

Challenging Lung Carcinoma with Coexistent Δ Np63/p40 and Thyroid Transcription Factor-1 Labeling Within the Same Individual Tumor Cells

Giuseppe Pelosi, MD, MIAC, *† Alessandra Fabbri, MD, * Elena Tamborini, DSc, *
 Federica Perrone, DSc, * Adele M. Testi, DSc, * Giulio Settanni, DSc, * Adele Busico, DSc, *
 Giovanni Centonze, LabTech, * Paola Braidotti, DSc, ‡ Gaetano Bulfamante, MD, ‡
 Filippo De Braud, MD, § Marina Garassino, MD, § and Ugo Pastorino, MD ||

We herein report on an unusual instance of non-small-cell lung carcinoma (NSCLC) with double squamous/basal-like and glandular differentiation within the same individual tumor cells as documented in a biopsy sample by means of immunohistochemistry (IHC), electron microscopy (EM), and molecular investigation. Coexistence of glandular and squamous traits within the same tumor cells has previously been described in NSCLC using EM¹ to testify the possibility of “amphicrine” biphenotypic tumors as an extreme consequence of intratumor heterogeneity in lung cancer. Currently, Δ Np63/p40 (henceforth simply p40) is thought to be the most specific forerunner of squamous differentiation in the lung,²⁻⁴ thereby supporting the use of a minimalist IHC panel approach encompassing thyroid transcription factor-1 (TTF1) in the diagnostic workflow of demanding NSCLC cases. However, there is very little experience on NSCLC simultaneously positive for both p40 and TTF1 IHC at the level of the same individual tumor cells.

A 77-year-old man, former smoker (pack-year 40) was admitted to hospital for an 8.5-sized left hilar tumor mass involving major bronchi and associated with ipsilateral pleural effusion. Bronchial biopsy showed a high-grade NSCLC with just focal hints to squamous differentiation (Fig. 1A and B). Because of demanding structure, p40 (clone BC28, Biocare Medical, Concord, CA) and TTF1 (clone 8G7G3/1, Dakopatts, Glostrup, Denmark) IHC was undertaken, with widespread and strong decoration being unexpectedly observed for both biomarkers at the level of the same individual tumor cells (Fig. 1C and D). A final diagnosis of NSCLC with adenosquamous immunophenotype was thus rendered, with a comment on the possibility of facing with adenosquamous carcinoma (ADSQC) and a recommendation to study

the tumor molecularly. To further unravel such an unusual case, an EM study was carried out on two cylindrical tissue cores as small as 1 mm in diameter retrieved from the original paraffin block by using microarray technology, analyzing several ultrastructural fields for consistency. EM revealed extracellular lumen formation with microvilli-like cytoplasmic protrusions and mucous granules along with abundant perinuclear tonofilaments consistent with both adenocarcinoma and squamous cell carcinoma differentiation at the level of the same individual tumor cells, respectively (Fig. 1E and F).

Molecular investigation was carried out by using the 50-gene Ion AmpliSeq Cancer Hotspot Panel v2 approach with the Ion-Torrent Personal Genome Machine platform (both from Life Technologies, Foster City, CA) and fluorescence in situ hybridization analysis for *FGFR1*. Mutations of *KRAS* and *TP53* were documented in 32% and 71% allelic DNA, respectively (Fig. 2A and B) and *FGFR1* gene amplification with an average copy number of 7.1 signals in all tumor cells (Fig. 2C), all consistent with molecular traits of either adenocarcinoma or squamous cell carcinoma. Because of the lack of *EGFR* mutation and the clinical presentation, neoadjuvant chemotherapy was planned but the patient's conditions rapidly worsened because of respiratory failure until death a month and a half after the first hospitalization.

This is the first instance, to the best of our knowledge, which documents by means of IHC, EM, and molecular assays that TTF1 and p40 coexpression at the level of the same individual NSCLC cells underpinned concurrent bipartite squamous/basal-like and glandular differentiation, probably as expression of stem/progenitor cells plasticity or microenvironmental influences. On the one hand, these findings reinforce the diagnostic validity of the axiom “no p40, no squamous.”³

*Department of Pathology and Laboratory Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy; †Department of Biomedical and Clinical Sciences Luigi Sacco, Università degli Studi di Milano, Milan, Italy; ‡Division of Pathology, San Paolo Hospital, Università degli Studi di Milano, Milan, Italy; §Department of Medical Oncology, and ||Division of Thoracic Surgery, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy.

Disclosure: The authors declare that they have no conflicts of interest.

Address for correspondence: Giuseppe Pelosi, MD, MIAC, Dipartimento di Patologia Diagnostica e Laboratorio, Fondazione IRCCS Istituto Nazionale dei Tumori, Via G. Venezian 1, I-20133 Milano, Italy. E-mail: giuseppe.pelosi@unimi.it

DOI: 10.1097/JTO.0000000000000553

Copyright © 2015 by the International Association for the Study of Lung Cancer

ISSN: 1556-0864/15/1010-1500

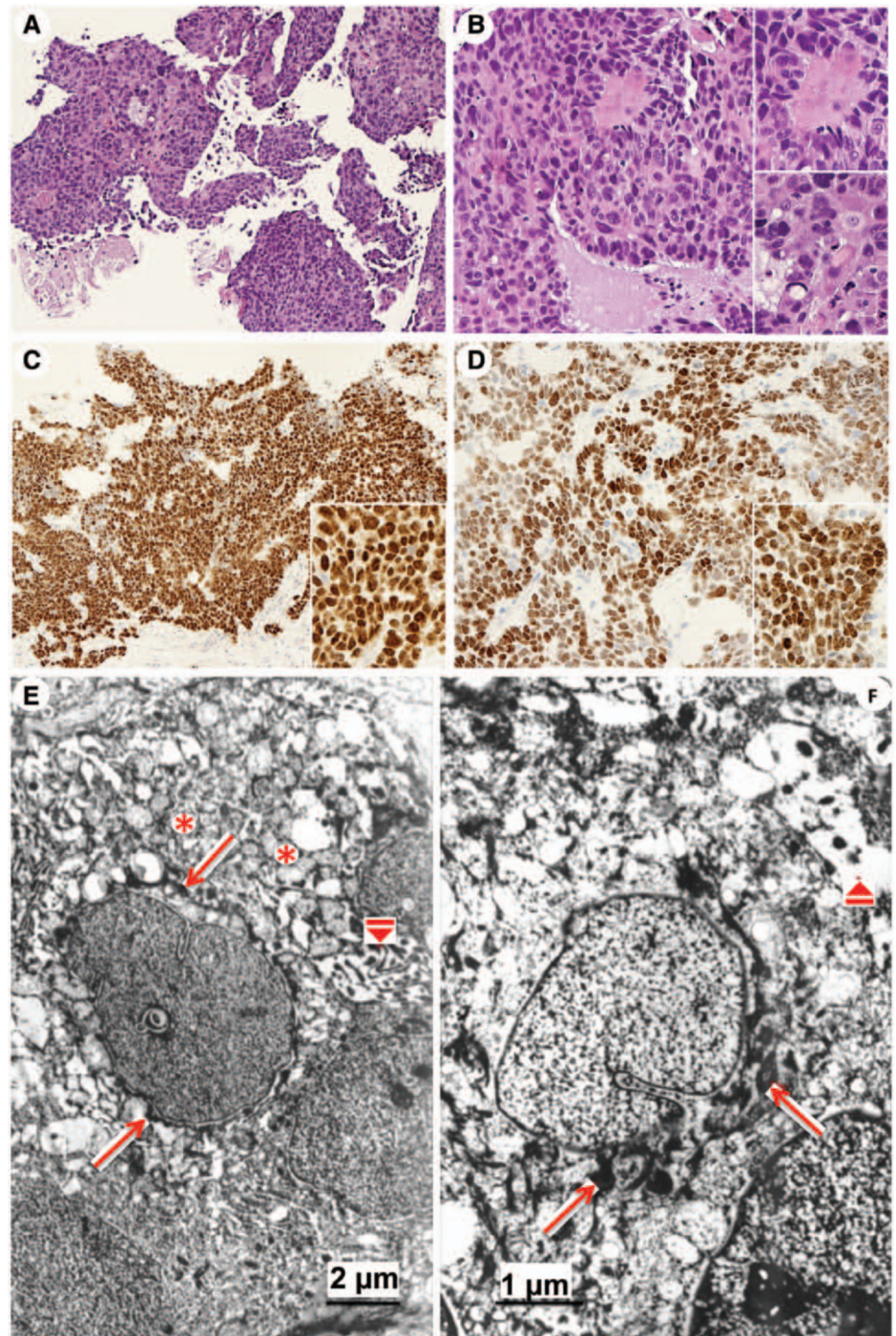


FIGURE 1. Biopsy tissue fragments showing poorly differentiated non-small-cell lung carcinoma (NSCLC) (A,B) with focal features suggestive of squamous cell differentiation (B, insets). Upon immunohistochemistry, a widespread and strong nuclear decoration was seen for both p40 (C, inset) and thyroid transcription factor-1 (TTF1) (D, inset), supporting a biphenotypic tumor at the level of the same individual tumor cells. Electron microscopy confirmed this dual concurrent differentiation at the level of the same individual cells showing (E) tumor cells partly lining lumen spaces with cytoplasm extensions (arrowhead), numerous mucous granules (asterisks) and abundant electron-dense thick tonofilaments around the nucleus (arrows), and (F) tumor cells with adjacent lumen (arrowhead) and bundles of electron-dense tonofilaments (arrows).

On the other hand, they challenge the notion that, if a case is positive for an adenocarcinoma marker such as TTF-1, the tumor should be tautologically classified as NSCLC favor adenocarcinoma, regardless of the concurrent expression for squamous markers.⁵ As a matter of fact it could be argued that whether it was more appropriate the term NSCLC favor adenocarcinoma as recommended by current guidelines⁵ or rather NSCLC favor ADSQC on the basis of this intriguing double lineage at the level of the same individual cells, although an

ultimate diagnosis of ADSQC would be only suggested in biopsy samples,⁵ as we really rendered in our pathology report. As some uncertainty still exists in the treatment of ADSQC, it is important for the pathologists to be aware of these challenging biomarker combinations (we observed a few other similar instances in either biopsy samples or surgical specimens). Therefore, we support the term ADSQC or, at least, NSCLC with adenosquamous immunophenotype for better witnessing about such an “amphicrine” biphenotypic tumor.

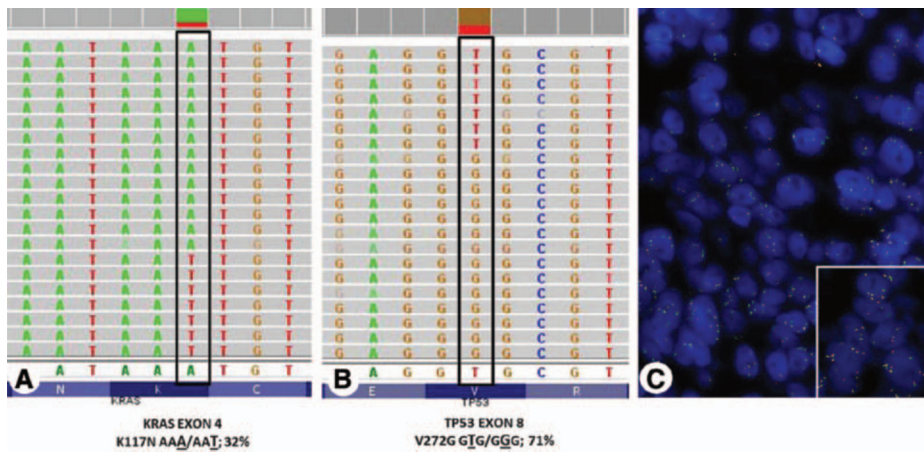


FIGURE 2. Mutations of *KRAS* (AAA>AAT, K117N, exon 4, 32% allelic DNA) (A) and *TP53* (GTG>GGG, V272G, exon 8, 71% allelic DNA) (B) gene are shown as derived from targeted next generation sequencing, which are either typical of or frequent in lung adenocarcinoma. Tumor cells showed also concurrent *FGFR1* gene amplification typical of squamous cell carcinoma, with an average copy number being 7.1 signals in all tumor cells (C, inset).

ACKNOWLEDGMENTS

This study was supported by LILT, Lega Italiana per la Lotta contro i Tumori, Section of Milan, and was dedicated to the memory of Carlotta, an extraordinarily lively girl who untimely died of cancer in the prime of life.

REFERENCES

- Müller KM, Fisseler-Eckhoff A. What's new in lung tumor heterogeneity? *Pathol Res Pract* 1988;184:108–115.
- Pelosi G, Fabbri A, Bianchi F, et al. Δ Np63 (p40) and thyroid transcription factor-1 immunoreactivity on small biopsies or cellblocks for typing non-small cell lung cancer: a novel two-hit, sparing-material approach. *J Thorac Oncol* 2012;7:281–290.
- Pelosi G, Rossi G, Cavazza A, et al. Δ Np63 (p40) distribution inside lung cancer: a driver biomarker approach to tumor characterization. *Int J Surg Pathol* 2013;21:229–239.
- Bishop JA, Teruya-Feldstein J, Westra WH, Pelosi G, Travis WD, Rekhtman N. p40 (Δ Np63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. *Mod Pathol* 2012;25:405–415.
- 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;137:668–684.