Characterization of Fibroblast Growth Factor Receptor 1 in Small-Cell Lung Cancer

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Introduction: There remains a significant therapeutic need for small-cell lung cancer (SCLC). We and others have reported high frequency of copy number gains in cytogenetic bands encoding fibroblast growth factor receptor 1 (FGFR1) in SCLC tumors and cell lines.

Methods: Thirteen SCLC cell lines and 68 SCLC patient tumor samples were studied for FGFR1 amplification. Growth inhibition assays were performed using PD173074, a pan-FGFR inhibitor to determine the correlation between FGFR1 expression and drug sensitivity.

Results: We did not detect *FGFR1* mutations in SCLC cell lines. Focal amplification of *FGFR1* gene was found in five tumor samples (7%), with high-level focal amplification in only one tumor sample (1%). Amplification owing to polysomy of chromosome 8, where FGFR1 locates, was observed in 22 tumor samples (32%). There was no correlation between *FGFR1* gene copy number and messenger RNA expression or protein expression in SCLC cells. *FGFR* inhibitor sensitivity correlated with *FGFR1* copy number determined by real-time polymerase chain reaction assay ($r = -0.79$; $p = 0.01$).

Conclusion: *FGFR1* gene mutations and focal amplification are rare in SCLC, but polysomy of chromosome 8 is relatively common. *FGFR1* copy number gain predicts sensitivity to FGFR inhibition, and FGFR expression correlates inversely with chemosensitivity.

Key Words: Small-cell lung cancer, FGFR1, Focal amplification, Copy number gain.

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Small-cell lung cancer (SCLC) comprises 13% of all lung cancers.[1] Despite extensive research over the last two decades, treatment for SCLC has not improved significantly and prognosis remains poor.[2] Genetic characterization of driver mutations could potentially offer effective drug targets and novel therapies in SCLC. Although previous studies have reported recurrent genetic alterations in SCLC,[3–5] therapeutically tractable targets have so far been elusive.

Fibroblast growth factors and fibroblast growth factor receptors (FGFR) play essential roles in regulation of normal cell proliferation, survival, migration, and differentiation.[6] The FGFR tyrosine kinase family comprises four kinases: FGFR1, FGFR2, FGFR3, and FGFR4. *FGFR1* amplification has been reported in 22% of squamous cell lung carcinoma and may predict sensitivity to FGFR inhibition.[7] The fibroblast growth factor signaling pathway may influence growth of SCLC. FGFR inhibition impairs SCLC growth in vitro and in vivo, reduces intratumor proliferation, and increases apoptosis.[8] We and others have reported high copy number gains (CNGs) in cytogenetic bands encoding FGFR1 in SCLC cell lines and tumors.[4,5] High CNGs in some of the samples suggest a role for FGFR1 as a potential therapeutic target in a subgroup of SCLC. In this study, we sought to further characterize the role of FGFR1 in SCLC.

PATIENTS AND METHODS

Tumor Samples, Tissue Microarray, and Cancer Cell Lines

Thirteen SCLC cell lines and 68 SCLC tumor samples were studied. Use of human samples was approved by the National Cancer Institute (NCI) Institutional Review Board. Details of cells and tumors are described in the Supplementary Materials and Methods (Supplementary Digital Content 1, http://links.lww.com/JTO/A534).

Array Comparative Genomic Hybridization Analysis

We performed array comparative genomic hybridization (aCGH) analysis on five patient samples from the NCI cohort. aCGH was performed and analyzed as described previously.[5] A reference genomic DNA was used for hybridization. Data analysis was performed using Nexus 4.0 software (Biodiscovery Inc., Hawthorne, CA). CNG and loss of the FGFR1 gene were defined

if the $\log_2$ ratio values of sample DNA/reference genomic DNA were more than 0.2 and less than −0.2, respectively.

Sequencing

Exons 2 to 18 of *FGFR1* gene were sequenced in 13 SCLC cell lines as described in the Supplementary Materials and Methods (Supplementary Digital Content 1, http://links.lww.com/JTO/A534).

Real-Time Polymerase Chain Reaction

The messenger RNA (mRNA) expression of *FGFR1* gene and the copy numbers of the *FGFR1* gene in SCLC cells were evaluated using the Taqman gene expression assay and Taqman copy number assay, respectively (Applied Biosystems, Foster City, CA), following manufacturer's instruction. Details of the method are described in the Supplementary Materials and Methods (Supplementary Digital Content 1, http://links.lww.com/JTO/A534).

Western Blot

Western blotting was performed as previously described.[9] Anti-FGFR1 antibody was obtained from Abcam (Cambridge, United Kingdom), anti-p-FGFR antibody was from Cell Signaling Technology (Danvers, MA), and anti-Actin antibody was from Sigma-Aldrich (St. Louis, MO). Semiquantification of signals of Western blot was analyzed by Image J software (National Institutes of Health, Bethesda, MD). FGFR1 protein expression was normalized by actin protein expression and then calibrated by the expression level of the H69 cells before further analysis.

Growth Inhibition Assays

The concentration that inhibits 50% to drugs in SCLC cells was determined as described in the Supplementary Materials and Methods (Supplementary Digital Content 1, http://links.lww.com/JTO/A534).

Fluorescence In Situ Hybridization for the *FGFR1* Gene

Fluorescence in situ hybridization (FISH) assays were performed on 5 μm formalin-fixed paraffin-embedded tumor sections using a laboratory standardized protocol with slight modifications.[10] Details of the method are described in the Supplementary Materials and Methods (Supplementary Digital Content 1, http://links.lww.com/JTO/A534). CNG was defined as red FGFR1 signals with the corresponding control chromosome enumeration probes (CEP8) more than or equal to four copies per cell in more than 20% of cells. Focal amplification was defined if the ratio of the *FGFR1* gene to the CEP8 was more than 2 (low-level amplification 2–4; high-level amplification >4).

Statistical Analysis

Correlation between variables was analyzed using Spearman's method, and p values less than 0.05 were regarded as significant.

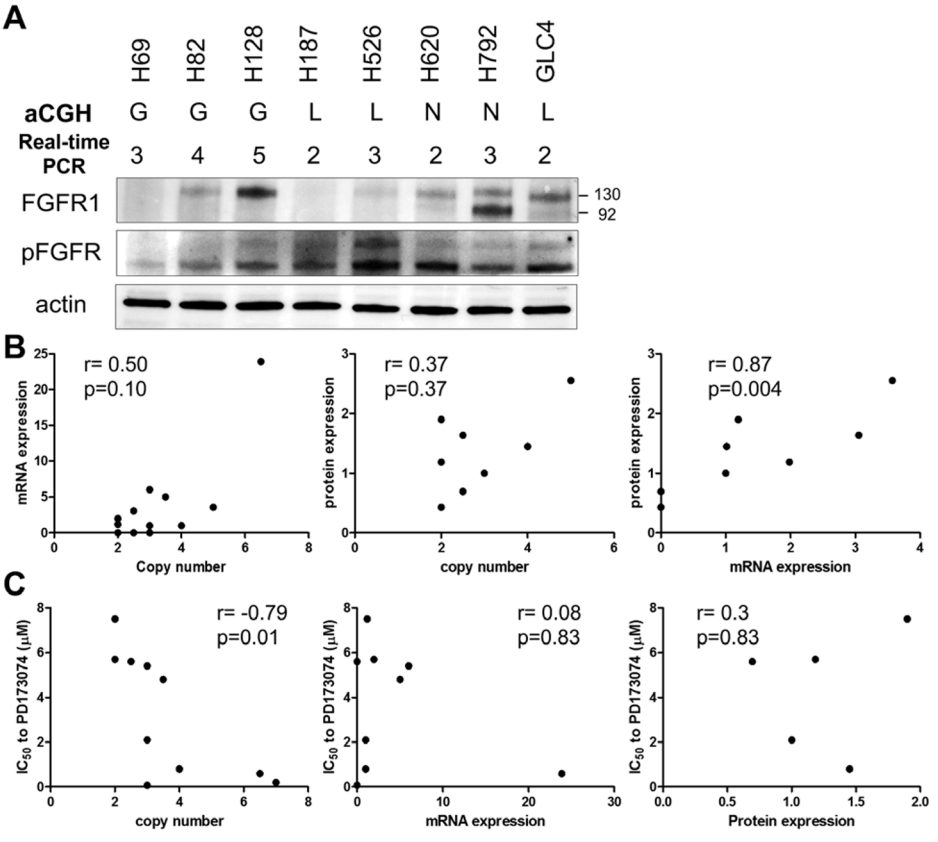

FIGURE 1. FGFR1 in SCLC cells. *A*, FGFR1 protein expression in SCLC cells. G, copy number gain; N, copy number normal; L, copy number loss. The numbers indicate the copy number of the *FGFR1* gene determined by the real-time PCR assay. *B*, Correlation of *FGFR1* copy number assessed by real-time PCR and mRNA expression and protein expression. *C*, Correlation of concentration that inhibits 50% to PD173074 and *FGFR1* copy number assessed by real-time PCR assay, FGFR1 mRNA expression, or FGFR1 protein expression. FGFR1, fibroblast growth factor receptor 1; SCLC, small-cell lung cancer; PCR, polymerase chain reaction; mRNA, messenger RNA.