Complementary Value of Electron Microscopy and Immunohistochemistry in the Diagnosis of Non-Small Cell Lung Cancer

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Background

Lung cancer is the most common cause of cancer-related mortality around the world, in spite of improvements in diagnostic techniques and new treatments. Over the last years, its pathological classification has been widely redefined by cytogenetics and molecular data. Consequently, therapeutic targets related to mutations of EGFR, KRAS, ALK, ROS1 and MET have been identified for pulmonary adenocarcinoma (ADK). Thus, it is crucial to accurately distinguish between ADK and squamous cell carcinoma (SQCC) in poorly differentiated cases. Immunohistochemistry (IHC) is a very helpful technique in making this differential diagnosis. However, there is a subset of cases that remains classified as Non-Small Cell Lung Carcinoma, NOS (NSCLC-NOS) after IHC. In this setting, Electron Microscopy (EM) can be a useful tool, as it objectively allows the identification of very small, inter or intracellular glandular lumina, that are the clue for ADK, even when they are not identified by light microscopy analysis. Furthermore, a very little amount of tissue is needed for EM and paraffin-embedded tissue can also be pretty informative in this differential diagnosis. These advantages are particularly relevant in this setting, as the pretreatment diagnosis of these cases is usually based on small biopsies or cell-blocks obtained from cytological preparations, from which all ancillary immunohistochemical and molecular studies must be derived.
The aim of this study was to determine the value of EM and IHC in the NSCLC-NOS subclassification.

Design

Forty-eight NSCLC-NOS cases analyzed by bronchoscopic or core needle biopsy were selected from the files of Parc de Salut Mar Biobank, Barcelona, Spain. A basic immunohistochemical panel containing TTF-1 and p40 antibodies, and p63 for older cases, was performed. From 1 to 5 pieces of tissue, 0.5 mm each, were retrieved from paraffin-embedded blocks and processed for EM. The results of each technique were compared to the final diagnosis (gold standard), which was derived from the combination of light microscopy, IHC, EM, cytogenetics, molecular studies and data of the resection specimen if available.

Results

IHC results concurred with the final diagnosis in 36 cases (75%) (Kappa=0.517) and the identification of ADK by IHC had a sensitivity of 73%, specificity of 100%, positive predictive value (PPV) of 100% and negative predictive value (NPV) of 52.4%. EM results agreed with the final diagnosis in 35 cases (72.9%) (Kappa=0.471). For the diagnosis of ADK, IHC failed to recognize 10 cases (TTF-1 and P40 negative) and in all of them EM was conclusive, while in 10 cases with inconclusive EM, IHC gave the diagnosis. Thus, the values obtained for EM were identical to those of IHC: sensitivity 73%, specificity 100%, PPV 100% and NPV 52.4%. Combining results of IHC and EM, 47 cases (97.9%) were coincident with the final diagnosis (Kappa=0.943). (See table). Illustrated examples of the combined use of IHC and EM are shown (Figures 1-3).

Table

	Kappa	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IHC	0.517	73	100	100	52.4
EM	0.471	73	100	100	52.4
IHC + EM	0.943	100	100	100	100

Concordance of the different techniques with the gold standard (integrated diagnosis)

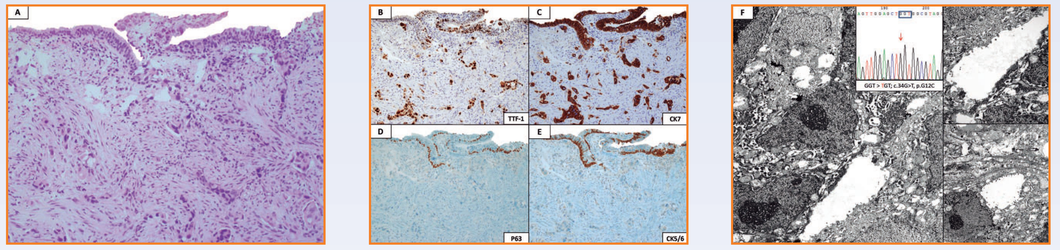

Figure 1. Bronchoscopic biopsy (A). Clusters of tumor cells with pleomorphic nuclei, conspicuous nucleoli and abundant pale cytoplasm. Neoplastic cells showing immunoreactivity for TTF-1 (B) and Cytokeratin 7 (C) and negativity for P63 (D) and Cytokeratin 5/6 (E). Electron microscopy (EM) revealed glandular differentiation (F). Small intracellular glandular lumina with short microvilli and occasional junctional complexes. Both EM and immunohistochemistry were typical of adenocarcinoma (ADK). Inset: A KRAS exon 2 (pG12C, c.34G>T) oncogene mutation was suspected by Sanger Sequencing method, which was finally confirmed by Quantitative Real-Time PCR-based analysis.

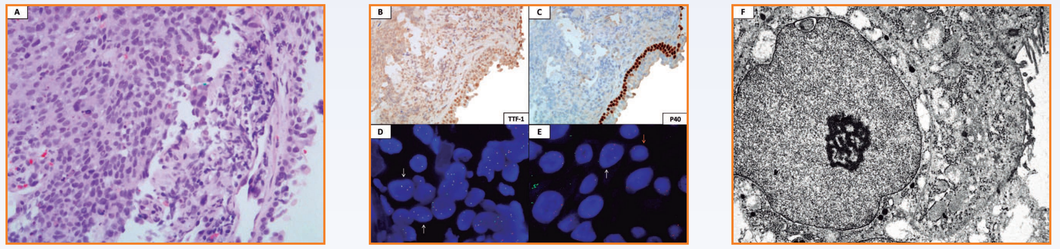

Figure 3. Bronchoscopic biopsy (A). Infiltrative proliferation of cohesive tumor cells growing in a solid-nested pattern, without evidence of glands or keratinization. Tumor cells did not show immunoexpression for TTF-1 (B) nor P40 (C). FISH for ALK and ROS1 gene rearrangements were negative. However, the analyzed interphase nuclei revealed copy number gain for ALK (3-4 copies) in 86% of nuclei (D) (white arrows) and for ROS1 (3-5 copies) in 35% of nuclei (E) (white arrow) and a monosomy in 31% (orange arrow). Electron microscopy (F). Scant well-developed microvilli could be seen in the apex of this tumor cell, which was attached to the two neighboring cells by junctional complexes. These findings were indicative of glandular differentiation, characteristic of ADK.

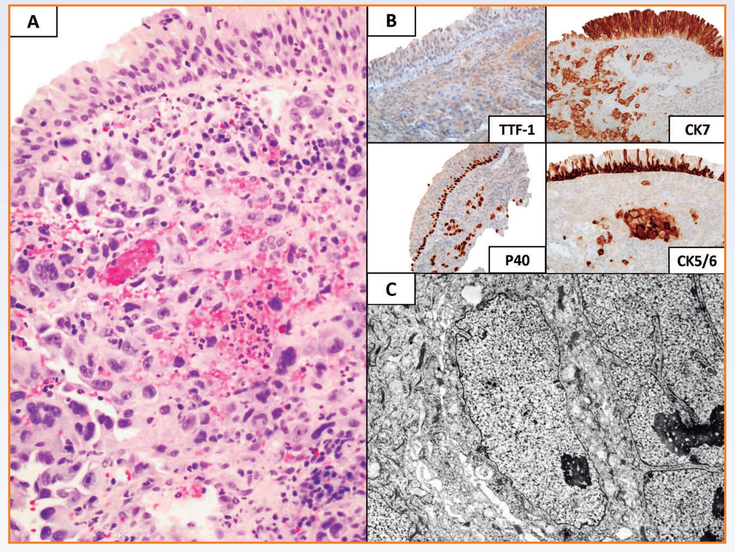

Figure 2. Bronchoscopic biopsy (A) revealing a proliferation of poorly differentiated carcinoma cells. Nuclei ranged from round to very irregular with prominent nucleoli. Cytoplasm was abundant, pale and finely granular. (B) Tumor cells were highlighted by immunostaining with P40, Cytokeratins 7 and 5/6. TTF-1 antibody was negative. Electron microscopy (C) showed moderately abundant tonofilament bundles and well-developed desmosomes, without glandular lumina, and this was highly consistent with squamous cell carcinoma.

Conclusion

The results of this retrospective study support that EM can provide useful information, in order to subtype NSCLC-NOS, mainly in recognizing poorly differentiated ADK, and that it has a particularly helpful role in cases in which IHC shows inconclusive results. Even in small samples retrieved from paraffin, EM is a cost-effective tool, very sensitive and extremely specific, that may have an indirect impact on prognostic and therapeutic decisions in this subset of patients.

Nothing to disclose – The authors of this abstract have indicated that they have no conflicts of interest that relate to the content of this abstract.

References

1. Pelosi G, Barbareschi M, Cavazza A et al. Large cell carcinoma of the lung: a tumor in search of an author. A clinically oriented critical reappraisal. Lung cancer 2015;87(3):226-31.
2. Cadioli A, Rossi G, Costantini M et al. Lung cancer histologic and immunohistochemical heterogeneity in the era of molecular therapies: analysis of 172 consecutive surgically resected, entirely sampled pulmonary carcinomas. Am J Surg Pathol 2014;38(4):502-9.
3. Rossi G, Mengoli MC, Cavazza A et al. Large cell carcinoma of the lung: clinically oriented classification integrating immunohistochemistry and molecular biology. Virchows Arch 2014;464(1):61-8.
4. Travis WD, Rekhtman N. Pathological diagnosis and classification of lung cancer in small biopsies and cytology: strategic management of tissue for molecular testing. Semin Respir Crit Care Med 2011;32(1):22-31.
5. Mukhopadhyay S, Katzenstein AL. Subclassification of non-small cell lung carcinomas lacking morphologic differentiation on biopsy specimens: Utility of an immunohistochemical panel containing TTF-1, napsin A, p63, and CK5/6. Am J Surg Pathol 2011;35(1):15-25.

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