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Fibroblast Growth Factor Receptor 1 and Related Ligands in Small-Cell Lung Cancer

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Introduction: Small-cell lung cancer (SCLC) accounts for 15% of all lung cancers and has been understudied for novel therapies. Signaling through fibroblast growth factors (FGF2, FGF9) and their high-affinity receptor has recently emerged as a contributing factor in the pathogenesis and progression of non–small-cell lung cancer. In this study, we evaluated fibroblast growth factor receptor 1 (FGFR1) and ligand expression in primary SCLC samples.

Methods: FGFR1 protein expression, messenger RNA (mRNA) levels, and gene copy number were determined by immunohistochemistry (IHC), mRNA in situ hybridization, and silver in situ hybridization, respectively, in primary tumors from 90 patients with SCLC. Protein and mRNA expression of the FGF2 and FGF9 ligands were determined by IHC and mRNA in situ hybridization, respectively. In addition, a second cohort of 24 SCLC biopsy samples with known *FGFR1* amplification by fluorescence in situ hybridization was assessed for FGFR1 protein expression by IHC. Spearman correlation analysis was performed to evaluate associations of FGFR1, FGF2 and FGF9 protein levels, respective mRNA levels, and *FGFR1* gene copy number.

Results: FGFR1 protein expression by IHC demonstrated a significant correlation with FGFR1 mRNA levels ($p < 0.0001$) and *FGFR1* gene copy number ($p = 0.03$). The prevalence of FGFR1 mRNA positivity was 19.7%. FGFR1 mRNA expression correlated with both FGF2 ($p = 0.0001$) and FGF9 ($p = 0.002$) mRNA levels, as well as with FGF2 ($p = 0.01$) and FGF9 ($p = 0.001$) protein levels. There was no significant association between FGFR1 and ligands with clinical characteristics or prognosis. In the second cohort of specimens with known *FGFR1* amplification by fluorescence in situ hybridization, 23 of 24 had adequate tumor by IHC, and 73.9% (17 of 23) were positive for FGFR1 protein expression.

Conclusions: A subset of SCLCs is characterized by potentially activated FGF/FGFR1 pathways, as evidenced by positive FGF2, FGF9, and FGFR1 protein and/or mRNA expression. FGFR1 protein expression is correlated with FGFR1 mRNA levels and *FGFR1* gene copy number. Combined analysis of FGFR1 and ligand expression may allow selection of patients with SCLC to FGFR1 inhibitor therapy.

Key Words: Small-cell lung cancer, Fibroblast growth factor receptor 1, Fibroblast growth factor 2, Fibroblast growth factor 9.

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Small-cell lung cancer (SCLC) comprises approximately 15% of all lung cancers with more than 30,000 new cases per year in the United States.[1] SCLC is an extremely aggressive malignancy, with less than 5% survival 3 years after diagnosis. No major therapeutic progress has been achieved in SCLC in the past decades. Identification of new therapies in SCLC is urgently needed.

Novel molecularly targeted therapies, such as epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors and anaplastic lymphoma kinase (ALK) inhibitors, have dramatically improved the clinical course for advanced non–small-cell lung cancer (NSCLC) patients with EGFR mutations and ALK rearrangements, respectively.[2,3] However, there are no approved molecularly targeted therapies for patients with SCLC. Part of the reason for the lack of improvement in care of patients with SCLC is that there is limited availability of tissue for molecular studies due to difficulties in obtaining sufficient tumor samples. This highlights the value of performing

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and reporting research on available SCLC tissue to advance the identification of novel therapeutically relevant genomic alterations in this disease.

This study focuses on defining the fibroblast growth factor (FGF)/fibroblast growth factor receptor (FGFR) signaling pathway as a target for drug therapy in patients with SCLC. FGFs comprise a complex family of signaling molecules that have been implicated in angiogenesis and inflammation in a wide variety of human disorders.[4] Activation of the FGFR1 signaling pathway is thought to drive epithelial-to-mesenchymal transition, transforming normal cells to tumor cells. Twenty-three FGFs and four FGFRs (FGFR1–FGFR4) have been identified. Results of several studies have demonstrated the coexpression of FGF2 and FGF9 ligands in association with FGFR1 in human lung cancers.[5,6] The binding of FGF ligands to FGFRs mediates signal transduction through induction of receptor dimerization and promotes a cascade of downstream Ras-dependent mitogen-activated protein kinase and Ras-independent phosphoinositide 3-kinase–Akt signaling pathways. Other pathways can also be activated by FGFRs, including signal transducer and activator of transcription (STAT)-dependent signaling.[7] The FGF/FGFR signaling pathway has been implicated as an autocrine signaling loop that leads to tumor proliferation and angiogenesis in a variety of NSCLC cell lines.[6]

The *FGFR1* gene is located on the short arm of chromosome 8 (8p12) and is a member of the FGFR family of tyrosine kinase receptors. *FGFR1* amplification, translocation, and point mutations have been described in several tumor types, including breast, prostate, esophageal, bladder, and endometrial cancers and recently in 13% to 25% of squamous-cell lung cancers (SqCCs).[7–10] In addition, *FGFR1* amplification/overexpression has also been found in a subset of patients with non-SqCC NSCLC.[11] Recent genomic analysis of a set of 29 SCLC samples has revealed focal *FGFR1* amplification among other molecular aberrations.[12]

FGF2 is a mitogen and a survival factor in many experimental models and is involved in neoangiogenesis in vivo. Evidence suggests that FGF2 induces proliferation and chemoresistance in SCLC cells.[13] High levels of serum FGF2 have been associated with poor prognosis in SCLC, possibly because of an FGF2-mediated cytoprotective effect, whereby the expression of antiapoptotic proteins is upregulated, promoting resistance to current anticancer treatment.[14] However, studies of the FGF2 and FGF9–FGFR1 signaling pathway have been typically performed on small series of SCLC tumor samples, likely due to limited availability of SCLC specimens.

Preclinical evidence suggests that SCLC patients may benefit from FGFR inhibitor therapy. Different FGFR inhibitors such as BGJ 398 (Novartis, Switzerland), AZD 4547 (AstraZeneca, UK), Ponatinib (Ariad, Cambridge, MA), and LY2874455 (Eli Lilly, Indianapolis, IN) are currently in phase I and II clinical trials.[7] The FGFR inhibitor PD173074 has blocked SCLC growth both in vitro and in vivo.[13] A small interfering RNA against FGF2 and FGF-ligand traps (FP-1039) have been developed to inhibit FGF ligands in vitro.[15,16] Thus, FGFR and its ligands are promising therapeutic targets.

The aim of this study was to describe the characteristics of the FGF–FGFR1 signaling pathway in SCLC. We first assessed the frequency of FGFR1, FGF2, and FGF9 protein and messenger RNA (mRNA) expression and *FGFR1* gene amplification from a series of 90 surgically resected primary SCLCs. Next, we assessed the frequency of FGFR1 protein expression in second cohort of 24 SCLC biopsy samples with known *FGFR1* amplification. Our goal with the second cohort was to confirm the ability of our selected FGFR1 antibody and methods to detect *FGFR1* amplification and to better understand the correlation between *FGFR1* amplification and protein expression. We analyzed the data to investigate associations among levels of FGFR1, FGF2, and FGF9 protein and respective mRNA levels, *FGFR1* gene amplification, and clinical characteristics. To our knowledge, this is the first study to provide a comprehensive analysis of the FGF2 and FGF9–FGFR1 signaling pathway in SCLC.

MATERIALS AND METHODS

Patient Population and Tumor Specimens

Two cohorts of SCLC specimens were studied sequentially. The first cohort was primary SCLC tumor specimens collected from a series of patients with limited disease who underwent pulmonary resection.[17] Archival formalin-fixed paraffin-embedded tumor samples were obtained from a unique series of 90 patients with SCLC who underwent pulmonary resection between 1982 and 2002 at the Medical University of Gdansk, Poland. In most patients, SCLC histology was established at the time of surgery. All primary diagnoses were reviewed by three experienced pathologists according to the 2004 World Health Organization criteria.[18] For all patients, medical records were reviewed to obtain clinical characteristics, including age, gender, tumor diameter, tumor, node, metastasis stage, and overall survival. In all patients, surgery was followed by standard chemotherapy. Median follow-up was 17.8 months (range, 1–212 mo), median survival was 18.7 months, and the probability of survival 2 years after diagnosis was 42%.

The second cohort of 24 SCLC biopsy cases was from the Institute of Pathology at the University Hospital Cologne, Germany, with known *FGFR1* amplification by fluorescence in situ hybridization (FISH).[19] All of these cases met our criteria for *FGFR1* amplification (*FGFR1* gene signals ≥ 6 per nucleus or *FGFR1*/CEN8 ratio ≥ 2).

Tissue Microarray Construction

Ninety surgically resected SCLC specimens from the first cohort were constructed into a tissue microarray (TMA) using a manual MTA-1 Beecher Instrument (Beecher Instruments, Inc, Sun Prairie, WI). Briefly, morphologically representative areas of SCLC were identified and annotated on a hematoxylin and eosin–stained slide under the microscope by a pathologist. The annotated slides were used to guide dissection of three 0.6-mm diameter cores from different tumor areas of the paraffin-embedded blocks. The triplicate cores were set into TMA blocks.

Immunohistochemistry

Immunohistochemistry (IHC) on 4-μm sections was performed using primary commercially available antibodies