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CASE REPORT

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Non-small cell lung carcinoma with focal coexpression of thyroid transcription factor-1 and Δ Np63/p40: A case report

Kazuhiro Terada[1] | **Toshi Menju**[2] | **Horoshi Date**[2] | **Hironori Haga**[1] | **Akihiko Yoshizawa**[1,3]

[1]Department of Diagnostic Pathology, Kyoto University Hospital, Kyoto, Japan

[2]Department of Thoracic Surgery, Kyoto University Hospital, Kyoto, Japan

[3]Department of Diagnostic Pathology, Nara Medical University, Kyoto, Japan

Correspondence
Kazuhiro Terada, Department of Diagnostic Pathology, Kyoto University Hospital, Kyoto, Japan.
Email: katerada@kuhp.kyoto-u.ac.jp

Abstract

Most lung carcinomas are subtyped by their morphologies; however, immunohistochemistry is usually performed when it is difficult to determine. The most reliable antibodies for distinguishing lung adenocarcinoma from squamous cell carcinoma are thyroid transcription factor-1 (TTF-1) and p40 (ΔNp63). In general, these markers are mutually exclusive in their expression of lung primary carcinoma; however, a few cases of non-small cell lung carcinoma (NSCLC) with coexpression of both markers have been reported. Examining a tissue microarray of 229 squamous cell carcinomas and 346 adenocarcinomas, we found one case of NSCLC with coexpression of TTF-1 and p40. Herein, we present a 71-year-old man, who had a mass lesion in the left lung apex. A transbronchial lung biopsy was performed, revealing NSCLC. He underwent left upper segmentectomy and lymph node dissection. Macroscopically, the mass showed a white-to-tan solid tumor on the cut surface. Microscopically, the tumor was composed of polygonal tumor cells which had round and vesicular nuclei with prominent nucleoli. They had an abundant amount of cytoplasm, which was slightly eosinophilic or amphophilic. Multinucleated cells with atypical nuclear features were observed to be scattered in some areas. Multifocal necrosis and hemorrhage were also noted. Distinct squamous features and obvious glandular features were absent. Immunohistochemically, the most tumor cells were coexpressed positive for both TTF-1 and p40. In our study, NSCLC with TTF-1 and p40 coexpression is rare; therefore, it is necessary to obtain further data and examine similar cases to establish more precise definitions and clinicopathological features.

KEYWORDS
case report, coexpression, lung, p40, TTF-1

INTRODUCTION

Most lung carcinomas are classified according to their morphology; however, some are nonetheless still difficult to characterize.[1] Such cases require immunohistochemical analysis using reliable antibody markers. The most reliable antigens for distinguishing lung adenocarcinoma (ADC) from squamous cell carcinoma (SQCC) are thyroid transcription factor-1 (TTF-1) and p40 (ΔNp63).[2,3] In general, TTF-1 and p40 are mutually exclusive in their expression in primary lung carcinoma; however, several cases of non-small cell lung carcinoma (NSCLC) with coexpression of both markers have also been reported.[4–9] We performed immunohistochemistry on existing tissue microarrays (TMAs) of 229 SQCCs and 346 ADCs to study the frequency of such cases, which resulted in the identification of one case.

CASE REPORT

A 71-year-old man presented with bloody sputum and left chest pain. He was a past smoker who had consumed 20 cigarettes per day for 59 years. Chest

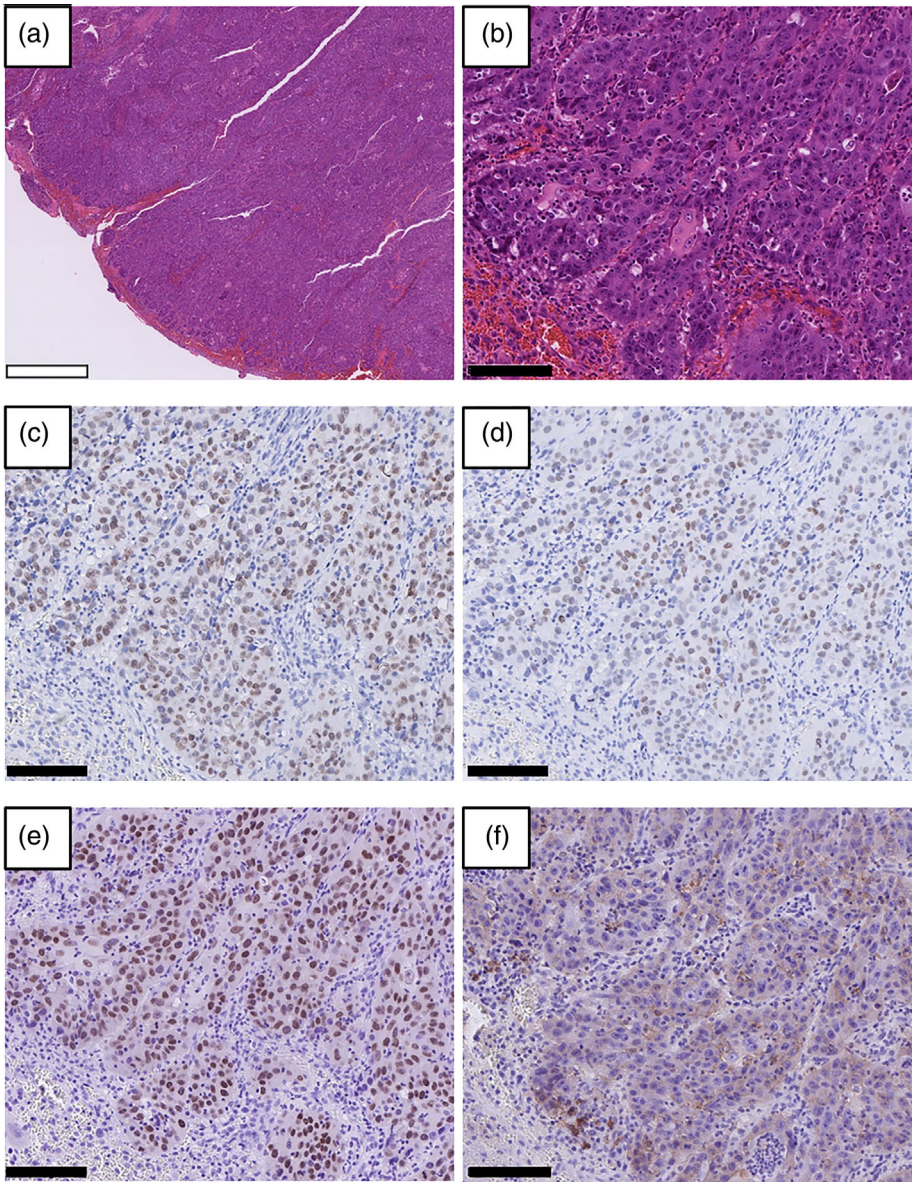

FIGURE 1 Representative images of NSCLC with TTF-1 and p40 coexpression. Hematoxylin and eosin (HE) images with low (a) and high (b) magnification, and immunohistochemical staining for p40 (c), TTF-1 (clone 8G7G3/1) (d), TTF-1 (clone SPT24) (e), and PD-L1 (22C3) (f). Most of the p40-positive tumor cells were positive for TTF-1 (clone SPT24). In contrast, there was weak TTF-1 (clone 8G7G3/1) staining of the tumor cells. The tumor proportion score for PD-L1 was 60%. The white and black scale bars indicate 1 mm and 100 μm, respectively. NSCLC, non-small cell lung carcinoma; TTF-1, thyroid transcription factor-1.

radiography revealed a mass at the apex of the left lung. A transbronchial lung biopsy revealed NSCLC. The patient underwent left upper segmentectomy and lymph node dissection.

Macroscopically, the mass was 6.0 cm in its greatest dimension and had a white-to-tan color on the cut surface. Microscopically, the solid tumor was composed of polygonal cells (Figure 1a, b). The tumor cells had round vesicular nuclei with prominent nucleoli as well as a slightly eosinophilic or amphophilic abundant cytoplasm. Scattered multinucleated cells, multifocal necrosis, and hemorrhage were also observed. No distinct squamous or glandular features were observed. No lymph node metastasis was detected (Table 1).

Immunohistochemically, approximately 80% of the tumor cells were stained with anti-p40 (BC28; Roche Diagnostics) (Figure 1c). In contrast, anti-TTF-1 (8G7G3/1; Dako) and anti-TTF-1 (SPT24; Novocastra) stained positively in 10% and 40% of cases, respectively (Figure 1d, e). Most TTF-1-positive samples (both clones) were also positive for p40, indicating coexpression of p40 and TTF-1. The TTF-1 staining intensity of SPT24 was stronger than that of 8G7G3/1. The tumor proportion score for PD-L1 (22C3 pharmDx KIT) was 60% (Figure 1f). Genomic analyses were performed using the Oncomine Dx Target Test (Thermo Fisher Scientific); however, no mutations were identified because of poorly preserved tumor DNA. No *ALK* or *ROS1* rearrangements were identified.

Lastly, we diagnosed the tumor as NSCLC with TTF-1 and p40 coexpression because there was no distinct glandular or squamous differentiation, and the immunohistochemistry yielded equivocal findings. Approximately four

TABLE 1 Comparison of clinicopathological findings between six reports and this case.

Author	Sex	Age	Smoking history	Specimen	Localization and tumor size	TNM classifiaction
Pelosi et al.[4]	Male	77	Former	Biopsy	Left hilar 8.5 cm in diameter involving major bronchi Ipsilateral pleural effusion	NA
Hayashi et al.[5]	Male	73	Former	Resection	Left upper lobe 1.9 cm in diameter	NA
Spinelli et al.[6]	Male	51	Current	Biopsy	Right upper lobe 3.1 cm in diameter Lymph node metastasis Brain metastasis	NA
Pelosi et al.[7] (2 cases)	(1) Female (2) Male	(1) 62 (2) 62	(1) Former (2) Former	(1) Resection (2) Biopsy	(1) Right upper lobe 4.5 cm in diameter Lymph node metastasis (2) Left side 4.7 cm in diameter Lymph node metastasis	(1)pT4 N2 (2)pT2 N2
Ying et al.[8]	Male	38	Never	Biopsy	right upper lung to mediastinum 4.5 cm in diameter	pT4 N3
Omid et al.[9] (14 cases)	Male 7 cases Female 7 cases	65-94	Current or former 8 or more cases	Biopsy or aspiration 9 cases Resection 5 cases	NA	pT1a, pT1c, pT2a, or more N0, N2, or more
Present case	Male	71	Former	Resection	Left upper lobe 6.0 cm in diameter pleural invasion	pT4 N0

Author	Histology	Definition	p40 clone	p40 ratio
Pelosi et al.[4]	Solid pattern focally suggestive of squamous cell differentiation	Both p40 and TTF-1 coexpression at the level of the same individual tumor cells	BC28	NA
Hayashi et al.[5]	Solid pattern necrosis and focal glandular pattern	Both p40 and TTF1 coexpression in the individual tumor cells	BC28	NA
Spinelli et al.[6]	Solid pattern predominantly no clear glandular differentiation and keratin formation	Both p40 and TTF1 coexpression in the same tumor cells	RP163-05	NA
Pelosi et al.[7] (2 cases)	(1) Solid pattern comedo-type necrosis peripheral palisading (2) Solid pattern suspection of squamous differentiation without horn pearls and intercellular bridges	Both p40 and TTF1 coexpression in the same individual tumor cells diffusely and strongly.	BC28	(1)NA (2)Consistent positivity
Ying et al.[8]	Solid pattern obvious atypia, a high nucleoplasmic ratio, variably sized nuclei, coarse chromatin, inconspicuous nucleoli abundant eosinophilic cytoplasm	Both p40 and TTF1 coexpression in the same tumor cells	BC28	50-60%
	Eosinophilic cytoplasm resembling nonkeratinizing squamous cell carcinoma	Both p40 and TTF-1 labeling same cell populations in	BC28	50-100%

(Continues)

TABLE 1 (Continued)

Author	Histology	Definition	p40 clone	p40 ratio
Omid et al.[9] (14	8 cases basaloid features 6 cases	consecutive sections		
Present case	Solid pattern prominent necrosis	Presence of both p40 and TTF1 coexpression in the same individual tumor cells.	BC28	80%

Author	TTF-1 clone	TTF-1 ratio	Electron microscopy	Genetic alternations
Pelosi et al.[4]	8G7G3/1	NA	Numerous mucous granules and abundant electron-dense thick tonofilaments	KRAS, TP53 mutation FGFR1 gene amplification
Hayashi et al.[5]	8G7G3/1	NA	NA	PTEN and TP53 mutation
Spinelli et al.[6]	SPT24	NA	NA	TP53 mutation
Pelosi et al.[7] (2 cases)	8G7G3/1	(1) NA (2) Consistent positivity	(1) Intercellular lumina bordered by microvilli, fascicles of keratin fibers, Clara cell granule, and desmosomes (2) Intracytoplasmic lumina, Clara cell-like granules, fascicles of keratin fibers, and desmosomes	(1) EGFR and TP53 mutation GF1R, MYC, CCND1 and CDK2 copy number variation (2) NF1 mutation
Ying et al.[8]	8G7G3/1	NA	NA	EML4-ALK and PIK3CA mutation
Omid et al.[9] (14 cases)	8G7G3/1	50-95%	Mixture of exocrine (mucous granules, intercellular lumina) squamous (keratin fibers, tonofilaments, desmosome) features at a single cell level (3 cases)	TP53 (7 cases), CDKN2A (3 cases), KRAS (2 cases), and EGFR (1 case) mutation FGFR1 (5 cases), MYC (3 cases), NKX2-1 (2 cases), and AKT1 (2 cases) amplification
Present case	8G7G3/1 SPT24	8G7G3/1: 10 % SPT24: 40 %	NA	No available mutations identified due to poorly preserved tumor DNA ALK and ROS1 rearrangement not identified

Note: Twelve patients were male and eight were female, and their ages ranged from 38 to 94. Most (13 or more) of these patients were smokers. The specimens were prepared from 12 biopsies, one aspiration, and seven resected materials. In the first four reports, three tumors were situated at the left side, and three tumors were at the right upper lobe. The tumor diameter ranged from 1.9 to 8.5 cm, with a mean of 4.5 cm. Three cases showed lymph node metastasis. Microscopically, all these tumor cells formed predominantly solid nests, with focal squamous differentiation in 10 cases and focal glandular pattern in one case. TTF-1 clones were 8G7G3/1 in 19 cases and SPT24 in one case, whereas p40 clones were BC28 in 19 cases and RP163-05 in one case. All the cases demonstrated coexpression as both p40 and TTF-1 positivity was observed in the individual cells. Electron microscopy revealed morphological changes such as desmosomes, fascicles of keratin fibers, mucous granules, intercellular lumina bordered by microvilli. The genetic alternation was variable; however, 11 cases demonstrated TP53 mutations.
Abbreviation: NA, not available.

months after the segmentectomy, recurrence in the lumbar vertebrae was observed. Despite postoperative chemoradiotherapy, the patient died of cancer nine months after the surgery.

DISCUSSION

The current WHO classification suggests that TTF-1 positivity indicates ADC, while diffuse p40 positivity indicates SQCC. However, NSCLC with TTF-1 and p40 coexpression has not been described to date as a subtype of lung cancer. In previous reports, NSCLCs with TTF-1 and p40 coexpression occurred more frequently in men and smokers.[4–9] Microscopically, the tumor cells tended to form solid nests with focal squamous or glandular differentiation, regardless of the specimen type. In most cases, TTF-1 (clone 8G7G3/1) and p40 (clone BC28) could be detected immunohistochemically, and TP53 mutations were often identified genetically. These cases met the criteria of SQCC only from an immunohistochemical perspective because more than 50% of p40 positivity was considered SQCC.[10] However, the TTF-1 expression levels were contradictory. For anti-TTF-1 staining, the 8G7G3/1, SPT24, and SP141 clones are generally used. ADCs exhibited 89%, 93%, and 93% positivity, respectively.[2] In contrast, SQCCs exhibited 0%, 6%, and 8% positivity, respectively. In our study, we used these clones, which resulted in different positivity rates.

Furthermore, there was a varying degree of labeling in previous reports regardless of the specimen type. Our case demonstrated >50% TTF-1 and p40 positivity in TMAs; however, it did not meet the criteria for resected specimens. Therefore, it is necessary to consider whether a diagnosis can be made based only on biopsy specimens.

The precursors of double-positive tumor cells are unknown. In normal lung tissues, TTF-1 is scattered in type 2 pneumocytes, the bronchial epithelium, and some basal cells.[11] In contrast, p40 expression is detected in tracheobronchial and bronchiolar basal cells. These findings indicate that some bronchial basal cells are precursors of double-positive tumor cells. NSCLCs with TTF-1 and p40 coexpression exhibited poorly differentiated characteristics, aggressive clinical behavior, and poor prognosis.

In conclusion, NSCLC with TTF-1 and p40 coexpression is rare, as there was only a single one case in the TMAs containing 575 NSCLCs. The diagnostic thresholds for TTF-1 and p40 positivity are poorly defined. Therefore, it is necessary to obtain further data and examine similar cases to establish more precise definitions and clinicopathological features.

AUTHOR CONTRIBUTIONS

T.N. stained the slide with TTF-1 (clone SPT24) immunohistochemicallly.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data sets used in this study are available from the corresponding author upon reasonable request.

ORCID

Kazuhiro Terada https://orcid.org/0009-0000-6381-8043
Akihiko Yoshizawa https://orcid.org/0000-0003-1462-7341

REFERENCES

1. WHO Classification of Tumours Editorial Board. Thoracic tumours. 5th ed. International Agency for Research on Cancer: Lyon; 2021.
2. Vidarsdottir H, Tran L, Nodin B, Jirström K, Planck M, Mattsson JSM, et al. Comparison of three different TTF-1 clones in resected primary lung cancer and epithelial pulmonary metastases. Am J Clin Pathol. 2018;150(6):533–44.
3. Tatsumori T, Tsuta K, Masai K, Kinno T, Taniyama T, Yoshida A, et al. p40 is the best marker for diagnosing pulmonary squamous cell carcinoma: comparison with p63, cytokeratin 5/6, desmocollin-3, and sox2. Appl Immunohistochem Mol Morphol. 2014;22(5):377–82.
4. Pelosi G, Fabbri A, Tamborini E, Perrone F, Testi AM, Settanni G, et al. Challenging lung carcinoma with coexistent ΔNp63/p40 and thyroid transcription Factor-1 labeling within the same individual tumor cells. J Thorac Oncol. 2015;10(10):1500–2.
5. Hayashi T, Takamochi K, Yanai Y, Mitani K, Tomita H, Mogushi K, et al. Non-small cell lung carcinoma with diffuse coexpression of thyroid transcription factor-1 and ΔNp63/p40. Hum Pathol. 2018;78:177–81.
6. Spinelli M, Khorshad J, Viola P. When tumor doesn't read textbook. Third case of TTF1 and p40 co-expression in the same tumour cells in a non-small cell carcinoma. A potential new entity to consider? Pathologica. 2019;111(2):58–61.
7. Pelosi G, Eng MB, Eng MV, Uccella S, Forest F, Leone G, et al. Coexpression of ΔNp63/p40 and TTF1 within Most of the same individual cells identifies life-threatening NSCLC featuring squamous and glandular Biphenotypic differentiation: Clinicopathologic correlations. JTO Clin Res Rep. 2021;2(11):100222.
8. Cai Y, Liu H, Chen X, Yang J, Huang B. P40 and TTF-1 double-expressing non-small cell lung cancer with EML4-ALK and PIK3CA gene mutations: a case report and review of the literature. Oncol Lett. 2022;25(2):59.
9. Savari O, Febres-Aldana C, Chang JC, Fanaroff RE, Ventura K, Bodd F, et al. Non-small cell lung carcinomas with diffuse coexpression of TTF1 and p40: clinicopathological and genomic features of 14 rare biphenotypic tumours. Histopathology. 2023;82(2):242–53.
10. Yatabe Y, Dacic S, Borczuk AC, Warth A, Russell PA, Lantuejoul S, et al. Best practices recommendations for diagnostic immunohistochemistry in lung cancer. J Thorac Oncol. 2019;14:377–407.
11. Cabibi D, Bellavia S, Giannone AG, Barraco N, Cipolla C, Martorana A, et al. TTF-1/p63-positive poorly differentiated NSCLC: a histogenetic hypothesis from the basal reserve cell of the terminal respiratory unit. Diagnostics. 2020;10:10.

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