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Is there a specific phenotype associated with the different subtypes of *KRAS* mutations in patients with advanced non-small-cell lung cancers?

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ABSTRACT

Objectives: KRAS mutations occur in 20 to 25% of non-small-cell lung cancers (NSCLC) and seem to predict a poor prognosis. There is heterogeneousness in the frequency and spectrum of *KRAS* mutations, which can be categorized in transitions and transversions. We wondered if subtypes of *KRAS* mutation were associated with specific clinical phenotypes and specific survival.
Materials and methods: Between July 2007 and May 2012, patients with advanced NSCLC and *KRAS* mutation diagnosed in two university hospitals were included. Clinical and histological characteristics, therapeutics and survival data were collected.
Results: Among 635 patients screened for *KRAS* mutations, 90 were found to be mutated and were included. Median age was 59 years (range: 54–69). Most were males (60%), current or former smokers (63% and 33%, respectively) and had an adenocarcinoma (ADC) (80%). Eighty patients were stage IV and 10 were stage IIIB. Eighty percent of the *KRAS* mutations were transversions and 20% were transitions. In uni- and multivariate analyses, there was a trend for fewer smokers among patients with transitions than among those with transversions (Odds Ratio [OR] = 0.28, 95% CI [0.079–0.999], $p = 0.05$). No significant difference was noted between transitions and transversions for other clinical characteristics. Patients with transitions had more frequently squamous-cell carcinoma (SCC) compared to those with transversions, who had more frequently adenocarcinomas (OR = 16.7, 95% CI [2.76–100.8], $p = 0.002$). Seventy-nine patients (86%) had received first-line chemotherapy. No significant difference was seen for disease-control rate, median progression-free survival or overall survival between transitions and transversions.
Conclusion: A higher proportion of non-smokers and SCC subtypes were observed in the transitions compared to transversions. This confirms the heterogeneity of *KRAS* mutations and could suggest to expand *KRAS* testing in SCC to assess impact of *RAS* in SCC, which remains poorly investigated.

1. Introduction

Lung cancer remains one of the leading causes of cancer-related death worldwide for both women and men, even though its incidence has been decreasing over the past five years [1]. Improvements in survival have been obtained through the efficacy of molecules such as tyrosine-kinase inhibitors (TKI) in those

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patients with an oncogenic mutation of the epidermal growth factor receptor (*EGFR*) or gene rearrangement of anaplastic lymphoma kinase (*ALK*). However, these molecular abnormalities are rare: only 10–15% of patients suffering from non-small-cell lung cancer (NSCLC) carry an *EGFR* mutation and 5% an *ALK* gene rearrangement [2].

One of the most frequent molecular abnormalities is *KRAS* mutation (i.e., 20–25%) but, unfortunately, no specific effective treatment has yet been developed. *KRAS* mutated lung cancers seem to be predictive of poor prognosis [3] in resected [4] or advanced disease, and more recently of resistance to *EGFR*-TKI such as erlotinib or gefinitib in advanced NSCLC [5,6]. The frequency of specific mutations of *KRAS* varies according to cancer type. A distinctive spectrum exists with transversions (substituting a pyrimidine for a purine, or a purine for a pyrimidine) and transitions (substituting a purine for a purine, or a pyrimidine for a pyrimidine). Transversions are usually associated with exposure to tobacco whereas transitions are not [7]; they are also the most prevalent subtype of *TP53* mutation in lung cancers in smokers [8,9]. In colorectal cancer, *KRAS* mutations occur in 30–40% of tumors [10]. The most frequent mutations are transitions [11] in codon 12 or 13 (56% of *KRAS* mutations) and are more often observed in nonsmoking patients. Moreover, *KRAS* mutations are predictive of poorer efficacy to anti-*EGFR* therapies such as panitumumab or cetuximab [10].

In NSCLC, most *KRAS* mutations occur in codon 12, whereas codons 13, 10, 61 and 146 are much less frequently mutated. The most common *KRAS* mutation is G to T (G > T) transversion (67% of *KRAS* mutations): their amino-acid replacements at codon 12 and/or codon 13 are a glycine (Gly or G) by a cysteine (Cys or C) (45%), a valine (Val or V), (21%) or a phenylalanine (Phe or F) (1%). G to C (G > C) transversions are less frequent (9% of *KRAS* mutations), with amino-acid replacement at codon 12 of glycine to an alanine (Ala or A) (7%) or an arginine (Arg or R) (2%). G to A (G > A) transitions, resulting in substitution of glycine to an aspartate (Asp or D) at codons 12 and 13, represents 24% of mutations [12]. Another way to explore the phenotypes of patients with *KRAS* mutations is to categorize them as G12C and G12V versus the others. Further recent studies have shown that signaling of downstream *RAS* was specific to mutation subtype [13]. *KRAS*–G12D preferably activates Akt signaling whereas *KRAS*–G12C and *KRAS*–G12V preferentially activates RalA/B signaling. Moreover, those last ones were associated with decreased progression free survival in the literature [13]. The substitution of different amino acids then induces heterogeneous behavior in *KRAS*, which implies that therapeutic interventions will need to take into account the specific mutant *KRAS* expressed by the tumor. For all these reasons, we wondered if *KRAS* mutations were associated with specific clinical phenotypes and survival. We analyzed the clinical and survival data from 90 patients with advanced NSCLC and a mutated *KRAS*, among a total of 635 patients screened.

2. Materials and methods

2.1. Patients

Patients with NSCLC and a *KRAS* mutation were retrieved from the archive database from the pathology departments at two university hospitals in Paris (France) (Tenon Hospital, APHP and Curie Institute) between July 2007 and May 2012. Clinical data from the medical charts (C.D.) were reviewed: gender, ethnicity, median age at diagnosis, smoking history, stage IIIB/IV at diagnosis or recurrence after surgery, performance status (PS), tumor histologic type and locations of metastatic sites. Smoking status was defined as never smoker (<100 lifetime cigarettes), former smoker (quit >1 year prior to diagnosis) or current smoker (still smoking or quit <1 year prior to diagnosis). Pack-years of smoking were defined as the number of cigarettes per day/20 × years of smoking.

Histological classification was determined based on the 2015 IASLC/ATS/ERS new classification of lung cancer [14]. Response criteria were defined according to the guidelines of Response Evaluation Criteria in Solid Tumors (RECIST, version 1.1) [15]. Data on progression-free survival (PFS) under first-line chemotherapy, best response to therapy, and overall survival (OS) were collected.

2.2. Biomarker analyses

Assessment of *KRAS* mutations has been routinely performed at Tenon Hospital since May 2007, according to the local guidelines, and at the Curie Institute since January 2010, according to the French National Cancer Institute (INCa) guidelines. Direct sequencing of exon 2 of the *KRAS* mutations was performed. DNA was extracted from areas of fresh formalin-fixed, paraffin-embedded tumor sections using a QIAmp DNA mini kit (Qiagen, Hilden, Germany) and analyzed for *KRAS* mutations (codon 12: c.34G > A/p.G12S, c.34G > C/p.G12R, c.34G > T/p.G12C, c.35G > A/p.G12D, c.35G > C/p.G12A, c.35G > T/p.G12V and codon 13: c.38G > A/p.G13D). Amplifications were done using a touchdown protocol between 56 and 52 °C. Aliquots of PCR products were examined by electrophoresis on 2% agarose gel containing ethidium bromide (Tenon Hospital) or with a fluorescent dye (Curie Institute). In both sites, PCR products were purified and directly sequenced using the Applied Biosystems Prism dye-terminator cycle-sequencing method on an ABI Prism 3100 Genetic Analyzer (Thermofisher, Applied Biosystems).

2.3. Pathological review

Due to the weak association reported in the literature between *KRAS* mutation and squamous NSCLC, all tumors with a diagnosis of SCC had been reviewed by a pathologist specialized in thoracic oncology (M.A.). Hematoxylin and Eosin-stained sections were reviewed to identify areas of tumor. Immunohistochemistry was performed with either thyroid transcription factor-1 (TTF1), p40, p63, or cytokeratin (CK) 5 and 6 and are shown in Supplementary Appendix 1.

2.4. Statistical analyses

The groups were defined as (i) transitions versus transversions, or (ii) G12C and G12V versus others. Associations were tested for qualitative variables using Pearson's chi-squared test or Fisher's exact test (if one group's effective was <5) whereas comparisons of continuous variables were made using the Mann–Whitney test (univariate analyses). Factors associated with the different groups of mutations were analyzed using a logistic regression model. Independent variables with $p < 0.150$ in the univariate analyses were included in multivariate analyses, with the final model chosen using a backward stepwise variable selection based on *p*-value as the candidate predictor. PFS and OS were estimated using the Kaplan–Meier method and were expressed as their median ± interquartile ranges (IQR). The censoring date was 01/09/2012. Results were considered significant if the *p*-value was <0.05. Cox's model was used for survival analysis in the multivariate analyses. Statistical tests were performed using SPSS 20.0 software (IBM Corporation, New York NY, USA).

2.5. Ethics

According to French national guidelines, as *KRAS* testing is a routine practice, no signed consent was required to research *KRAS* mutations. However, all patients had given signed consent for