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Original Article

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?

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Summary

Introduction. The 2011 WHO Classification for lung adenocarcinoma enlightened the need for a wise use of immunohistochemistry to preserve tissue for both diagnosis and molecular studies. The current recommendation is to use a panel comprising TTF1 and p40 to classify tumors with no clear squamous or glandular differentiation as many studies have showed the higher specificity of p40 over p63 as marker of squamous differentiation. However, the co-expression of both markers opens a new scenario with subsequent classification and potentially treatment issues.

Materials and methods. We report a case of a non-small lung cell carcinoma (NSCLC) with coexistent expression of TTF1 and p40 in the same tumour cells. To our knowledge, this peculiar immunohistochemical profile is very rare, and thus a review of the clinical and molecular features including molecular variances of the tumour was performed. Review of the pertinent literature was also carried out.

Results. Two additional articles describing unusual cases of NSCLC with coexistent expression of TTF1 and p40 were found and compared to our case. Interestingly, they all carried out aberrant mutation in TP53 oncogene and were of advance stage.

Conclusion. The positivity for both "squamous" and "adenocarcinomatous" markers and mutations of TP53 could be the expression of a not fully recognized variant of NSCLC with possible implications for classification, diagnosis and therapy.

Key words

Lung squamous cell carcinoma • Lung adenocarcinoma • TP53 mutations • TTF-1 • P40

In 2011 the International Association for the Study of Lung Cancer (IASLC), the American Thoracic Society (ATS) and the European Respiratory Society (ERS) have jointly proposed a new classification for lung adenocarcinomas [1]. This classification, which also includes molecular features, stressed the need to optimize the management of the tissue available in order to render both diagnosis and molecular studies [2].

An algorithm based on morphological and immunohistochemical features recommends thyroid transcription factor-1 (TTF1) and p63 as markers for adenocarcinoma (ADC) and squamous cell carcinoma (SCC) respectively. Recently p40, an isoform of p63, has shown greater sensitivity and specificity in identification of SCCs when compared to p63. Bishop et al. [3] demonstrated that although p40 and p63 have the same sensitivity, polyclonal p40 has higher specificity as p63 antibody can stain up to 20-30% of ADCs leading to confusion in some poorly differentiated tumors. The recommendation is to use a panel comprising TTF1 and p40 which are generally mutually exclusive [3] to classify tumors with no clear squamous or glandular differentiation and with solid/pseudosquamoid histology. In fact, they can be misclassified as SCCs with severe treatment implication: the exclusion from molecular testing and potentially lethal pulmonary hemorrhage in patients treated with bevacizumab [2,3].

However, Pelosi in 2015 [4] and Hayashi in 2018 [5] have both described unusual cases of NSCLC with co-expression of TTF1 and p40 in the same cells. Interest-

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ingly both cases have a similar molecular signature harboring TP53 mutation. We reported the third case with similar features.

A 51-years-old male smoker patient (the number of cigarettes was not specified) was referred to our hospital for persistent headaches and a head RMI revealed multiple metastases in his brain. A 3.1 cm lung mass with associated mediastinal lymphadenopathy and adrenal lesion was subsequently discovered on CT scan and biopsy was performed (stage cT2a N3 M1c). The core of lung tissue showed a NSCLC with morphology slightly favoring squamous differentiation with occasional intercellular bridges and dense eosinophilic cytoplasm. Tumor cells showed strong and diffuse positive staining for p40 (Diagnostic BioSystem), TTF1 (Clone SPT24 Leica Biosystem Newcastle) and Napsin-A in the same tumor cells (Fig. 1). Our clone was SPT24 ready to use, while the other reports used the other clone so this means that more then one antibody highlights these type of tumours. In view of the positivity for adenocarcinoma markers, the sample was sent for molecular testing, ALK (negative) and PD-L1 (strong positive) testing. ALK was analyzed by immunohistochemistry using VENTANA ALK (D5F3) CDx Assay intended for the qualitative detection of the anaplastic lymphoma kinase (ALK) protein in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung carcinoma (NSCLC) tissue stained with a BenchMark XT or BenchMark ULTRA automated staining instrument. It is considered positive if there is presence of strong granular cytoplasmic staining in tumor cells (any percentage of positive tumor cells). Positive control used was the presence of strong granular cytoplasmic staining in ganglion cells in appendix. PDL1 was analyzed by immunohistochemistry using VENTANA PD-L1 (SP263) Assay intended for the qualitative detection of the programmed death ligand 1 (PD-L1) protein in formalin-fixed, paraffin-embedded (FFPE) NSCLC tissues stained with OptiView DAB IHC Detection Kit on a BenchMark IHC/ISH instrument. It was considered positive if there is presence of any amount of membranous staining in tumor cells of any intensity (percentage of positive tumor cells and their intensity recorded). Positive control used was the presence of membranous staining in placenta.

ROS-1 was not tested.

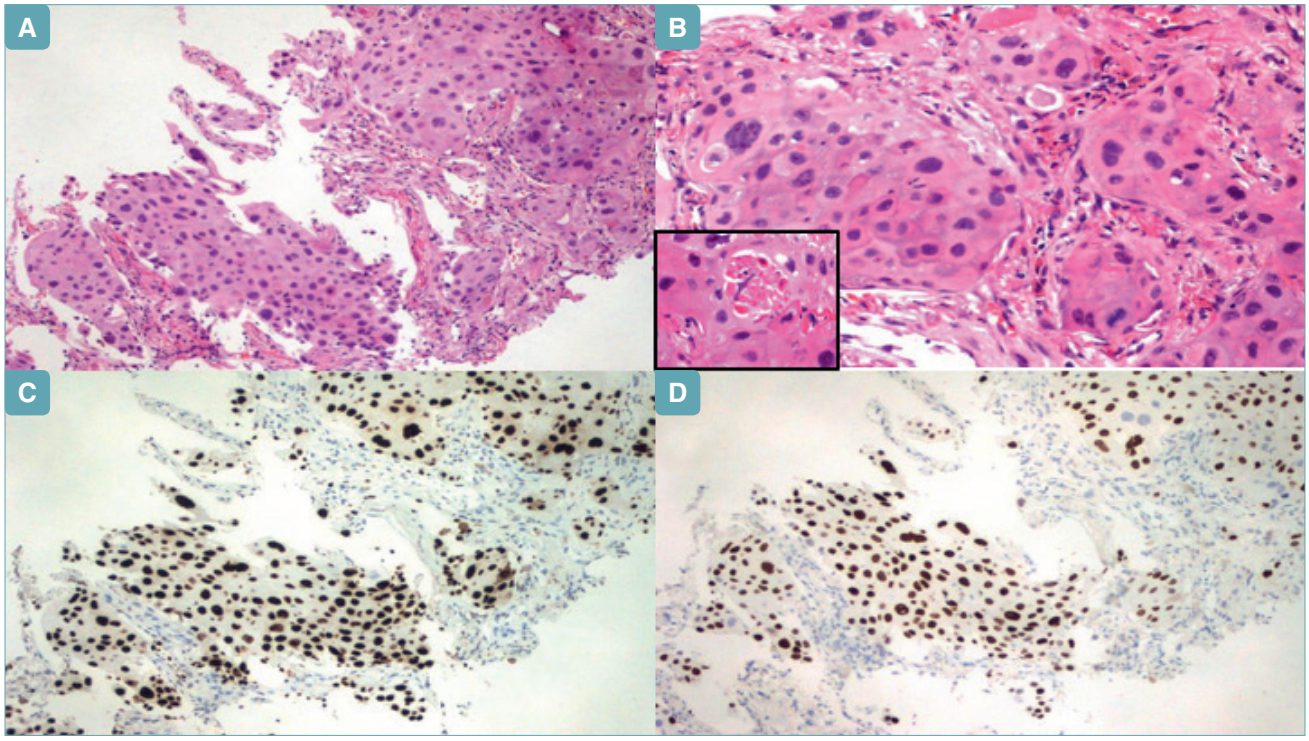

Fig. 1. (A) Lung biopsy showing a non-small cell carcinoma with predominantly solid pattern and no clear glandular differentiation or keratin formation (H&E, 10X). (B) Close up of the tumor featuring abundant eosinophilic cytoplasm and pleomorphic nuclei, hint of intercellular bridges is questionable (H&E, 20X). (C) Tumour cells show strong nuclear staining for TTF-1 (10X). (D) Strong nuclear immunoreactivity for p40 in the same tumor cells. The decoration pattern is identical for both markers (10X).

Mutational screening was performed by next generation sequencing using the Ion Torrent Cancer Hotspot panel v2. This assay comprises 207 amplicons in 50 oncogenes frequently mutated in solid tumors. DNA was extracted from paraffin embedded tissue using the Qiagen QIA symphony DSP DNA Mini Kit. The results showed no actionable mutations but a polymorphism in *TP53* (TP53):c.215C > G (p.Pro72Arg) which is currently associated with inherited cancer predisposition syndrome.
These features suggests that there is a still small but significant group of NSCLS with coexpression of of TTF-1 and p40 in the same cells; in order to further characterize these tumors and best classify them as more comparable to adenocarcinomas or squamous cell carcinomas, we suggest a panel of markers including TP53, Napsin-A, ALK and PD-L1.
The new WHO classification of lung cancer contains recommendations to provide the most accurate diagnosis for every type of sample (biopsy, resection or cytology). The need to identify non-small/non-squamous carcinoma has become essential in order to test these cases for actionable mutations. The more squamous-specific marker p40 has been used in few studies to correctly re-classify solid/pseudosquamoid tumors showing co-expression of TTF1 and p63 [3]. However, these three cases open a new horizon for identification and classification of peculiar multi-phenotypic tumors. Pelosi in 2015 [4]. reported an "amphicrine" biphenotypic tumor on a lung biopsy. The biopsy contained a high grade NSCLC with focal areas suggestive for squamous differentiation. Immunohistochemistry showed strong and diffuse positivity for both p40 and TTF-1 and electronic microscopy confirmed these features. The lesion also showed a *TP53* mutation and a gene amplification of *FGFR-1*.
In 2018 Hayashi et al. reported a case of NSCLC with strong and diffuse positivity for p40 and TTF-1 together with mutations on the allelic DNA for *TP53* and *PTEN* genes [5]. The authors speculated that mutations in the key genes such as *TP53, PTEN, FGFR-1* and others would promote the selection of peculiar stem cells leading to poorly differentiated and multi-phenotypic tumors.
TP53 is commonly mutated in many tumors [9]. TP53 executes its tumor-suppressive phenotype through controlling the transcription of many target genes in response to stress signals such as DNA damage, environmental hazards, toxins and oncogene activation [6]. The mutated TP53 loses its oncosuppressor function.
Using *in vitro* and *in vivo* models, Jeong et al. in 2017 [7] demonstrated that the deletion of *TP53* in tracheal epithelial cells promotes self-renewal and development of tumor cells with features similar to squamous cell carcinoma, while the same deletion in peripheral lung cells lead to adenocarcinoma-like cell formation. The type of lung cancer formed depends on the cell type targeted by deletion of *TP53*.
Mutations of *TP53* occur frequently in NSCLC: over 75% of SCCs and over 55% of ADCs [8]. Lung ADCs not harboring *TP53* mutations usually show that the gene is altered by ubiquitination – which leads to its degradation – and/or accumulation. Even wild-type TP53 can play a role in the development of ADCs with no evidence of mutations [9]. Clinically, mutations of *TP53* are associated with higher tumor size, stage and lymph node metastases [10]. The patient of the present case underwent whole brain radiotherapy followed by chemotherapy and showed a partial reduction of size of brain metastases, but the disease is progressing rapidly, and the patient is deteriorating at the time of writing.

Tab. I. Non-small lung cell carcinoma with co-expression of TTF-1 and p40: cases reported in literature.

	Gender/age	Smoking history	Imaging	Histology	IHC	Molecular
Pelosi 2015	Male/ 77yrs	Ex-smoker (40 pack year)	Left hilar tumour (85mm)	High grade NSCLC with hints of squamous differentiation	P40 (clone BC28 Biocare Medical Concorde CA) and TTF1 (clone 8G7G3/1, Dakopatts, Glostrup, Denmark) positive	K-RAS (AAA>AAT K117N exon4), TP53(GTG>GGG, V272G exon 8)
Hayashi 2018	Male/ 73yrs	Ex-smoker (141 pack year)	Left upper lobe tumour (19mm)	NSCLC with hints of glandular differentiation and areas negative for mucin stain	P40 (clone BC28 Biocare Medical Concorde CA) and TTF1 (clone 8G7G3/1, Dakopatts, Glostrup, Denmark) positive	PTEN (pHis123Asp), TP53 (pVal272Leu)
Present case	Male/ 51yrs	Current smoker	Right upper lobe tumour (31mm)	High grade NSCLC with hints of squamous differentiation	P40 (Diagnostic Biosystem RP163-05) and TTF1 (Bond ready to use primary antibody clone SPT-24, Leica biosystem, Newcastle Ltd) positive	(TP53):c.215C>G(p. Pro72Arg)

In general, since patients with lung carcinomas featuring *TP53* mutations have poorer outcomes, the gene may be used as prognostic marker in clinical practice and could also represent a target for cancer molecular therapy [6].

At present IASLC, ATS and ERS guidelines do not mention the co-expression of both markers 40 and TTF-1 in the same tumor cells. The case hereby presented, together with the two previously reported, has this peculiar immunohistochemical profile associated with an allelic mutation of oncosuppressor gene *TP53*. We could speculate that these combined features – positivity for both "squamous" and "adenocarcinomatous" markers and mutations of *TP53* could be the expression of an aggressive, not yet recognized variant of lung adenocarcinoma or adenosquamous carcinoma which could be considered for further classification, specific diagnostic approach and possibly targeted therapy in the near future.

Conflict of interest statement

None declared.

References

1 Travis WD, Brambilla E, Noguchi M, et al. *International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of Lung Adenocarcinoma*. J Thorac Oncol 2011;6:244-85. doi:10.1097/JTO.0b013e318206a221.

2 Travis WD, Brambilla E, Noguchi M, et al. *Diagnosis of lung cancer in small biopsies and cytology: implications of the 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification*. Arch Pathol Lab Med 2013;137;668-84. doi: 10.5858/arpa.2012-0263-RA.

3 Bishop JA, Teruya-Feldstein J, Westra WH, et al. *p40 (ΔNp63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma*. Mod Pathol 2012;25:405-15. doi: 10.1038/modpathol.2011.173.

4 G Pelosi G1, Fabbri A, Tamborini E, 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;10:1500-2. doi:10.1097/JTO.0000000000000553.

5 Hayashi T, Takamochi K, Yanai Y, et al. *Non-small cell lung carcinoma with diffuse coexpression of thyroid transcription factor-1 and ΔNp63/p40*. Hum Pathol 2018;78:177-81. doi:10.1016/j.humpath.2018.01.023.

6 Turrell FK, Kerr EM, Gao M, et al. *Lung tumors with distinct p53 mutations respond similarly to p53 targeted therapy but exhibit genotype-specific statin sensitivity*. Genes Dev 2017;31:1339-53. doi:10.1101/gad.298463.117.

7 Jeong Y, Hoang NT, Lovejoy A, et al. *Role of KEAP1/NRF2 and TP53 mutations in lung squamous celcarcinoma development and radiotherapyresponse prediction*. Cancer Discov 2017;7:86-101.

8 Smardova J, Liskova K, Ravcukova B, et al. *Complex analysis of the p53 tumor suppressor in lung carcinoma*. Oncol Rep 2016;35:1859-67. doi:10.3892/or.2015.4533.

9 Taguchi A1, Delgado O, Celiktaş M, et al. *Proteomic signatures associated with p53 mutational status in lung adenocarcinoma*. Proteomics 2014;14:2750-9. doi:10.1002/pmic.201400378.

10 Bian C, Li Z, Xu Y, et al. *Clinical significance of mutant p53 protein expression in lung adenocarcinoma*. Chinese J Lung Cancer 2015;18:23-8. doi:10.3779/j.issn.l009-3419.2015.01.04.

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