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Case Report

Pulmonary squamous cell carcinoma with lepidic growth pattern: new insights into lung cancer classification

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Abstract: Lung squamous cell carcinoma (SCC) has always been considered a monomorphic entity, different from lung adenocarcinoma which is known to be a very heterogeneous tumor from morphological and molecular point of view. Just two histological subtypes of SCC are recognised, the basaloid and lymphoepithelioma-like histotypes, as in other sites different from the lung. Recently, different studies tried to expand the classification of SCC by adding different subtypes based on morphological characteristics (such as keratinization or clear cell features) or different growth patterns (papillary or basaloid). We report a case of squamous cell carcinoma with a previously unreported, distinctive and predominant "lepidic" growth pattern, with its immunophenotypical and molecular characterization.

Keywords: Lung pathology, squamous cell carcinoma, lepidic growth pattern

Introduction

Squamous cell carcinoma (SCC) accounts for 25-30% of all non-small cell lung cancers, often linked to a history of smoking [1]. During the past century, SCC was the most common subtype of NSCLC, but starting from '70 s a decrease of SCC prevalence relative to adenocarcinomas of the lung was noted and, today, adenocarcinoma of the lung is the most common subtype of NSCLC [2]. Histologically, SCC is a malignant epithelial tumour composed of cells with large and abundant cytoplasm and irregular, hyperchromatic nuclei with small nucleoli showing some degrees of keratinisation, pearls formation and intercellular bridges [3].

Histological variants of SCC are not fully evaluated and just two subtypes are recognised, the basaloid and lymphoepithelioma-like subtypes, as in other sites different from the lung [2].

According to the previous literature, only a minority of cases arises in small peripheral airways [4], although some recent studies suggest a proportional increase of peripheral type carcinoma. Funai et al [5] subdivided peripheral type squamous cell carcinoma according to the growth pattern into three types: the alveolar space-filling type, in which tumoral cell nests grow filling the alveolar space separated by thin preexisting septa; the expanding type, showing solid growth, destroying or compressing the surrounding parenchyma, and the combined type.

The molecular landscape of SCCs appears to be characterized by different driver mutations respect to lung adenocarcinomas (ADCs). Oncogenic activation of *PIK3CA*, *AKT1* and *DDR2* genes occur respectively in 9%, 7.1% and 3.8% of SCC, while *EGFR*, *KRAS*, *MEK1*, *ERBB2* and *ALK* activating mutations are more frequently involved in ADCs [6, 7].

We report a case of squamous cell carcinoma with a previously unreported, distinctive predominant "lepidic" growth pattern.

Materials and methods

A 73-year-old male patient with an history of HCV-related hepatopathy, hypertension, subrenal abdominal aortic aneurysm, chronic ob-

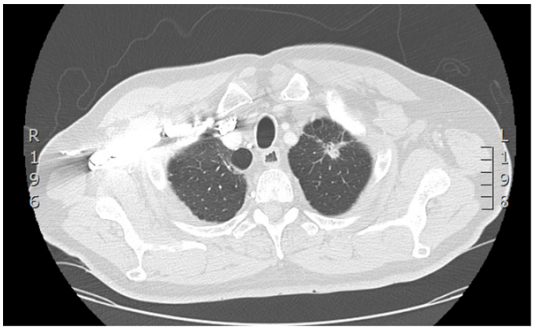

Figure 1. High-resolution computed-tomography (CT) scan of the chest and the abdomen showing a nodule nodule measuring 1.5 cm in the upper left pulmonary lobe.

structive pulmonary disease (COPD) due to tabagism and left radical nefrectomy for renal cell carcinoma came to our attention for dizziness and collapse.

A routine chest X-ray revealed a mass on the right lung, and high-resolution computed-tomography (CT) scan of the chest and the abdomen confirmed a nodule measuring 2.5 cm in the middle right pulmonary lobe, another nodule measuring 1.5 cm with speculated, irregular and infiltrative margins in the upper left pulmonary lobe and the presence of right hilar and mediastinal lymphadenopathy (**Figure 1**).

The PET/CT scan indicated an intense 18F-FDG activity in the middle right lobe (Standard Uptake Value = 5) and in the upper left lobe (SUV = 3, 4).

No other abnormal 18F-FDG uptake and no other localization of disease were identified at the clinical and imaging staging. The blood tumor markers were all within the normal ranges. Conventional bronchoscopy with flexible endoscope did not show pathological findings and bronchoalveolar lavage (BAL) was not diagnostic.

Left pulmonary nodule was not candidated for CT-guided percutaneous biopsy because of difficult lesion accessibility without a safe window for needle entry. In consideration of the clinical and imaging findings, the patient was candidate for a surgical lung biopsy of the left pulmonary solid nodule. Video-assisted thoracic surgery (VATS) with wedge resection of the upper left lobe was performed. The surgical thoracoscopic procedure was performed with the use of three ports: the pulmonary wedge resection of the left lung apex was made by the use of an endo-GIA linear stapling device removing the nodule completely. No additional lymph node sampling was performed considering the presence of controlateral pulmonary disease. A PET/CT scan performed forty days after surgery detected no more metabolic activity in the middle right pulmonary lobe suggesting the resolution of a flogistic disease; therefore non additional surgery procedure and no adjuvant therapy for lung cancer was indicated by oncologists.

Formalin-fixed, paraffin-embedded surgical specimens were stained with haematoxylin and eosin, and immunohistochemical stainings with antibodies directed against cytokeratin 7 (clone SP52), cytokeratin 20 (clone Ks20.8), cytokeratin 5-6 (clone D5/16 B4), p40 (clone BC28), TTF-1 (clone SP141) and Napsin A (clone MRQ40) were performed.

Immunohistochemistry was performed using the automatic system BenchMark XT (Ventana Medical Systems, Inc. Tucson, Arizona, USA). Reactions were revealed using the UltraViewTM Universal DAB, a biotin-free, multimer-based detection system, according to the manufacture's instruction.

Tumour genotyping by MassARRAY

In order to characterize the molecular profile of the tumor, a panel of mutations, insertions and deletions of the genes most frequently involved in NSCL genetic alterations was investigated. The Catalogue of somatic mutations in cancer (COSMIC) database was consulted to select the hotspot regions to analyze. Tumor DNA was isolated from formalin fixed paraffin embedded (FFPE) tissue sections, using the Biostic FFPE tissue DNA isolation Kit (Mo Bio Laboratories, Carlsbad, USA), following the manufacturer's instructions. The MALDI-TOF (Matrix Assisted Laser Desorption Ionization Time-of-Flight) analysis was performed on 30 ng of DNA. MassARRAY Analyzer 4 was employed (Agena Bioscience, Hamburg, Germany), according to the iPLEX Pro application guide, using Complete iPLEX Pro Genotyping Reagent Set and SpectroCHIP II Arrays and Clean Resin Kit (Agena Bioscience, Hamburg, Germany). MassARRAY Typer 4.0 software was used for data analysis.

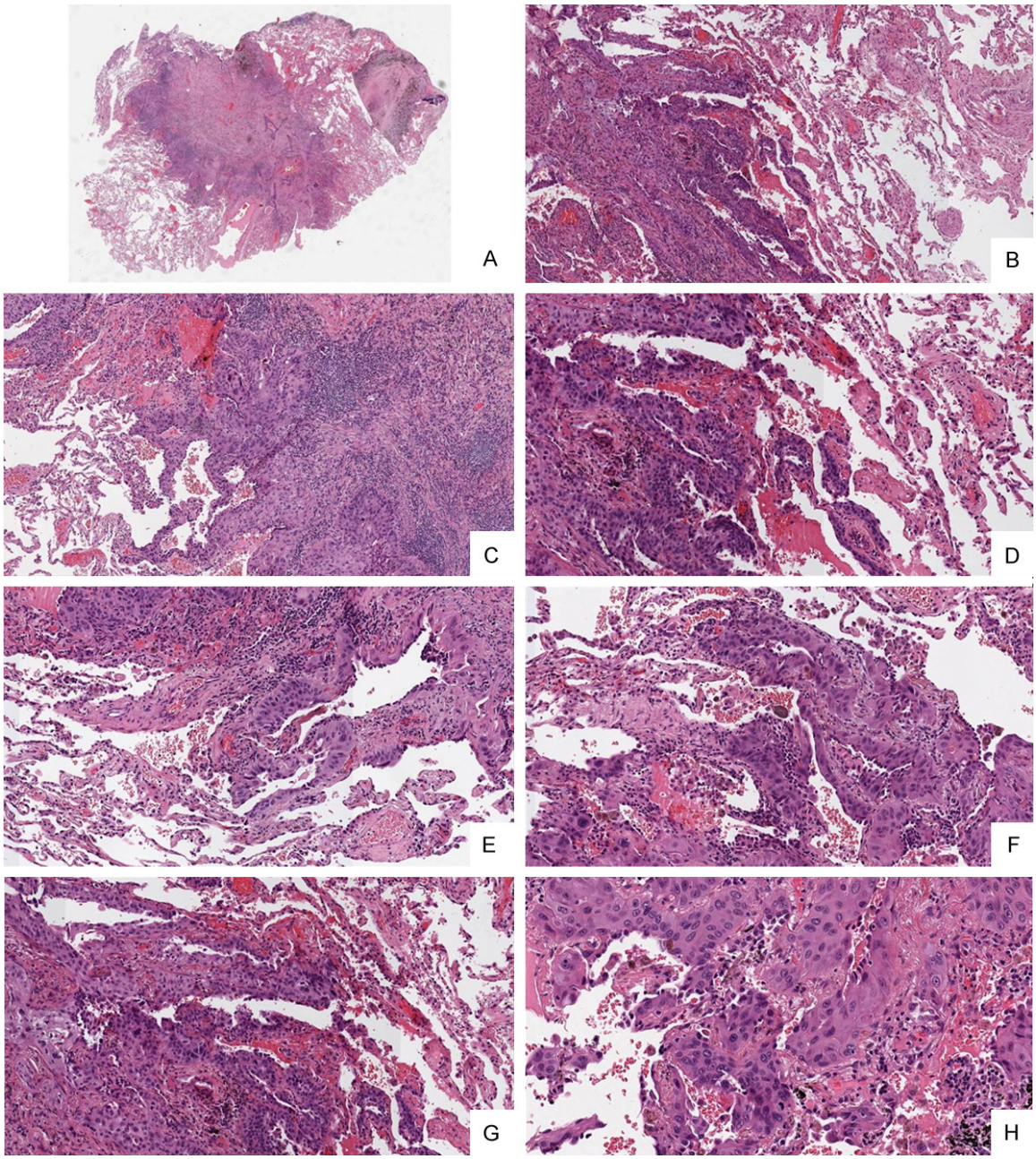

Figure 2. Morphological features of the nodule. Low-power magnification showed a central, solid scar with peripheral lepidic growth pattern (A. 0.5x). At a higher magnification, lepidic growth pattern can be better appreciated, with squamous cell neoplastic population growing over the pre-existing alveolar septa, without destruction of the parenchyma (B, C. 5x; D-G. 10x). Cytological details of the neoplastic population morphologically indicate squamous differentiation (H. 20x).

Results

Histopathological findings

Macroscopically, the nodule measured 1.3 cm and, on cut sections, it had a grayish appearance, with ill-defined margins and a sub-pleural localization. All the nodule was sampled and paraffin-embedded for histological examination.

Microscopical examination showed a moderately differentiated (G2) squamous cell carcinoma, exhibiting focal but clearly visible kerati-

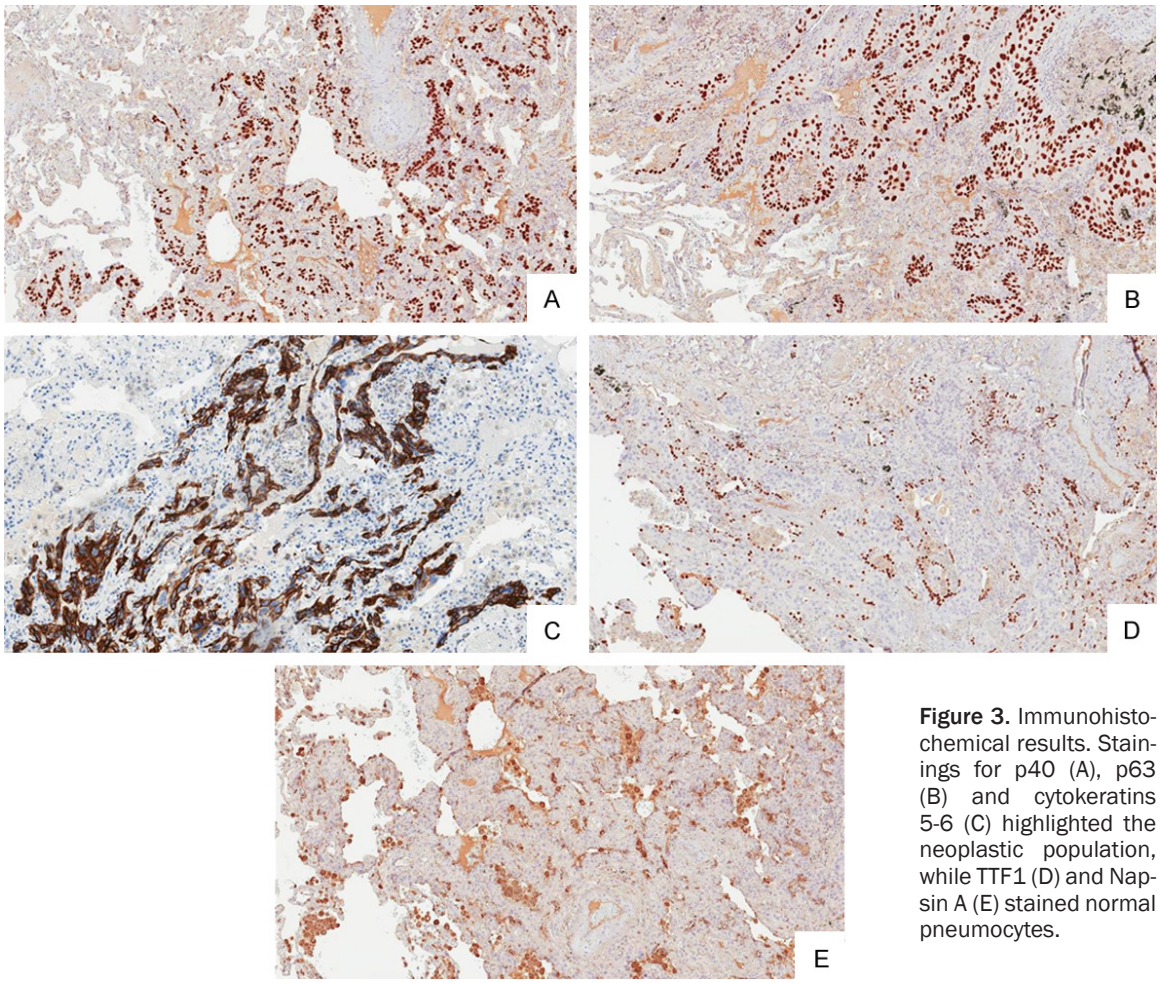

Figure 3. Immunohistochemical results. Stainings for p40 (A), p63 (B) and cytokeratins 5-6 (C) highlighted the neoplastic population, while TTF1 (D) and Napsin A (E) stained normal pneumocytes.

nization, characterized by an extensive lepidic growth: together with a central scar-like component, with an infiltrative growth pattern in the centre of the lesion, some areas showed malignant squamous cells growing along preexisting alveolar walls, preserving parenchymal architecture, frequently in multiple layers, displacing pneumocytes toward the central portions of alveolar lumen (**Figure 2**).

Strong immunoreactivity for p40 (**Figure 3A**), p63 (**Figure 3B**) and cytokeratins 5-6 (**Figure 3C**) confirmed the squamous nature of the lesion, while TTF-1 (**Figure 3D**) and Napsin A (**Figure 3E**) highlighted the intact frame of alveolar pneumocytes, with no staining in the neoplastic elements. Other immunohistochemical showed negativity in tumoral cells for cytokeratin 7 and 20.

Molecular findings

We genotyped the tumor investigating the most frequent driver mutations of NSCLC.

We did not find any mutations in *PIK3CA*, *AKT1* and *DDR2,* which are typical of SCCs, either in *EGFR*, *KRAS*, *MEK1*, *ERBB2* and *ALK*, generally found in ADCs.

Discussion

If primary lung adenocarcinoma is known to be a very heterogeneous tumor in many aspects with different morphological subtypes and molecular mutations, on the contrary squamous cell carcinoma has always been considered a “monomorphic”, solid entity [3].

Funai et al [5] tried to investigate different subtypes also in SCC, identifying in peripheral tumors three different growth pattern, the alveolar space-filling type, the expanding type and the combined type.

The alveolar space-filling type of SCC is characterized by filling of the alveolar space without destroying alveolar septa and it has the most favorable clinical prognosis. The expanding

type corresponds to the classic tumor nodule with infiltrative margins and with destruction of the pre-existing parenchyma and the combined type is a mixture of these two entitites.

Alveolar space-filling type can be considered a squamous evolution, a more advanced phase, of lepidic growth type, which is peculiar of adenocarcinoma: in lepidic-growth adenocarcinoma, the neoplasm arises from cells lining the alveoli and spreads along their walls, but in alveolar space-filling type squamous cell carcionoma, the neoplastic elements are not limited to cover septa, but they fill entirely the alveolar spaces.

In addition, a recent work by Kadota et al [8] indicates for SCCs five different subtypes: keratinizing, non-keratinizing, clear cell (which are not properly a growth pattern at all), basaloid and papillary type, but this is simpler and far from the complex classification of adenocarcinoma.

In our case, in the majority of the tumor nodule we did not have a proper alveolar filling but just only a lepidic growth along alveolar walls and we can speculate that the presence of this pattern can be explained with a squamous metaplastic change of the alveolar epithelium that has subsequently undergone cancerization and invasion.

Moreover, immunohistochemical of the lesion confirmed the squamous nature of the neoplasia.

Despite its relatively monomorphic presentation, molecular alterations in squamous cell lung cancers have not been comprehensively studied, and no targeted therapies have been developed.

Significantly altered pathways includes NFE2L2 and KEAP1 in 34%, squamous differentiation genes in 44%, PI3K pathway genes in 47%, and CDKN2A and RB1 in 72% of tumours [9].

In our opinion, the discovery of new subtypes within squamous cell carcinomas can have significant clinical impact, since research of molecular mutations, in mixed forms, should be guided by the morphology and could lead to new therapeutic strategies in these patients. This is well known in adenocarcinoma, where tumor heterogeneity influences evaluation of prognostic biomarkers [10].

We investigated the molecular profile of the tumor in order to identify the typical driver mutation of SCCs, but no alterations were found.

To our knowledge this is the first reported, morphologically and molecularly documented case of lung squamous cell carcinoma with this peculiar histological presentation, and its detection confirms the recent studies indicating that squamous cell carcinoma is an entity which may arise with different subtypes and histological growth patterns.

To highlight this tumor heterogeneity, a further measure should be to do a high number of samples of the nodules. In our case, the relatively small size of the nodule has allowed a sample of the tumor in its entirety.

Disclosure of conflict of interest

None.

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