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Original contribution

A comparison of *EGFR* and *KRAS* status in primary lung carcinoma and matched metastases

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Summary Epidermal growth factor receptor (*EGFR*) and v-Ki-ras 2 (KRAS; viral Kirsten rat sacoma 2 oncogene homolog) oncogenes are predictors of response to *EGFR*-targeted therapy in lung carcinomas. Morphologic heterogeneity of lung carcinomas is reflected at the molecular level and may confound interpretation of immunohistochemistry, fluorescence in situ hybridization, and mutational assays, which are all used for analysis of *KRAS* and *EGFR* genes. Furthermore, molecular characteristics may differ between the primary tumor and corresponding metastases. The aim of this study was to determine if the *KRAS* and/or *EGFR* status of primary and metastatic lung carcinoma differs. Three hundred thirty-six cases of primary lung carcinomas were tested for *EGFR* and *KRAS*, and 85 cases had a metastasis (25%). Of the 40 cases (47%) with sufficient material for *EGFR* and *KRAS* mutational analysis, there were 11 (27.5%) primary tumors and 4 (10%) metastases identified with a *KRAS* mutation. Of the cases with *EGFR* fluorescence in situ hybridization results, there were 3 (8%) primary tumors and 8 (24%) metastases that were fluorescence in situ hybridization positive. Overall, there were 9 cases (22.5%) with discordant *KRAS* status and 11 cases (32.5%) with discordant *EGFR* fluorescence in situ hybridization status. Our results suggest that the *EGFR* and *KRAS* status of primary lung carcinomas may not predict the status in the corresponding metastases. This observation may have important implications for molecular testing for targeted therapies.

1. Introduction

Lung cancer is the leading cause of cancer deaths in men and women [1,2], and of the histologic subtypes of lung carcinoma, adenocarcinoma is the most common [3]. Traditionally, patients with non-small cell lung carcinomas (NSCLCs), including adenocarcinomas, have been treated by surgical resection with or without chemotherapy or other adjuvant treatment. However, because of the high mortality of lung carcinoma and the variable response rates to treatment, there has been a great deal of research investigating different molecular markers of NSCLC that could serve as potential therapeutic targets [4-9].

The epidermal growth factor receptor (*EGFR*) superfamily has been an area of particular interest because of the finding that *EGFR* is frequently overexpressed in NSCLC [4,5]. In particular, the development of small molecule inhibitors of *EGFR* (gefitinib, erlotinib) resulted in clinical trials that have shown a high response rate in lung adenocarcinomas with somatic mutations in exons 18 to 21 of the tyrosine kinase domain of *EGFR* [6-9]. These mutations correlated with the observed clinical characteristics of responders to tyrosine kinase inhibitors (TKIs)

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including female sex, Asian ethnicity, absent or low smoking history, and the diagnosis of lung adenocarcinoma [8,10-14].

It was also discovered that *KRAS* (v-Ki-ras 2; viral Kirsten rat sacoma 2 oncogene homolog) mutations occur in up to 30% of lung adenocarcinomas and are predominantly seen in smokers. The *KRAS* mutations have been shown to be a negative prognostic factor and a predictor of failure of *EGFR* TKI therapy [6,7,9]. In addition, studies have shown that *EGFR* and *KRAS* mutations have a mutually exclusive relationship [15-17].

Testing for *EGFR* and *KRAS* status in lung carcinoma has involved a variety of different methodologies, including DNA mutational analysis, fluorescence in situ hybridization (FISH), and immunohistochemistry. In addition to the variety of testing methods, there is a lack of standardization for the methodologies and the criteria for interpretation. There is also ongoing discussion about the most appropriate sample (surgical specimens versus cytology specimens) that should be submitted for analysis. Although *KRAS* and *EGFR* mutational status have frequently been analyzed on surgical resections of primary lung tumors, there are no consensus guidelines regarding whether to test the primary tumor and / or the metastasis. Hence, it is uncertain which result should be used in selecting the appropriate patients for TKI therapy.

Only a few studies investigated *KRAS* and *EGFR* status in primary lung tumors and their metastases [18-24]. In addition, the literature on the clinical relevance of mutational status in metastases is sparse [25].

The aim of our study was to compare the *KRAS* and *EGFR* status between primary lung carcinomas and their corresponding metastases to investigate whether the *KRAS* and *EGFR* status is stable in both synchronous and metachronous metastases.

2. Materials and methods

2.1. Clinicopathologic characteristics

Three hundred thirty-six cases of primary lung carcinomas were analyzed for *EGFR* and *KRAS* mutations, in addition to *EGFR* FISH in most cases, between January 1, 2005, and August 17, 2007, at the University of Pittsburgh Medical Center (Pittsburgh, PA). A total of 85 cases (25%) were identified with a metastasis in a period of follow-up ranging from 6 to 42 months. Forty of these cases (47%) had available surgical or cytologic material in a sufficient quantity for testing. We excluded cases that were unavailable in our files, in addition to cases with a small amount of available tumor tissue, in an effort to avoid false-negative results. The metastases were considered synchronous if they occurred before or during resection of the primary tumor or within 12 months from the surgical procedure. All metastases identified more than 12 months after the primary resection were considered metachronous, as defined by prior studies [26-28]. All histologic and cytologic slides were reviewed by 2 pathologists (SM and SD), and the tumors were classified according to the World Health Organization Classification of tumors of the lung and pleura [29]. Angiolymphatic invasion, pleural invasion, and tumor grade were assessed in each case. Clinicopathologic characteristics including tumor size, patient age, sex, tumor stage, and smoking history were obtained from the available records. The study protocol was reviewed and approved by the institutional review board of the University of Pittsburgh Medical Center (institutional review board no. PRO08040162).

2.2. *KRAS* and *EGFR* mutation analysis

For surgical and cytologic samples, the formalin-fixed paraffin-embedded block with at least 75% of viable tumor was chosen. In each case, tumor was microdissected from five 4-μm-thick unstained histologic sections under direct visualization using a stereoscopic microscope. DNA was extracted by proteinase K digestion and DNeasy DNA isolation kit (Qiagen, Valencia, CA) according to the manufacturer instructions.

Direct DNA sequencing of codons 12 and 13 of exon 2 of the *KRAS* gene and exons 19 and 21 of the *EGFR* gene was performed as previously described using the BigDye Terminator kit (Applied Biosystems, Inc, Foster City, CA) on ABI 3730 (Applied Biosystems). Forward and reverse sequences were analyzed using Mutation Surveyor V3.01 software (SoftGenetics, State College, PA). Each case was classified as positive or negative for the *KRAS* or *EGFR* mutation based on the comparison of the sequence to the wild-type sequence.

2.3. Fluorescence in situ hybridization

FISH analysis of *EGFR* was performed using standard method with the dual-color *EGFR* SpectrumOrange/CEP7 SpectrumGreen probe (Vysis, Inc, Downers Grove, IL) and paraffin pretreatment reagent kit (Vysis, Inc) as previously described [30]. In brief, paraffin sections were de-paraffinized, dehydrated in ethanol, and air-dried. Sections were digested with protease K (0.5 mg/mL) at 37°C for 28 minutes. The slides were denatured at 75°C for 5 minutes and dehydrated in ethanol. The probes were denatured for 5 minutes at 75°C before hybridization. Slides were hybridized overnight at 37°C and washed in 2× SSC (300 mM sodium chloride, 30 mM sodium citrate; pH 7.0)/0.3% NP40 (Nonidet P-40; Abbott Molecular 07J05-001; Abbott Park, IL) at 72°C for 2 minutes. Nuclei were counterstained with DAPI/antifade 1 (Vysis, Inc). Each FISH assay included normal lung tissue sections as a negative control and sections of lung NSCLC previously identified as being positive for *EGFR* gene amplification as a positive control. Analyses were performed using a fluorescence microscope (Nikon Optiphot-2 [Nikon, Melville, NY] and Quips Genetic Workstation [Vysis, des Plains, IL]) equipped with Chroma