Clinical significance of TTF-1 protein expression and TTF-1 gene amplification in lung adenocarcinoma

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Received: June 20, 2008; Accepted: October 22, 2008

Abstract

The majority of lung adenocarcinomas express the lineage-specific thyroid transcription factor-1 (TTF-1). We recently reported that in a subset of lung adenocarcinomas the TTF-1 gene is amplified. Although the prognostic significance of TTF-1 expression has been previously investigated, the significance of TTF-1 amplification has not been established. We studied 89 consecutive patients with lung adenocarcinomas treated by surgery at Brigham and Women’s Hospital between 1997 and 1999 and performed immunohistochemical analysis for TTF-1 expression and fluorescence in situ hybridization for TTF-1 amplification. We investigated associations between clinical-pathological characteristics, TTF-1 expression, TTF-1 amplification and overall survival. TTF-1 expression was categorized as high (48%), low (24%) or absent (28%). TTF-1 was amplified in 7% of cases. Patients with adenocarcinomas with low or high TTF-1 expression had a significantly better outcome than those with absent TTF-1 expression (median overall survival times of 72.4, 77.8 and 30.5 months, respectively, P = 0.002). In contrast, patients with adenocarcinomas with TTF-1 expression had a worse outcome if TTF-1 was amplified (median overall survival time 39.5 versus 87.5 months). In multivariate analysis, improved overall survival was independently predicted by TTF-1 expression in combination with no TTF-1 amplification (P < 0.001). In patients with lung adenocarcinoma, TTF-1 expression is a predictor of good outcome. Patients with no TTF-1 expression or TTF-1 expression and TTF-1 gene amplification tend to have a significantly worse prognosis than patients with TTF-1 expression and no TTF-1 gene amplification.

Keywords: lung adenocarcinoma • TTF-1 • survival

Introduction

Lung cancer is the leading cause of death from cancer in both men and women [1]. Despite advances in treatment, the 5-year overall survival rate is approximately 15.7% [2]. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of lung cancer cases [3]. In the last several decades adenocarcinoma has become the predominant type of NSCLC [4, 5]. Currently, the pathological stage is the most important system to predict survival in patients with NSCLC [6] and to define groups with similar treatment strategies [7]. However, additional prognostic markers are needed in order to refine prognostic profiles that can potentially select patients who will benefit most from established treatments and save others from toxicity of ineffective treatments. Moreover, the discovery of novel molecular alterations may help identify potential therapeutic targets that would be more efficient and less harmful than current treatments.

Thyroid transcription factor-1 (TTF-1), also known as NKX2-1, is a 38-kD transcription factor that is normally expressed in adult thyroid and lung tissue [8] and is essential in lung development [9–11]. It is expressed in type II pneumocytes in the normal adult lung [8] and in approximately 75% of non-mucinous lung
adenocarcinomas [12]. Currently, testing for TTF-1 expression by
immunohistochemistry is commonly used to confirm the pulmonary
origin of a carcinoma [12, 13]. Several studies have evaluated the
prognostic significance of TTF-1 expression in NSCLC [14–20]
and lung adenocarcinoma specifically [13, 21–23]. Although stud-
ies reporting the prognostic significance of TTF-1 are conflicting,
most have shown that TTF-1 is a favourable prognostic factor for
survival in patients with lung adenocarcinoma [15].

We recently have shown that the gene that encodes TTF-1,
located on chromosome 14q13.3, is amplified in approximately
12% of lung adenocarcinomas [24]. Furthermore, amplification of
the TTF-1 gene is the single most common focal genetic event
assessed by high-resolution copy-number analysis of lung adeno-
carcinoma [24]. The clinical significance of TTF-1 amplification
and the relationship with TTF-1 expression have not been established.

We therefore conducted a retrospective study of the clinical
and pathological factors associated with TTF-1 expression and
TTF-1 amplification in a cohort of 89 consecutive patients with
lung adenocarcinoma and related the findings to survival.

### Materials and methods

#### Patient characteristics

The study group included 89 consecutive patients with lung adenocarci-
noma treated with surgery alone or surgery and post-operative adjuvant
therapy at Brigham and Women’s Hospital between March 1997 and
December 1999 (Table 1). The group was identified through a search of the
Department of Pathology Registry database maintained by the Department
of Pathology and included location and type of the primary tumour. Each
resection specimen had been evaluated with standard pathological meth-
ods as described in the Surgical Pathology Dissection Manual of the
Department of Pathology [25]. The cases were reviewed and staged
according to Sixth Edition of the American Joint Committee on Cancer
manual [6].

Patients were selected for study with the following inclusion criteria:
lung adenocarcinoma; first treatment by surgery alone; no other malignant
tumours in 5 years prior to the diagnosis of lung adenocarcinoma except
squamous cell or basal cell carcinoma of the skin or carcinoma in situ of
the uterine cervix; and no deaths in the perioperative period less than 30
days after surgery.

The clinical characteristics ascertained were sex, age, smoking status,
cigarette consumption and type of surgery (Table 1). The pathological
characteristics were anatomic location of the primary tumour, tumour size,
pathologic stage, tumour and lymph node status (Table 1) and
histopathological subtype of adenocarcinoma.

#### Histological analysis

For each case, one slide of tumour was reviewed simultaneously by
two pathologists (J.A.B. and L.R.C.) at a multi-head viewing microscope
and classified according to WHO criteria [26, 27]. Cases with significant

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**Table 1 Clinicopathological characteristics of patients with lung adenocarcinoma**

| Characteristic | No. of patients n = 89 |
|---------------|------------------------|
| Sex           |                        |
| Male          | 32 (36%)               |
| Female        | 57 (64%)               |
| Age           |                        |
| Median        | 67 years               |
| Range         | (34–84)                |
| Smoking status*|                       |
| Nonsmoker     | 11 (14%)               |
| Smoker        | 67 (86%)               |
| Pack-years: median | 46.5                |
| Range         | (5–150)                |
| Resection type|                        |
| Wedge resection| 26 (29%)             |
| Lobectomy     | 61 (69%)               |
| Pneumonectomy | 2 (2%)                 |
| Tumour location|                       |
| RUL           | 32 (36%)               |
| RML           | 6 (7%)                 |
| RLL           | 7 (8%)                 |
| LUL           | 30 (34%)               |
| LLL           | 14 (16%)               |
| Tumour size   |                        |
| Median        | 2.5 cm.                |
| Range         | (0.6–7.0)              |
| Pathologic stage|                      |
| IA            | 22 (25%)               |
| IB            | 32 (36%)               |
| IIA           | 3 (3%)                 |
| IIB           | 9 (10%)                |
| IIIA          | 7 (8%)                 |
| IIIB          | 7 (8%)                 |
| IV            | 9 (10%)                |
| Tumour status |                        |
| pT1           | 32 (36%)               |
| pT2           | 44 (49%)               |
| pT3           | 3 (3%)                 |
| pT4           | 10 (11%)               |
| Node status** |                        |
| Positive      | 23 (29%)               |
| Negative      | 57 (71%)               |

*Smoking status known for 78 patients.
**Eighty patients had surgically resected lymph nodes.
differences between the two reviewers were evaluated by a third pathologist (W.D.T.). Poorly differentiated adenocarcinomas with a pure solid growth pattern were confirmed by a positive mucicarmine stain and negative p63 immunohistochemical stain.

**Tissue microarray (TMA) construction**

A TMA was constructed from a representative block from formalin-fixed, paraffin-embedded archival tissue specimens. Three tumour samples (0.6-mm cores) from each case were included into paraffin donor blocks using a manual arrayer (Beecher Instruments, Inc., Sun Prairie, WI, USA). The three cored areas on each donor block were randomly selected from different parts of the tumour tissue based on a histological characterization of the haematoxylin and eosin stained slide. For all cases, if at least one core was present, the core findings were used. Only for cases that we had no results for the tumour on the array, did we use whole mounts.

**Immunohistochemical analysis for TTF-1 expression**

After construction of the TMA blocks, 4-μm sections were placed on charged polylysine-coated slides. Slides were deparaffinized, placed in 3% hydrogen peroxide for 5 min. and washed. Heat-induced epitope retrieval was performed using 0.001 mol/l ethylenediaminetetraacetic acid for 50 min. in a steamer (Model HS80, Black & Decker, Shelton, CT, USA). Slides were cooled for 10 min., washed, placed in Tris buffer pH 7.6, then incubated for 1 hr with monoclonal antibody to TTF-1 (clone 8G7G3/1, Dako, Carpinteria, CA, USA) at a 1:200 dilution. Slides were then washed and incubated with mouse PowerVision detection system (ImmunoVision Technologies, Daly City, CA, USA) for 30 min. Antibody localization was effected using a peroxidase reaction with DAB+ (Dako) as chromogen. Staining intensity was enhanced with DAB enhancer (Zymed Lab, South San Francisco, CA, USA). Slides were counterstained with haematoxylin and mounted.

TTF-1 immunohistochemical staining was assessed by two pathologists (J.A.B. and L.R.C.), who reviewed the cases simultaneously at a multi-head viewing microscope, without access to clinical data or TTF-1 amplification status and recorded the staining intensity relative to the strong staining intensity of type II pneumocytes as: no expression (absent staining) or high expression (strong staining intensity = 2). There is a direct correlation between the intensity TTF-1 immunohistochemical staining and the levels of TTF-1 protein (data not shown) [24].

**Tissue microarray dual-colour interphase fluorescent in situ hybridization (TMA-FISH)**

FISH analysis was performed with a bacterial artificial chromosome (BAC) probe containing NKK2-1 (Fig. 1) on lung adenocarcinoma
samples from TMAs or full-mount sections from paraffin blocks in cases with insufficient probe hybridization or lack of viable tumour on the TMA slide. A Biotin-14-dCTP labelled BAC clone RP11–1083E2 (conjugated to produce a red signal) was used for the NKX2–1 probe and a DigoxindUTP labelled BAC clone RP11–72J8 (conjugated to produce a green signal) was used for the reference probe [24]. Tissue hybridization, washing and colour detection were performed as described previously [28–30]. The BAC clones were obtained from the BACPAC Resource Center, Children’s Hospital Oakland Research Institute (CHORI, Oakland, CA, USA). Prior to tissue analysis, the integrity and purity of all probes were verified by hybridization to metaphase spreads of normal peripheral lymphocytes. The samples were analysed under a 60× oil immersion objective using an Olympus BX-51 fluorescence microscope equipped with appropriate filters, a CCD (charge coupled device) camera and the CytoVision FISH imaging and capturing software (Applied Imaging, San Jose, CA, USA). Semi-quantitative evaluation of the tests was independently performed (A.J.I., S.P., L.A.J. and M.A.R.) and the samples were recorded as amplified or non-amplified; at least 100 nuclei for each case were analysed when possible. In cases where a discrepancy in the result interpretation was noted, a group consensus was reached. TTF-1 was considered amplified if there was a significant gain in TTF-1 as compared to the reference probe.

Statistical analysis

Fisher’s exact test was used to compare categorical data for clinicopathological characteristics between expression and amplification subgroups, and Wilcoxon rank-sum test was used to compare continuous data. Overall survival was calculated from time of surgery to time of death from any cause or to time of last follow-up, at which point the data were censored. Overall survival curves were constructed using the Kaplan–Meier method, and log-rank test was used to evaluate the differences between expression and amplification subgroups. The independent prognostic significance of the TTF-1 expression and amplification status was determined using Cox proportional hazards model for multivariate analysis.

Results

Patient demographics and pathological characteristics

The clinicopathological characteristics of the patients with lung adenocarcinoma included in this study are summarized in Table 1.

Thirty-two (36%) patients were male, and 57 patients (64%) were female. The median age was 67 years (range 34–84 years). Pathological stage was IA in 22 patients (25%), IB in 32 patients (36%), IIA in 3 patients (3%), IIB in 9 patients (10%), IIIA in 23 patients (28%), IIIB in 7 patients (8%) and IV in 9 patients (10%). The tumour status was pT1 in 32 patients (36%), pT2 in 44 patients (49%), pT3 in 3 patients (3%) and pT4 in 10 patients (11%). Of 80 patients (90%) who had surgically resected lymph nodes, involvement by adenocarcinoma was present in 23 patients (N1 and N2 status, 29%) and absent in 57 patients (N0 status, 71%).

TFF-1 expression and TTF-1 amplification status of lung adenocarcinomas (Table 2, Fig. 2)

TTF-1 staining was homogeneous in 45 cases (51%) and heterogeneous in 44 cases (49%, Table 2). The predominant staining score was 0 in 25 cases (28%), 1 in 21 cases (24%) and 2 in 43 cases (48%). TTF-1 protein was more likely to be expressed in smaller tumours (P = 0.007), and in adenocarcinomas from female patients (P = 0.026, Table 3). No association was observed with tumour or node status, pathological stage or other patient characteristics (Table 3).

TTF-1 gene amplification was assessable in all 89 cases (Table 2). The TTF-1 gene was amplified in 6 cases (7%) and non-amplified in 71 cases (80%). TTF-1 polysomy was detected in 12 cases (13%). TTF-1 protein expression was not associated with TTF-1 gene amplification status (P = 1.0). Furthermore, no correlation was found between TTF-1 gene polysomy and TTF-1 expression (Spearman r = 0.008, P = 0.47). Tumours with TTF-1 gene amplification had a strong TTF-1 expression in three cases (50%), weak in one case (17%) and absent in two cases (33%). The two cases with TTF-1 amplification but no TTF-1 staining showed cytoplasmic but no nuclear staining for TTF-1 with appropriate internal positive controls. One case had adenocarcinoma with a predominantly solid pattern and the patient died of disease after 30.5 months; and the second had adenocarcinoma with a predominantly acinar growth pattern and the patient is alive after 105.6 months of follow up.
**Adenocarcinoma subtypes**

The predominant pattern was acinar in 49 cases (55%), papillary in 13 cases (15%), solid in 25 cases (28%), bronchioloalveolar in 2 cases (2%). TTF-1 protein was more likely to be expressed by tumours with dominant acinar, papillary or bronchioloalveolar subtypes \( (P = 0.017) \). Of the six lung adenocarcinomas with TTF-1 amplification, two (33%) were classified as acinar, three (50%) as solid and one (17%) as papillary subtype.

**Survival analysis**

The median follow-up period was 87 months (range 4–110 months) among the 37 patients still alive. The overall survival time was significantly longer for patients with adenocarcinomas with high (median 72.4 months) or low TTF-1 expression (median 77.8 months), than for patients with adenocarcinomas with absent TTF-1 expression (median 30.5 months) \( (P = 0.002, \text{Fig. 3A}) \). Overall survival did not differ significantly between patients with adenocarcinomas with low TTF-1 expression and patients with adenocarcinomas with high TTF-1 expression \( (P = 0.995) \). A multivariate model confirmed that patients with absent TTF-1 expression have a significantly worse prognosis \( \text{[Hazard Ratio (HR) = 2.6, } P = 0.003] \) compared to patients with high or low TTF-1 expression. The model was adjusted for tumour size, dominant subtype and gender as potential confounders due to their associations with TTF-1 expression in Table 3.

In contrast, there was no difference in overall survival between patients with or without TTF-1 gene amplification (median 39.5 versus 67.1 months, \( P = 0.508, \text{Fig. 3B} \)).

**Presence of TTF-1 amplification in patients with adenocarcinomas with TTF-1 expression**

Within our cohort, we found a group of 64 patients with adenocarcinomas with TTF-1 expression and an assessable TTF-1 gene amplification status. We identified two categories based on the FISH amplification status of the TTF-1 gene: patients with adenocarcinomas with TTF-1 expression and TTF-1 gene amplification (4 patients, 6%), and patients with adenocarcinomas with TTF-1 expression with TTF-1 non-amplified (60 patients, 94%, Table 4) There were no statistically significant differences in age, sex and clinicopathological characteristics between the two groups, except for pathological stage and tumour status (Table 5). TTF-1 gene amplification was more likely to be associated with a higher
### Table 3 Characteristics of the patients with lung adenocarcinoma according to TTF-1 expression*

| Characteristic                      | Adenocarcinoma without TTF-1 expression (n = 25) | Adenocarcinoma with TTF-1 expression (n = 64) | P-value |
|-------------------------------------|-----------------------------------------------|---------------------------------------------|---------|
| **Sex**                             |                                               |                                             |         |
| Male                                | 14 (56%)                                      | 18 (28%)                                    | 0.026   |
| Female                              | 11 (44%)                                      | 46 (72%)                                    |         |
| **Age**                             |                                               |                                             | 0.313   |
| Median                             | 64 years                                      | 68 years                                    |         |
| Range (years)                       | (34–77)                                       | (36–84)                                     |         |
| **Smoking status†**                 |                                               |                                             | 0.717   |
| Non-smoker                          | 2 (10%)                                       | 9 (16%)                                     |         |
| Smoker                              | 19 (90%)                                      | 48 (84%)                                    |         |
| Pack-years: median                  | 50                                             | 40                                          | 0.334   |
| Range (pack-years)                  | (10–150)                                      | (5–110)                                     |         |
| **Resection type**                  |                                               |                                             | 0.608   |
| Wedge resection                     | 6 (24%)                                       | 20 (31%)                                    |         |
| Lobectomy/pneumonectomy             | 19 (76%)                                      | 44 (69%)                                    |         |
| **Tumour location**                 |                                               |                                             | 0.638   |
| Left side                           | 11 (44%)                                      | 33 (52%)                                    |         |
| Right side                          | 14 (56%)                                      | 31 (48%)                                    |         |
| **Tumour size**                     |                                               |                                             | 0.007   |
| Median                             | 3.0 cm.                                       | 2.3 cm.                                     |         |
| Range (cm.)                         | (1.1–7.0)                                     | (0.6–6.0)                                   |         |
| **Pathological stage**              |                                               |                                             | 0.611   |
| IA                                  | 4 (16%)                                       | 18 (28%)                                    |         |
| IB                                  | 9 (36%)                                       | 23 (36%)                                    |         |
| IIA                                 | 1 (4%)                                        | 2 (3%)                                      |         |
| IIB                                 | 3 (12%)                                       | 6 (9%)                                      |         |
| IIIA                                | 4 (16%)                                       | 3 (5%)                                      |         |
| IIIIB                               | 2 (8%)                                        | 5 (8%)                                      |         |
| IV                                  | 2 (8%)                                        | 7 (11%)                                     |         |
| **Tumour status**                   |                                               |                                             | 0.229   |
| pT1                                 | 6 (24%)                                       | 26 (41%)                                    |         |
| pT2                                 | 14 (56%)                                      | 30 (47%)                                    |         |
| pT3                                 | 2 (8%)                                        | 1 (2%)                                      |         |
| pT4                                 | 3 (12%)                                       | 7 (11%)                                     |         |
| **Nodal status‡**                   |                                               |                                             | 0.411   |
| Negative                            | 14 (64%)                                      | 43 (74%)                                    |         |
| Positive                            | 8 (36%)                                       | 15 (26%)                                    |         |
| **Dominant subtype**                |                                               |                                             | 0.017   |
| Bronchioloalveolar/acinar/papillary | 13 (52%)                                      | 51 (80%)                                    |         |
| Solid                               | 12 (48%)                                      | 13 (20%)                                    |         |

* Due to rounding, not all values total 100%.
† Exposure data was available in 78 patients.
‡ Nodal status was available in 80 patients.
pathological stage ($P = 0.019$) and more advanced tumour status ($P = 0.005$) among patients with adenocarcinomas expressing TTF-1. The overall survival was worse for patients with adenocarcinomas with TTF-1 expression and TTF-1 gene amplification than for patients with adenocarcinomas with TTF-1 expression and TTF-1 non-amplified (median 39.5 versus 87.5 months, $P = 0.113$, Fig. 3C). However, overall survival was similar between the patients with TTF-1 expression and TTF-1 gene amplification and the patients with no TTF-1 expression ($P = 0.990$). These combined subgroups were associated with significantly worse overall survival compared to patients with TTF-1 expression and TTF-1 non-amplified ($P < 0.001$). A multivariate model confirmed that patients with no TTF-1 expression or TTF-1 expression and TTF-1 gene amplification have a significantly worse prognosis ($P = 0.002$) than patients with TTF-1 expression and no TTF-1 gene amplification after adjusting for the clinicopathological factors found to be associated with gene amplification among patients with TTF-1 expression in Table 5. In addition to tumour status and pathological stage, we also controlled for the factors showing a trend towards association ($P = 0.200$), namely tumour location and dominant subtype. The estimated hazard ratio of 2.8 suggests that patients with no TTF-1 expression or TTF-1 expression and TTF-1 gene amplification have a risk of death almost three times higher relative to patients with adenocarcinomas with TTF-1 expression and without TTF-1 amplification.

Discussion

Amplification of the gene that encodes the lineage-specific transcription factor TTF-1, located at 14q13.3, was recently identified as a novel molecular abnormality in 12% of lung adenocarcinomas [24, 31]. The clinical significance of TTF-1 gene amplification and the relationship with TTF-1 protein expression have not been established. In this study, we investigated TTF-1 expression and TTF-1 amplification in lung adenocarcinomas from a cohort of 89 consecutive patients. We found that TTF-1 is expressed in 72% and the TTF-1 gene is amplified in 7%. We evaluated the prognostic significance of TTF-1 gene amplification and the relationship with TTF-1 protein expression in patients with lung adenocarcinoma.
Table 5 Characteristics of the patients with TTF-1 expression in the lung adenocarcinoma according to TTF-1 amplification*

| Characteristic            | TTF-1 Non-amplified (n = 60) | TTF-1 Amplified (n = 4) | P-value |
|---------------------------|-------------------------------|-------------------------|---------|
| Sex                       |                               |                         | 0.313   |
| Male                      | 16 (27%)                      | 2 (50%)                 |         |
| Female                    | 44 (73%)                      | 2 (50%)                 |         |
| Age                       |                               |                         | 0.674   |
| Median                    | 68 years                      | 59.5 years              |         |
| Range                     | 36–84                         | 38–77                   |         |
| Smoking status†           |                               |                         | 0.507   |
| Non-smoker                | 8 (15%)                       | 1 (25%)                 |         |
| Smoker                    | 45 (85%)                      | 3 (75%)                 |         |
| Pack-years: median        | 40                            | 45                      | 0.869   |
| Range                     | (5–110)                       | (10–66)                 |         |
| Resection type            |                               |                         | 0.300   |
| Wedge resection           | 20 (33%)                      | 0                       |         |
| Lobectomy/pneumonectomy   | 40 (67%)                      | 4 (100%)                |         |
| Tumour location           |                               |                         | 0.114   |
| Right side                | 31 (52%)                      | 0                       |         |
| Left side                 | 29 (48%)                      | 4 (100%)                |         |
| Tumour size               |                               |                         | 0.721   |
| Median                    | 2.25 cm.                      | 2.5 cm.                 |         |
| Range                     | (0.6–6.0)                     | (1.6–2.5)               |         |
| Pathological stage        |                               |                         | 0.019   |
| IA                        | 18 (30%)                      | 0 (0%)                  |         |
| IB                        | 23 (38%)                      | 0 (0%)                  |         |
| IIA                       | 2 (3%)                        | 0 (0%)                  |         |
| IIB                       | 5 (8%)                        | 1 (25%)                 |         |
| IIIA                      | 3 (5%)                        | 0 (0%)                  |         |
| IIIB                      | 3 (5%)                        | 2 (50%)                 |         |
| IV                        | 6 (10%)                       | 1 (25%)                 |         |
| Tumour status             |                               |                         | 0.005   |
| pT1                       | 26 (43%)                      | 0                       |         |
| pT2                       | 29 (48%)                      | 1 (25%)                 |         |
| pT3                       | 1 (2%)                        | 0                       |         |
| pT4                       | 4 (7%)                        | 3 (75%)                 |         |
| Nodal status‡             |                               |                         | 0.273   |
| Negative                  | 41 (76%)                      | 2 (50%)                 |         |
| Positive                  | 13 (24%)                      | 2 (50%)                 |         |
| Dominant subtype          |                               |                         | 0.181   |
| Bronchioloalveolar/acinar/papillary | 49 (82%) | 2 (50%) |         |
| Solid                     | 11 (18%)                      | 2 (50%)                 |         |

*Due to rounding, not all values total 100%.
†Exposure data was available in 57 patients.
‡Nodal status was available in 58 patients.
TTF-1 expression was high in 48%, low in 24%, and absent in 28% of cases. Similar to recently published studies, we found that patients with lung adenocarcinomas with TTF-1 expression had a better outcome than patients with lung adenocarcinomas with no TTF-1 expression (median overall survival times of 77.8 and 30.5 months, respectively, P = 0.002) [15, 21, 23]. We also found that patients with low or high TTF-1 expression have a similar overall survival (P = 0.995). Although TTF-1 gene amplification was not a predictor of overall survival in our entire patient group (P = 0.508), in patients with adenocarcinomas with TTF-1 expression, TTF-1 gene amplification was associated with poor outcome (median overall survival 39.5 versus 87.5 months, P = 0.113). The finding that TTF-1 amplification is a potential indicator of poor prognosis is consistent with the findings of Kendall et al. who reported that focal amplification of TTF-1 correlated with a higher stage at diagnosis and recurrence [31]. The prevalence of TTF-1 amplification in our study focused only on a cohort of patients with well-characterized lung adenocarcinomas and excluded other types of non-small cell lung carcinomas, and differences in TTF-1 amplification in different tumour types might explain the reported discrepancies from previously published analyses [24, 31]. Patients with lung adenocarcinomas with TTF-1 expression and TTF-1 amplification have similar overall survival as patients with adenocarcinomas with no TTF-1 expression (P = 0.990).

In our study there were two cases harbouring TTF-1 amplification that had no nuclear TTF-1 expression by immunohistochemical staining. Kendall et al. reported amplification of 14q13.3 without TTF-1 protein expression in the squamous cell cancer cell line NCI-H2170 [31]. They found that the RNA message of TTF-1 was undetectable in this cell line, suggesting gene silencing at the transcription level. In the two cases in our study that had TTF-1 amplification but no nuclear TTF-1 expression, there was cytoplasmic TTF-1 staining suggesting that although TTF-1 is expressed, it is aberrantly localized to the cytoplasm. While TTF-1 gene amplification has been shown to have a role in oncogenesis, we expect that this would be true only for cases that have TTF-1 amplification and nuclear TTF-1 protein expression since TTF-1 functions as a transcription factor. Lack of nuclear expression for two cases with amplification in this study indicates that TTF-1 staining by immunohistochemistry must be performed in conjunction with analysis for TTF-1 gene amplification in evaluating for the biologic or prognostic significance of TTF-1 amplification.

Our study is limited by the small number of patients with adenocarcinomas TTF-1 gene amplification. In addition, we were able to examine only three randomly selected tissue cores from each tumour. Additional studies are needed to validate the potential diagnostic utility of the characteristics we report, including determinations of intraobserver and interobserver variation in their evaluation; their sensitivity, specificity, predictive values for the presence of TTF-1 positivity and correlation between the different growth patterns, TTF-1 gene expression and TTF-1 amplification; their intratumoural heterogeneity and role of sampling in evaluation and the findings in lung adenocarcinomas. However, the results of our study support previous findings that TTF-1 expression is a positive prognostic factor in patients with lung adenocarcinomas. Additionally, this is the first report to suggest that not all cases of adenocarcinoma that harbour TTF-1 amplification express nuclear TTF-1 protein, indicating the importance of evaluating protein expression levels in conjunction with TTF-1 amplification status.

Our study provides support for the concept of tumour heterogeneity in lung adenocarcinoma. Therefore, our findings suggest that lung adenocarcinoma with TTF-1 expression in combination with no TTF-1 amplification represents a distinct subgroup of lung adenocarcinoma with characteristic pathological and clinical features, and improved overall survival.

Acknowledgements

This study was supported by CA90578, CA074386 and CA092824 from the National Institutes of Health. We are indebted to Ms. Laura Kwan for secretarial support.

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