ORIGINAL ARTICLE

Thyroid transcription factor 1 in pulmonary adenocarcinoma

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Aims: To discover whether variations in thyroid transcription factor 1 (TTF-1) staining in different subtypes and patterns of pulmonary adenocarcinoma are related to the putative origin of the tumour. In addition, to confirm the specificity of TTF-1 for pulmonary (as opposed to other sites) adenocarcinoma, to examine the possible prognostic relevance of TTF-1 positivity in lung cancer, and to review this laboratory's experience of TTF-1 in diagnostic practice.

Materials/Methods: In total, 128 primary lung adenocarcinomas, 106 primary non-pulmonary adenocarcinomas, and 37 pulmonary non-adenocarcinoma tumours were studied. In addition, 100 cases where TTF-1 was used in routine surgical pathology practice were investigated. Immunoperoxidase staining was performed on formalin fixed, paraffin wax embedded sections using anti-TTF-1 antibody. Staining was evaluated semiquantitatively using the frequency and intensity of nuclear positivity.

Results: None of the 106 non-pulmonary adenocarcinomas expressed TTF-1 and only three of the 37 non-adenocarcinoma lung cancers, all neuroendocrine carcinomas, were positive. Of the pulmonary adenocarcinomas, 75% were strongly positive for TTF-1. Mucinous (two of six) and poorly differentiated adenocarcinomas (four of 10) were less likely to stain. Of the peripheral adenocarcinomas, 33 of 37 were positive, whereas only seven of 14 of those of bronchial origin stained strongly. Atypical adenomatous hyperplasia strongly expressed TTF-1. No "false positives" were encountered in the 100 routine diagnostic cases.

Conclusion: Positive TTF-1 staining is useful in the differential diagnosis of pulmonary adenocarcinomas. TTF-1 may be a lineage marker for tumours arising from the peripheral airway or alveolar epithelium and has no prognostic relevance.

Lung cancer is a common, frequently fatal, malignancy and the adenocarcinoma subtype is rising in incidence in many countries. It is more prevalent in women and, overall, is now the most common form of lung cancer in many parts of the world.^{1–2} Adenocarcinomas of the lung readily metastasise and, as the diversity of chemotherapeutic agents increases, with differential efficacy against cancers from different sites, the importance of the histopathologist being able to ascertain the primary source of a metastatic tumour has increased.

Thyroid transcription factor 1 (TTF-1) is a tissue specific, homeodomain containing transcription factor, which is found predominantly in normal type II alveolar pneumocytes. It is also present in thyroid tissue and has been identified during the embryological development of human lung and brain.^{3–5} TTF-1 plays a role in epithelial morphogenesis,⁶ stimulates the synthesis of pneumocyte surfactant proteins, and regulates secretory product gene transcription in Clara cells.^{6–7}

Among non-small cell carcinomas of lung, TTF-1 has been found in up to 76% of adenocarcinomas but rarely, if ever, in squamous carcinomas.^{8–11} In several tissues and sites where metastatic carcinoma has been found in biopsy material, anti-TTF-1 immunohistochemistry has been useful in the differentiation of metastatic, morphologically similar, adenocarcinomas of pulmonary origin from other unknown primary sites.^{12–16} The lung is also a common site for metastases, and solitary metastatic deposits may be resected, as targeted treatment for limited metastatic disease, or in the belief that the tumour is primary in the lung.

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In addition to the expected expression in thyroid tumours, TTF-1 has also been demonstrated in small cell carcinomas and other neuroendocrine tumours.^{17–21} Although most studies have suggested that TTF-1 can be found in neuroendocrine tumours of both pulmonary and extrapulmonary origin,^{11–18–19} some have found the marker largely confined to those arising in the lung.^{20–21}

In our study, we examined TTF-1 reactivity in 165 pulmonary carcinomas and 106 non-pulmonary adenocarcinomas. Specifically, we considered the different architectural subtypes (as described in the recent World Health Organisation (WHO)/International Association for the Study of Lung Cancer (IASLC) classification,²²) exhibited by the adenocarcinomas, to ascertain whether or not TTF-1 expression varies between them. Given the propensity for TTF-1 staining in alveolar pneumocytes and Clara cells, when compared with the bronchial epithelium,⁵ we also hypothesised that peripheral-type adenocarcinomas would show more positivity than central bronchial-type adenocarcinomas.^{23–24} Furthermore, we reviewed the usefulness of anti-TTF-1 staining in 100 consecutive diagnostic surgical histopathology/cytopathology cases, where its use was aimed

Abbreviations: AAH, atypical adenomatous hyperplasia; BAC, bronchioloalveolar carcinoma; TTF-1, thyroid transcription factor 1; WHO, World Health Organisation

at the identification of pulmonary adenocarcinoma in a range of clinicopathological situations.

MATERIALS AND METHODS

Study material

TTF-1 reactivity was studied in 271 primary tumours. Of 165 resected primary pulmonary tumours, 128 were adenocarcinomas, 29 were squamous, four were mixed tumours, one was large cell undifferentiated, two were large cell neuroendocrine carcinomas, and one was a typical carcinoid tumour. A further 106 primary non-pulmonary adenocarcinomas of the pancreas (seven), endometrium (25), ovary (15), stomach (24), and colon (35) were also studied. In each case, the archival surgical histopathological material was examined and an appropriate tissue block selected for sectioning and staining. In the case of the pulmonary adenocarcinomas, in the section selected for immunostaining, the range of different tumour patterns present, as defined in the 1999 WHO/IASLC classification,²² namely solid, tubular, papillary, mucinous, and bronchioloalveolar (BAC), was recorded and, using information available in the original report and by study of all the available sections, a judgement was made as to whether the tumour was of peripheral parenchymal type, central bronchial type, or unclassifiable.^{23, 24} Central bronchial-type adenocarcinomas frequently arise in conjunction with a large airway, are large, often well circumscribed tumours with focal necrosis, and may have an endobronchial component. Histologically, they consist of sheets or lobules of large cuboidal or columnar malignant cells, often with an acinar or cribriform pattern. Better differentiated tumours show round acini, and columnar cells with basal nuclei and eosinophilic cytoplasm. Mucin is variable, may be abundant, and some tumours have mucin containing signet ring cells. Parenchymal-type adenocarcinoma is usually a peripheral, subpleural lesion of variable size, often with pronounced pleural puckering and a central anthracotic scar. A peripheral BAC component is very common, whereas the invasive tumour is usually a heterogeneous mixture of irregular acini in a fibrous stroma, papillary tumour or more solid, poorly differentiated adenocarcinoma. The cell type is also a more heterogeneous mixture, including mucin secreting cells, goblet cells, columnar cells, and various rounded, ovoid, tongue shaped, and "peg" cells corresponding to Clara cell and type II pneumocyte differentiation. Occasionally, a focus of tumour with a central-type morphology may be seen.

A further 100 cases encompassed numerous tissues from biopsy material, postmortem tissues, and cytological preparations encountered in local routine diagnostic practice. Anti-TTF-1 was used, in conjunction with a range of other antibodies, appropriate to the particular clinical problem, where lung adenocarcinoma entered the differential diagnosis. Note was taken of all clinical information available, up until the time of our study.

Immunohistochemical staining

Immunohistochemical staining was carried out on 4 µm thick sections cut from formalin fixed, paraffin wax embedded tissue, using microwave antigen retrieval and a standard streptavidin-biotin based technique.²⁵ The anti-TTF-1 antibody (8G7G3/1, mouse monoclonal antibody; Dako, Ely, Cambridgeshire, UK) was used at a dilution of 1/100, with a positive and negative control for each assay. Immunoreactivity was evaluated semiquantitatively according to the percentage of cells displaying nuclear staining (0; +, ≤ 10% cells positive; ++, 10–50% positive; +++, > 50% positive) along with the intensity of that staining (graded + to +++). Scoring was carried out independently by two of us (GS and KMK). These scores were then reviewed, a

consensus was agreed upon in any case where there was disagreement, and a decision taken to allocate an overall score of low/negative or high staining. Cytoplasmic staining was not scored as positive. In each case of primary lung cancer examined, particular attention was paid to ensure that entrapped normal alveolar walls within the tumour were not erroneously scored as positive tumour.

Length of postoperative survival was available on all patients with primary lung carcinoma from our own lung cancer data base and the Scottish Cancer Registry.

Statistics

The χ^2 test was used to examine the association between TTF-1 staining in the bronchial and peripheral-type adenocarcinomas. The Kaplan–Meier method was used to test for differences in postoperative survival using log rank to indicate significance.

RESULTS

None of the 106 adenocarcinomas of non-pulmonary origin showed nuclear staining. Weak cytoplasmic positivity was seen in five cases (three gastric and two endometrial adenocarcinomas). Similarly, of the 37 pulmonary carcinomas, which were not adenocarcinoma, only the three neuroendocrine tumours (two large cell and one typical carcinoid tumour) showed evidence of strong nuclear staining.

Of the 128 primary pulmonary adenocarcinomas, 96 (75%) had high level nuclear staining for TTF-1. Of the remaining 32 tumours (25%), 14 showed weak positivity whereas 18 were completely negative, giving an overall positivity rate of 86%. Table 1 shows the expression of TTF-1 by overall tumour type and table 2 displays staining by tumour location. Peripheral, parenchymal tumours and those with prominent BAC or papillary patterns stained most strongly. Central, bronchial tumours and those with the poorest differentiation stained the least. Analysis of the patterns of primary lung adenocarcinoma present in the 128 cases showed only one scorable pattern in 76 sections, a further 44 revealed two patterns, and eight had three. Table 3 shows the results of TTF-1 staining of the various patterns of adenocarcinoma. The mucinous carcinomas appear to stain less well for TTF-1, and three mucinous BACs were also negative. High positivity was noted in only four of the 10 poorly differentiated areas of tumour and was relatively low in "solid" adenocarcinoma, whereas the better differentiated patterns showed more staining (fig 1A, B). Areas with a non-mucinous bronchioloalveolar pattern and papillary tumour showed the strongest and most abundant staining for TTF-1 (fig 1C).

Those bronchial-type adenocarcinomas (n = 14) were positive in half the cases, with generally only moderate staining, whereas 33 of the 37 peripheral tumours were positive (p < 0.02). In this last case, there was frequently widespread intense staining, often reflecting the presence, almost by definition, of BAC-like areas of tumour. Fortuitously, several sections of pulmonary adenocarcinoma also had foci of atypical adenomatous hyperplasia (AAH) in adjacent lung. Each of these five separate AAH lesions was strongly positive for TTF-1 (fig 1D).

Survival analysis for those patients with primary lung cancer showed no relation between TTF-1 expression and length of postoperative survival.

Specimens that had been stained for TTF-1 in routine surgical histo/cytopathology practice comprised 76 surgical histopathology cases from lung, bronchus, pleura, intestine, bone marrow, lymph node, brain, peritoneum and omentum, together with 20 cytopathology specimens of pleural or peritoneal fluid, or fine needle aspiration biopsy of several sites. Four postmortem cases were also investigated. To the

Figure 1 (A) Invasive acinar pattern of adenocarcinoma showing strong staining for thyroid transcription factor 1 (TTF-1). (B) Solid, more poorly differentiated adenocarcinoma exhibiting a high degree of staining. (C) Abundant staining in non-mucinous bronchioloalveolar carcinoma. (D) Strong positivity in atypical adenomatous hyperplasia. (E) Alveolar pneumocytes and bronchiolar epithelium are positive for TTF-1. In bronchioles staining is much stronger where the epithelium shows less stratification. (F) Central bronchial epithelium negative for TTF-1.

best of our knowledge, using all the information available to us, no inappropriate “false positives” occurred in the adenocarcinomas. There were 15 positive cases where the diagnosis was pulmonary adenocarcinoma. Nine positive tumours, which were patently not primary pulmonary adenocarcinoma, were shown to be of thyroid origin or neuroendocrine carcinomas by other methods. Staining was absent in tumours from two primary lung adenocarcinomas, one of which was poorly differentiated, the other probably of bronchial origin. Seventy four cases were judged to be “true

negative” cases; that is, TTF-1 negative and the absence of lung adenocarcinoma.

DISCUSSION

Overall, 75% of primary pulmonary adenocarcinomas showed strong staining for TTF-1, and a further 11% showed weak staining. Apart from a few neuroendocrine tumours, none of the other primary lung cancers and none of 106 non-pulmonary adenocarcinomas showed staining for TTF-1. In lung adenocarcinomas, the papillary and BAC patterns were more likely to stain for TTF-1, whereas poorly differentiated and solid adenocarcinomas were least likely.

Our score for substantial TTF-1 staining in 75% of lung adenocarcinomas agrees well with the findings of several previous reports, where the range of positivity was 72–80%,^{9 10 26 27} although there are exceptions, with one study

Table 1 TTF-1 staining in different tumour subtypes	
Overall adenocarcinoma subtype (WHO)	High TTF-1 staining
Mixed pattern	55/65 (85%)
Non-mucinous BAC	9/9 (100%)
Acinar, papillary, or tubular	10/14 (71%)
Solid with mucin	14/25 (56%)
Variants	
Mucinous (including mucinous BAC)	3/6 (50%)
Signet ring cell	1/1 (100%)
Clear cell	1/2 (50%)
Pleomorphic/sarcomatoid with adenocarcinoma	3/6 (50%)
In the mixed category, a minimum of 10% of each component was present in the tumour (n = 128). BAC, bronchioloalveolar carcinoma; TTF-1, thyroid transcription factor 1; WHO, World Health Organisation.	

Table 2 Thyroid transcription factor 1 (TTF-1) staining by tumour origin, either central, peripheral parenchymal, or indeterminate (n = 128)	
Parenchymal v bronchial	High TTF-1 staining
Peripheral, parenchymal type	33/37 (89%)
Indeterminate group	56/77 (73%)
Central, bronchial type	7/14 (50%)

Table 3 Number of areas, identified in 128 different adenocarcinomas, showing a specific architectural subtype, with number showing high positive staining for TTF-1

Subtype pattern	No. of areas	High positive
NMBAC	33	32 (97%)
MBAC	4	1 (25%)
Papillary	11	11 (100%)
Pap/Tub	18	15 (83%)
Tubular	48	40 (83%)
Solid	58	42 (72%)
PD	10	4 (40%)
Mucinous	6	2 (33%)
Total	188	147 (78%)

MBAC, mucinous bronchioloalveolar carcinoma; NMBAC, non-mucinous bronchioloalveolar carcinoma; Pap/Tub, admixed papillary and tubular patterns, where the papillary and tubular components could not be separated for the purpose of scoring; PD, poorly differentiated (including pleomorphic and sarcomatous areas).

reporting only 25% positivity.¹¹ It is likely that this unusual finding results from technical differences in the anti-TTF-1 antibody and staining methodologies used. Our data also reinforce the almost universal finding that anti-TTF-1 only stains adenocarcinomas that are primary in the lung and thyroid gland.^{10 27 28}

The issue of the staining pattern and its relation to tumour type and differentiation has been addressed to a variable extent in some previously published reports. Several studies have reported that there was no correlation between TTF-1 staining and tumour growth pattern or differentiation.^{9 11 26} Our data suggest that poorly differentiated tumours are less likely to be strongly positive than relatively well differentiated lesions showing complex architecture such as glands, tubules, papillae, or a BAC-like growth pattern. Care was taken throughout our study to ensure that issues such as poor fixation in the centre of a large tumour did not account for a false negative result. There are reports showing that mucinous tumours of the lung do not express TTF-1. Some have found, as we did, that mucinous BAC is usually negative for TTF-1,^{28 29} whereas invasive mucinous tumours show little positivity.¹⁰ Interestingly, the rare signet ring cell mucin secreting carcinoma seems to express abundant amounts of TTF-1.^{30 31} Our finding that 50% of pleomorphic, sarcomatoid tumours are also positive is in keeping with a previous study.³²

"Our data suggest that poorly differentiated tumours are less likely to be strongly positive than relatively well differentiated lesions showing complex architecture"

Most adenocarcinomas are believed to arise in the peripheral parts of the lung, from alveolar or bronchioloalveolar epithelium, possibly through a preneoplastic precursor lesion, such as AAH.^{33 34} A smaller number are thought to arise centrally from bronchial epithelium.^{23 24 35} Our attempts to classify these tumours according to the bronchial versus peripheral division resulted in a large proportion of unclassifiable cases (60%), much higher than Edwards' figure of 20%. Our proportion of bronchial-type cases was similar to Edwards. It is probable that many of our indeterminate group are peripheral-type tumours (this may be inferred in part by the fact that 73% of these showed abundant TTF-1 staining), which were excluded by strict interpretation of the criteria. Although TTF-1 expression is universal and strong in alveolar epithelium and generally strong and frequent in more peripheral bronchiolar epithelium, it is generally absent in larger bronchioles and in the central

Take home messages

- Staining for thyroid transcription factor 1 (TTF-1) is useful in the differential diagnosis of pulmonary adenocarcinomas if other tumours known to express this protein (thyroid and neuroendocrine tumours) are excluded
- TTF-1 may be a lineage marker for tumours arising from the peripheral airway or alveolar epithelium and reflects the degree of tumour differentiation
- TTF-1 had no prognostic relevance in our series

bronchial epithelium (fig 1E, F).^{5 10} This reflects the association between TTF-1 and both alveolar pneumocyte and Clara cell secretory activity.^{6 7} The finding of greater staining in non-mucinous BAC-type areas of tumour and peripheral parenchymal-type adenocarcinomas, when compared with those deemed to be of central bronchial type, seems to reflect the types of cytodifferentiation prevalent in different tumour types and, perhaps, the probable origin of the tumour. This supports the assertion of Yatabe *et al*, who suggested that TTF-1 expression could be used as a lineage marker for the terminal respiratory unit.³⁶ Thus, it is not surprising that the five examples of AAH stained by us showed strong expression of TTF-1.

We were unable to show that absence of TTF-1 staining was related to worse survival in a substantial group of surgically resected patients. Such an association has been demonstrated by Haque *et al*.²⁶ It is not clear whether this is a true relation or a finding compounded by tumour size and grade.

Our review of the usefulness of TTF-1 as a marker of pulmonary adenocarcinoma in surgical histo/cytopathological diagnosis has shown that it is indeed extremely useful, with no known case of a false positive. This is reflected in the literature.^{8 13-15} A few false negative cases occurred in our review, but this is not unexpected given the possibility of sampling problems and our finding that poorly differentiated, solid pattern and central bronchial-type adenocarcinomas stain less well for TTF-1.

TTF-1 is a useful and reliable marker for pulmonary adenocarcinoma, in circumstances where other tumours known to express this protein (thyroid and neuroendocrine tumours) are excluded. TTF-1 expression does, to some extent, reflect the pattern(s) and degree of differentiation evident in the tumour and may well be dependent on the site of origin and histogenesis of the tumour.

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