (Dako, Ely, Cambridge, UK) using Envision Peroxidase (Dako). For antigen retrieval, the slides were microwaved under pressure in 1 mmol/l EDTA, pH 8.0, for 5 min. Appropriate control material (according to the manufacturer's instructions) was used for each run. For negative controls, the primary antibody was omitted. After immunohistochemistry the samples were assessed by light microscopy for nuclear staining.

RESULTS

Three of the six colonic adenocarcinoma specimens showed focal nuclear positivity for TTF-1, including sections from two resections and one biopsy. The other three cases (two resections and one biopsy) were negative.

DISCUSSION

Immunohistochemical staining for TTF-1 is one of the most reliable markers for distinguishing primary adenocarcinoma of the lung from metastatic adenocarcinoma. Positive immunostaining for TTF-1 has been reported in 74–92% of primary lung adenocarcinomas, and antibodies to TTF-1 are considered to be a useful addition when investigating malignancies of unknown origin.^{3–7} Despite its high sensitivity and specificity, positive staining for TTF-1 in other tumours is occasionally

Abbreviations: CK7, cytokeratin 7; CK20, cytokeratin 20; CDX-2, the homeobox gene product; TTF-1, thyroid transcription factor-1

Figure 1 (A) Infiltrating adenocarcinoma in the bronchial biopsy specimen displaying morphological features in keeping with those of a primary colorectal adenocarcinoma ($\times 100$; haematoxylin–eosin staining). (B) Immunohistochemical staining of the adenocarcinoma shown in (A). Focal nuclear staining of tumour cells for thyroid transcription factor-1 TTF-1; ($\times 250$; anti-TTF-1) is present. (C) Primary adenocarcinoma of the sigmoid colon ($\times 100$; haematoxylin–eosin staining). (D) Immunohistochemical staining of the adenocarcinoma shown in (C). Focal nuclear staining of tumour cells for TTF-1 ($\times 100$; anti-TTF-1) is present.

Take-home messages

- The SPT24 antibody clone to TTF-1 may show nuclear positivity in adenocarcinomas of colorectal origin, which is not seen with the 8G7G1 clone.
- In small biopsy specimens, focal staining for TTF-1 should be interpreted with caution.
- Immunohistochemical staining should always be interpreted in conjunction with histological appearances and clinical history.

reported.^{5–6} We identified focal nuclear positivity for TTF-1 in a significant 57% (4/7) of colonic adenocarcinomas.

In this study, immunohistochemical staining for TTF-1 was carried out according to the manufacturer's instructions, with the exception of the use of EDTA buffer for antigen retrieval rather than citrate buffer. We have since repeated this study using citrate buffer for antigen retrieval, with identical results. Recently, Comperat *et al.*⁷ have also reported positivity for TTF-1 in 10% of metastatic and 5% of primary colonic adenocarcinoma specimens. Although this ratio is much lower than that in our study, Comperat *et al.* had a much larger sample, with 41 colorectal metastases and 90 primary colonic adenocarcinomas. They attributed this positive staining to the SPT24 clone, also used in our study, because staining of colonic adenocarcinomas with the 8G7G1/1 clone from Dako was negative. Although the SPT24 clone was less specific, it showed greater sensitivity than the 8G7G1/1 clone for pulmonary adenocarcinoma. TTF-1 immunostaining on our samples by using the 8G7G1/1 clone gave negative results, confirming the results by Comperat *et al.*⁷

In conclusion, the SPT24 antibody clone to TTF-1 may show nuclear positivity in adenocarcinomas of colorectal origin. Hence, focal staining in small biopsy specimens should be interpreted with caution. Positive staining is not

seen with the 8G7G1/1 clone, although this clone is less sensitive. The case reported reaffirms the importance of interpreting immunohistochemical staining in conjunction with histological appearances and clinical history.

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