**FGFR1 Amplification in Squamous Cell Carcinoma of the Lung with Correlation of Primary and Metastatic Tumor Status**

Sara E. Monaco, MD, Erika F. Rodriguez, MD, PhD, Alyssa L. Mahaffey, and Sanja Dacic, MD, PhD

From the Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA.

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**ABSTRACT**

**Background:** The FGFR1 gene can be amplified in squamous cell carcinoma of the lung (SqCC). The aim of this study was to compare FGFR1 status with stage and matched primaries with metastases.

**Methods:** Cases with FGFR1 fluorescence in situ hybridization (FISH) testing performed from 2000 to 2013 were evaluated for amplification status and clinicopathologic features.

**Results:** Of the 336 cases tested by FGFR1 FISH, 52 (15%) were positive for amplification. Eight (13%) of 60 N0 cases and eight (17%) of 46 N1 or N2 cases were amplified, with no statistically significant difference. Of the 24 cases with matched primary and metastatic tumors, 22 (92%) were synchronous and one (4%) had discordant amplification.

**Conclusions:** Frequency of FGFR1 amplification is similar in SqCC with and without lymph node metastases, but status in metastatic sites may be discordant from the primary in a small subset of cases, which may affect the decision to perform testing of metastatic SqCCs.

Lung cancer is the most common cause of cancer-related death in the United States and worldwide, and squamous cell carcinoma (SqCC) comprises 25% to 30% of these pulmonary cancers. Although there has been an increasing emphasis on molecular testing in non–small cell carcinomas, this has typically been confined to patients with adenocarcinoma. Patients with SqCC do not routinely undergo the extensive molecular testing because SqCCs do not show a response with EGFR-tyrosine kinase inhibitors or the addition of the antifolate agent, pemetrexed (Alimta).

In recent years, major advances in our understanding of the molecular genetics of non–small cell lung carcinoma have been achieved. The fibroblast growth factor receptor 1 (FGFR1), encoded by the FGFR1 gene located in chromosomal region 8p11, has emerged as an important oncogene in SqCC. FGFR1 is activated by a variety of mechanisms, including gene fusions and mutations, which results in activation of downstream signaling pathways, leading to increased cell proliferation and survival in a variety of tumors. In lung cancer, recent studies have described amplification of this gene in a subset of SqCCs. However, little is known about the correlation of FGFR1 amplification with clinicopathologic features and the findings in metastatic tumors, which may be helpful in characterizing these patients and guiding molecular testing in these cases. The aim of this study was to compare FGFR1 gene status with clinicopathologic variables and to look at the status in matched primary tumors and metastases.
Materials and Methods

Patient Selection

 Archived cases with a diagnosis of lung non–small cell carcinoma and SqCC that were tested for FGFR1 amplification by fluorescence in situ hybridization (FISH) were retrieved from the electronic records of the Department of Pathology at the University of Pittsburgh Medical Center from 2000 to 2013, and the clinicopathologic features were correlated. Of these cases, those with testing performed on both the primary and synchronous or metachronous metastatic lesions were also reviewed to look at the FGFR1 amplification status, clinical characteristics, and histologic diagnosis.

FGFR1 gene amplification was evaluated by FISH on formalin-fixed, paraffin embedded tissue sections from cytology cell blocks, tissue biopsy specimens, or surgical resections, using previously published methods. FISH analysis of FGFR1 amplification was performed using a standard method with a three-color combined break-apart and amplification probe for FGFR1 (Cytocell; Rainbow Scientific, Windsor, CT). FGFR1 (8p11.23-p11.22) appears as red and green signals, while the chromosome 8 centromere probe (blue signal) serves as a control probe. At least 60 cells were scored for each case and control. FGFR1 gene amplification was defined as a ratio between FGFR1 gene copy numbers and chromosome 8 greater than 2.

Statistical Analysis

 Categorical variable correlations were performed using χ² or Fisher exact test. Wilcoxon test was used to evaluate continuous variables. All tests were two-sided with P values less than .05 considered statistically significant. Statistical tests were performed using JMP software version 10 (SAS Institute, Cary, NC).

Results

Patient Demographics

 A total of 336 cases were successfully tested for FGFR1 amplification by FISH. The patients had a median age 67 years and a median primary tumor size of 2.4 cm. There were 147 (44%) female and 189 (56%) male patients. A total of 180 patients had a known smoking status in our records, of which 169 (94%) reported having a history of smoking, including 33 (100%) smokers of the 33 amplified cases with known smoking status, and 136 (93%) smokers of the 147 non-amplified cases with known smoking status. The clinicopathologic features of all the cases with FGFR1 FISH results are summarized in Table 1.

FGFR1 FISH Results

 A total of 52 cases (15%) were FGFR1 amplified and 284 (85%) were not amplified. FGFR1-amplified tumors occurred in younger patients (median age at diagnosis 65 years in amplified tumors and 69 years in nonamplified tumors; P = .04). There were no statistically significant differences for history of smoking or tumor size (P > .05). Of the 60 cases with documented N0 status, eight (13%) were amplified, and 52 (87%) were not amplified. Of the 46 cases with N1 or N2 disease identified, eight (17%) had gene amplification and 38 (83%) were not amplified in the primary tumor. There were no statistically significant differences for FGFR1 amplification between the tumors with N0 and N1/ N2 lymph node metastases (P = .59, Fisher exact test).

 A total of 24 patients had FGFR1 FISH performed on primary and metastatic tumors, of which 22 (92%) were synchronous and two (8%) were metachronous. The FGFR1 testing was performed on primary tumor specimens from 17 (71%) cases with lobectomy, six (25%) cases with pneumonectomy, and one (4%) case with a transbronchial biopsy. All of the synchronous metastases occurred in lymph nodes removed for staging purposes at the time of surgery, including, 15 (68%) hilar lymph nodes (level 10), six (27%) subcarinal lymph nodes, and one (5%) paratracheal lymph node (level 4). The metachronous metastases occurred 5 and 39 months after the primary diagnosis and were in the brain and adrenal, respectively. These 24 patients had a mean age of 68 years and included 10 (42%) females and 14 (58%) males. A summary of the clinicopathologic features of these 24 cases is seen in Table 2. Of these cases, four (17%) were positive for the FGFR1 amplification in the primary tumor with a median ratio of 2.6, while 20 (83%) of the primary tumors were negative for the FGFR1 amplification with a median ratio of 1.08. In these 24 cases with matched primary tumors and metastases, one (4%) metastatic lesion had a discordant amplification status while 23 (96%) had a concordant amplification status, including three (13%) cases that were positive in both the primary tumor and metastasis. Twenty (83%) cases were negative in both the primary tumor and metastasis Table 3, Image 1, and Image 2. The single discordant case had the primary tumor positive for the FGFR1 amplification (ratio = 3.26) and the synchronous lymph node metastasis negative for the FGFR1 amplification (ratio = 1.07) (Image 2). This discordant case had tumors that were morphologically similar and removed at the same time (synchronous). Primary tumor section that was submitted for FISH-FGFR1 analysis showed uniform distribution of FGFR1 gene amplification. However, the primary tumor in this case did measure 5 cm and thus was not submitted in its entirety, which may mean that there was some heterogeneity that was unsampled.
Discussion

The relatively recent discovery of FGFR1 amplification in SqCCs of the lung has given hope for promising targeted therapies in these tumors, as several FGFR1 kinase inhibitors have been in use in clinical trials for SqCCs and may offer an efficacious option for this tumor.10-12 These discoveries affect the way in which we triage SqCCs in pathology by limiting immunostains and conserving tissue for FISH testing, in an analogous fashion to what has been done with adenocarcinomas of the lung.3 In addition, questions arise when patients have metastatic SqCC of the lung, with regard to whether the primary or metastatic tumor should be tested for FGFR1 amplification. In general, the issue is based on whether the FGFR1 status can differ between the primary and metastatic tumors. Thus, the current study retrospectively looks at the overall results from FGFR1 FISH testing in a large series of tumors and explores the correlation between the FGFR1 amplification status in matched primary and metastatic SqCCs.

Overall, FGFR1 amplification was seen in 15% of cases, which is similar to other studies showing 10% to 21% of tumors testing positive by FISH.6-8,13 This makes it more common than ALK rearrangements, similar in prevalence to EGFR mutations, and less common than KRAS mutations in adenocarcinomas in white patients.14-20 Most studies have also shown that no specific clinicodemographic features correlate with FGFR1 amplification, which supports testing in all advanced-stage SqCCs of the lung in which targeted therapies may be considered.8,21 Some recent studies have shown that FGFR1 amplification may be an independent adverse prognostic factor, particularly if there is a 3.5-fold amplification.13,22,23 The FGFR1-amplified tumors in our
study tended to occur more often in slightly younger pa-
tients with a trend toward tumor larger size. When correlat-
ing the FGFR1 FISH results in matched primary and
metastatic tumors, the data show that discordance is rare
(4%) and that most synchronous and all metachronous cases
had concordant status. However, our samples were largely
from synchronous tumors diagnosed on the initial resection
specimen prior to treatment, which may enrich for tumors
that are genetically similar with minimal external factors se-
lecting for different clones. In studies looking at more meta-
chronous tumors, or matched cases occurring before and
after treatment, there may be more tumor heterogeneity, and
subsequently, it is plausible that the discordance rate could
be higher. Furthermore, other studies have shown that dis-
cordance may also be attributed to different tumors or other
factors, particularly in the setting of a temporal lapse of
more than 2.5 years, which is another argument to retest re-
currences or metastases regardless of the primary tumor
status.24

The discordance between molecular testing in primary
and metastatic tumors has been reported before and has
largely focused on the mutation testing in adenocarcinomas

Image 1 A-D. Metachronous primary and metastatic tumors with concordant FGFR1 fluorescence in situ hybridization (FISH)
status. The primary tumor was an invasive moderately differentiated squamous cell carcinoma of the right upper lobe that was
found to be positive for FGFR1 amplification with a ratio 2.39 (A, H&E, ×200; B, FGFR1 FISH). The patient was subsequently
found to have metastatic moderately differentiated squamous cell carcinoma involving the left adrenal gland, which was also
positive for FGFR1 amplification (ratio = 12.2). FGFR1 gene is shown as a red signal and/or fusion yellow signal due to juxta-
position of green and red FGFR1 probes, while the chromosome 8 centromere probe is shown as the blue signal.
of the lung, in addition to human epidermal growth factor receptor 2 (HER2) assessment in breast cancer and mutation testing in colorectal adenocarcinoma.\(^3\),\(^2\,5\,2\,6\,2\,8\,3\,\text{2}\) In adenocarcinomas of the lung, it has been shown that EGFR mutation status differs between primary tumors and corresponding metastases in about 5% cases.\(^3\),\(^2\,6\,2\,8\,2\) and KRAS mutation status has been shown to have a greater discordance in up to 22.5% cases.\(^2\,5\,2\,9\,3\,0\) Recent studies suggested that heterogeneous distribution of EGFR mutations is extremely rare. It was implied that when there is a combination of EGFR gene mutation and heterogeneously distributed amplification, usually the mutant allele is amplified, resulting in pseudoheterogeneity of EGFR mutations.\(^2\) Furthermore, the next-generation sequencing approach demonstrated that the key driver oncogenic mutations are constant during metastatic progression and show high levels of concordance for recurrent alterations between primary and metastatic tumors, although passenger alterations may be higher in metastases.\(^3\,3\) Therefore, the sensitivity of mutation assay and enrichment of the sample by tumor cells become very important. Currently, the molecular testing guidelines for patients with lung cancer state that primary tumors and metastatic lesions are equally suitable for testing, and in general, the quality of

Image 21 A-D. Synchronous primary and metastatic squamous cell carcinoma of the lung with discordant FGFR1 fluorescence in situ hybridization (FISH) status. A moderately differentiated squamous cell carcinoma of the left upper lobe lung, measuring 5.0 cm, was found to be positive for FGFR1 amplification (ratio = 3.26). However, testing of a synchronous lymph node metastasis was negative for FGFR1 amplification (ratio = 1.07). FGFR1 gene is shown as a red signal and/or fusion yellow signal due to juxtaposition of green and red FGFR1 probes, while the chromosome 8 centromere probe is shown as the blue signal.
sample should guide the specimen selection for molecular testing.\(^3\)

The choice of the sample for the assessment of gene amplification seems to be more challenging, as demonstrated in breast carcinoma, where discordance in HER2/neu status can be seen in up to 10% to 20% cases.\(^{25,31,34,35}\) Gene amplification is generally considered a late event in the development of lung carcinoma, and the possible role of chemotherapeutic interventions in gene amplification status is relatively uncertain.\(^3\) The data in this study support that discordant FGFR1 amplification status can rarely occur. This argues that testing of a primary tumor and subsequent metastases should be strongly considered for patients with metastatic SqCC.

One of the important issues to consider is that many metastatic SqCCs of the lung will be detected by cytology samples in solid organ metastases (eg, computed tomography–guided liver fine-needle aspirations), lymph node metastases (eg, endobronchial ultrasound-guided transbronchial needle aspirations), and pleural fluid specimens, which are relatively minimally invasive procedures for acquiring tumor cells for testing.\(^25\) Since FGFR1 amplification can be detected using FISH, this is easily applicable to cytology specimens given that there is a lower cellularity requirement for FISH studies in comparison to various mutation assays and can eliminate the need for larger tissue biopsies. For this reason, cytology specimens are ideal specimens for providing convenient metastatic tumor samples for FGFR1 testing in patients with metastatic SqCC to confirm metastatic disease and to look for a potentially discordant FGFR1 status that could affect treatment.

Overall, the current study looks at a large series of lung SqCCs tested for FGFR1 amplification and shows that amplification occurs in approximately 15% of cases with only 4% of matched primary and metastatic cases showing discordant status. In future studies, it will be helpful to look at the concordance and clinical follow-up in a larger series of cases. For now, the results suggest that testing of metastatic lesions may rarely show discordant results, which supports the practice of repeat testing for biomarkers on metastatic or recurrent tumors given that it could influence clinical management and may affect prognosis.

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**Corresponding author:** Sara E. Monaco, MD, University of Pittsburgh Medical Center, 5150 Centre Ave, PO Box 2, Suite 201, Pittsburgh, PA 15232; monaco@upmc.edu.

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**References**


