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Targeted DNA sequencing for assessing clonality in multiple lung tumors: A new approach to an old dilemma

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ABSTRACT

Background: The differential diagnosis between multiple primary lung cancer (MPLC) and advanced lung cancer has traditionally relied on conventional radiology and pathology. However, the outcomes of traditional diagnostic workup are often limited, and staging is uncertain. Increasing evidence suggests that next-generation sequencing (NGS) techniques offer the possibility of comparing multiple tumors on a genomic level.
Objectives: The objective of this study is to assess the clinical impact utility of targeted sequencing in patients presenting with multiple synchronous or metachronous lung tumors.
Materials and methods: We describe the diagnostic workup conducted in a patient with three lung tumors, where we used a targeted 50-gene DNA sequencing panel (Ion AmpliSeq TM Cancer Hotspot Panel v2) to assess clonality and establish an accurate lung adenocarcinoma stage. Positive results were confirmed by pyrosequencing or Sanger sequencing.
Results: Three surgically resected lung tumors were submitted for targeted sequencing. The tumor from the upper right lobe was positive for a *TP53* c.659A > G mutation and native for *KRAS*. The tumor from the upper left lobe was positive for *TP53* c.725G > T and *KRAS* c.35G > T mutations. The tumor from the lower left lobe was positive for *TP53* c.1024C > T and *KRAS* C.34G > T mutations. Results and reviewed literature in the field support the diagnosis of MPLC instead of a single advanced lung cancer.
Conclusion: Targeted DNA sequencing significantly increases diagnostic accuracy in patients with multiple lung tumors. NGS panels should be available for patients presenting with multiple lung tumors.

1. Introduction

Among the myriad of clinical scenarios in which lung adenocarcinoma arises, patients presenting with multiple lung lesions remain a diagnostic challenge with definite prognostic and therapeutic implications. It is estimated that multiple primary lung cancer (MPLC) is present in 1–8% of newly diagnosed carcinomas of the lung [1], while many more patients develop metachronous lung tumors.

The differential diagnosis between MPLC and metastatic lung cancer is not new to clinicians. In 1975, Martini and Melamed published their recommendations on the diagnosis of multiple lung tumors based on conventional pathology. More recently, in 2009, Girard et al. defined a comprehensive histologic assessment pursuing the same diagnostic goal [2] that included a semi-quantitative grading system evaluating growth patterns and cytologic features. This group became pioneers in the use of genomic testing in combination with a thorough histologic assessment to establish tumor clonality.

According to the American College of Chest Physicians (ACCP) guidelines, clinical staging should be guided by clinical and imaging characteristics [3], which often excludes pathological and molecular testing from the first steps of therapeutic decision-making. The International Association for the Study of Lung Cancer (IASLC) Criteria to Identify Separate Primary Lung Cancers [4], however, take into account genetic characterization in addition to conventional studies.

Since 2016, increasing evidence has been published on the role of NGS in this setting, showing that accurate differential diagnosis

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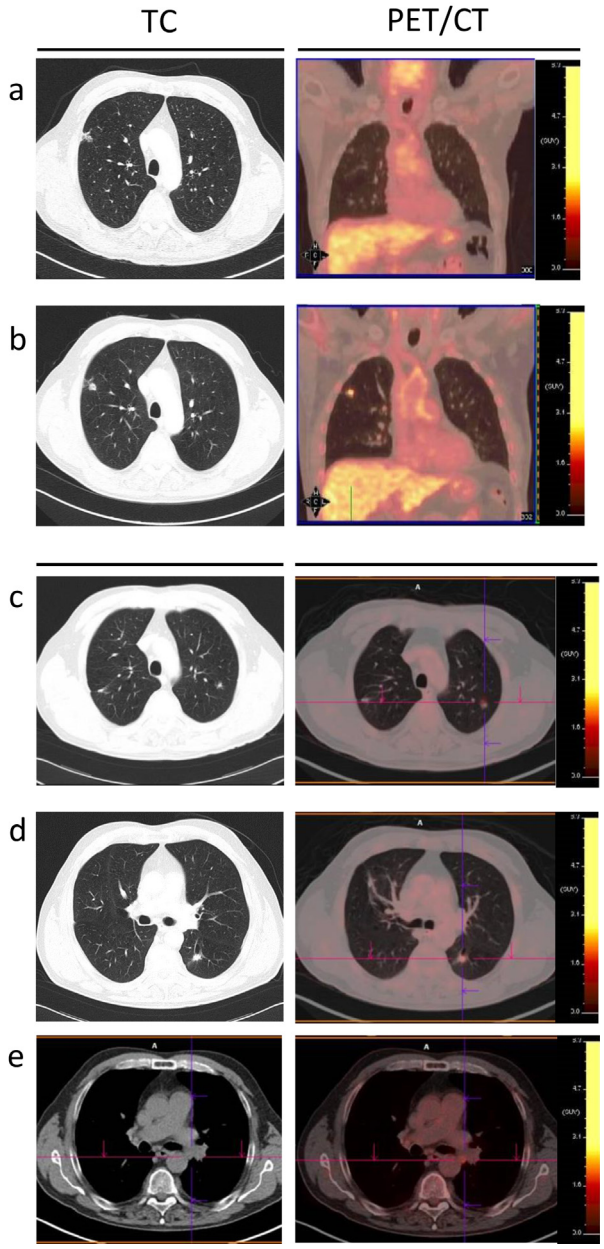

Fig. 1. Comparative CT and PET-CT scan images of resected lung nodules. A : CT and PET-CT scans performed in April 2011 during rectal cancer staging. The suspicious non-solid tumor did not show FDG uptake; B: CT and PET-CT scans performed in February 2012 before wedge resection of the same lesion. The solid component of the nodule and its enhanced metabolic activity prompted surgical resection; C: CT and PET-CT scan images of the upper left lobe nodule. D: CT and PET-CT scan images of the lower left lobe nodule. Note its proximity to an emphysematous bulla. E: CT and PET-CT scan images showing pathologic metabolic enhancement of an ipsilateral hilar lymph node. The latter had not been detected by CT imaging.

between MPLC and the metastatic spread of a single lung neoplasia is still an unmet need. As a means of better defining which diagnostic tests should be performed in this clinical context, we present a clinical case in which targeted genomic testing was conclusive in determining whether the patient should be diagnosed with MPLC or treated according to an advanced lung cancer diagnosis protocol.

2. Case study

2.1. Clinical description

A 58-year-old male former smoker consulted because of rectal tenesmus in April 2011. A diagnosis of moderately differentiated adenocarcinoma of the rectum was made. Endoscopic ultrasonography established a uT2 uN0 stage tumor; in addition, a computed tomography (CT) scan of the chest showed a 15 × 9 mm ground-glass opacity in the upper right lung lobe (Fig. 1a). Positron emission tomography (PET-CT) was performed, which was negative for distant pathologic fluorodeoxyglucose (FDG) uptake (Fig. 1a). Neoadjuvant chemo-radiotherapy was administered. Subsequently, this patient underwent surgical resection in July 2011 (pT1pNx).

In February 2012, a CT scan showed that the nonsolid lesion located in the upper right lung lobe had evolved into an 11 mm solid nodule (Fig. 1b). A FDG PET-CT evidenced suspicious metabolic activity (Fig. 1b). Due the tumor's small size and potentially metastatic nature, a wedge resection was performed. The pathological report described a poorly differentiated lung adenocarcinoma, immunohistochemically positive for CK-7 and TTF-1 and negative for CK-20, P-63 and CDX-2. It was classified as stage IA (pT1 cN0 cM0) Non-Small-Cell Lung Cancer (NSCLC) according to the 7th IASLC's TNM staging version, and no adjuvant treatment was administered.

In July 2015, a chest CT scan showed two new pulmonary nodules, one in the upper left lobe (Fig. 1c) and the other in the lower left lobe (Fig. 1d). Because of their spiculated appearance, two different early lung cancers were suspected. A new FDG CT-PET scan was performed showing metabolic enhancement of both nodules (Fig. 1c and d), as well as an enhancement of a left hilar lymph node (Fig. 1e). The patient underwent wedge resection of both lesions and hilar-mediastinal lymph node sampling. The pathologic diagnosis confirmed moderately differentiated adenocarcinoma in both tumors with a positive hilar node. All foci were immunohistochemically positive for TTF-1.

At this point, additional information was needed to define any clonal relationship between all three resected lung tumors and to ultimately establish a correct cancer stage, which would guide future clinical decisions.

2.2. Materials and methods

In order to answer this relevant question, a genomic analysis was performed to compare the three lung tumors. Samples were submitted for NGS, using a 50-gene panel (Ion AmpliSeq TM Cancer Hotspot Panel v2). DNA was obtained from formalin-fixed paraffin-embedded material. Informative results were confirmed by either pyrosequencing or Sanger sequencing.

2.3. Results

NGS results are summarized in Table 1.

The patient was diagnosed with three different early-stage lung adenocarcinomas. Based on the stage of the most advanced cancer (IIA), adjuvant platinum-based chemotherapy was administered. Currently, the patient is being followed-up without evidence of disease relapse.

3. Discussion

According to the ACCP [3], it is difficult to determine whether a metachronous lung lesion is a second primary tumor or a metastasis when the disease-free interval is between 2 and 4 years. However, the probability of the disease being staged M1a should be considered low in the absence of mediastinal nodal involvement. Nevertheless, surgical resection is recommended for potential T4 disease (if both left lung nodules were suspected to be clonal), based on the survival benefit seen in historical series [3].