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Functional cooperation between HIF-1α and c-Jun in mediating primary and acquired resistance to gefitinib in NSCLC cells with activating mutation of *EGFR*

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ABSTRACT

Objective: Hypoxia-inducible factor 1 (HIF-1) and activator protein 1 (AP-1) are important transcription factors regulating expression of genes involved in cell survival. HIF-1α and c-Jun are key components of HIF-1 and AP-1, respectively, and are regulated by epidermal growth factor receptor (EGFR)-mediated cell signaling and tumor microenvironmental cues. The roles of HIF-1α and c-Jun in development of resistance to EGFR tyrosine kinase inhibitor (TKI) in non-small cell lung cancer (NSCLC) with activating mutation of *EGFR* have not been explored. In this study, we investigated the roles of HIF-1α and c-Jun in mediating primary and acquired resistance to gefitinib in NSCLC cells with activating mutation of *EGFR*.

Materials and methods: Changes in HIF-1α protein and in total and phosphorylated c-Jun levels in relation to changes in total and phosphorylated EGFR levels before and after gefitinib treatment were measured using Western blot analysis in NSCLC cells sensitive or resistant to gefitinib. The impact of overexpression of a constitutively expressed HIF-1α (HIF-1α/ΔODD) or a constitutively active c-Jun upstream regulator (SEK1 S220E/T224D mutant) on cell response to gefitinib was also examined. The effect of pharmacological inhibition of SEK1-JNK-c-Jun pathway on cell response to gefitinib was evaluated.

Results: Downregulation of HIF-1α and total and phosphorylated c-Jun levels correlated with cell inhibitory response to gefitinib better than decrease in phosphorylated EGFR did in NSCLC cells with intrinsic or acquired resistance to gefitinib. Overexpression of HIF-1α/ΔODD or SEK1 S220E/T224D mutant conferred resistance to gefitinib. There exists a positive feed-forward regulation loop between HIF-1 and c-Jun. The JNK inhibitor SP600125 sensitized gefitinib-resistant NSCLC cells to gefitinib.

Conclusions: HIF-1α and c-Jun functionally cooperate in development of resistance to gefitinib in NSCLC cells. The translational value of inhibiting HIF-1α/c-Jun cooperation in overcoming resistance to EGFR TKI treatment of NSCLC cells with activating mutation of *EGFR* deserves further investigation.

1. Introduction

Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) have been used worldwide to treat patients with advanced non-small cell lung cancer (NSCLC) since the first-generation EGFR TKIs (gefitinib and erlotinib) were approved in early 2000s. In NSCLC patients with tumors harboring *EGFR* activating mutations in exons 19–21 of EGFR kinase domain, treatment with a first-generation EGFR TKI prolonged progression-free survival time by about 9.7 months [1].

However, not all NSCLC patients with the *EGFR* activating mutations in their tumors have such favorable outcomes in response to EGFR TKIs. About 20%–30% of NSCLC patients with the *EGFR* activating mutations in their tumors have no objective tumor-regressive response to initial treatment with a first-generation EGFR TKI [1–4]. A number of mechanisms can cause intrinsic (primary) resistance of NSCLC to EGFR TKIs, including EGFR downstream pathway redundancy (activated by overlapping signal pathways), pathway reactivation (independent of EGFR due to oncogenic mutations or mutational inactivation of key

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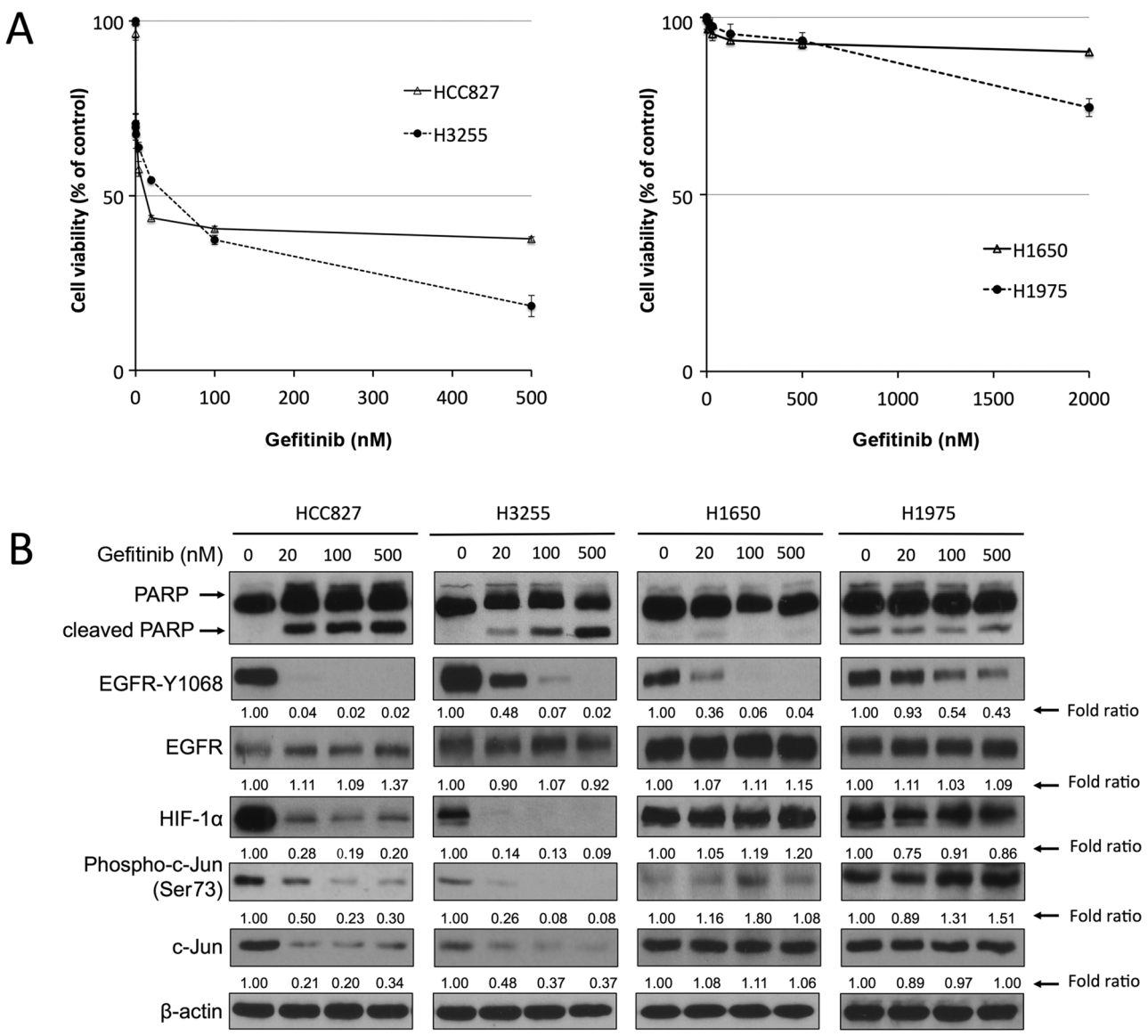

Fig. 1. Downregulation of HIF-1α and c-Jun protein correlates with cell inhibitory response to gefitinib treatment in NSCLC cells.
A. HCC827 and H3255 cells (left panel) and H1650 and H1975 cells (right panel) were treated with the indicated concentrations of gefitinib for 5 days. Cell survival was measured by MTT proliferation assay. **B.** HCC827, H3255, H1650, and H1975 cells were treated with the indicated concentrations of gefitinib for 24 h. Cells were then harvested, and equal amounts of protein lysates were subjected to Western blot analysis with the indicated antibodies. The level of β-actin was used as a reference of lysate protein loading control of each cell line.

signaling molecules, such as Ras [5,6] and PTEN [7,8]), and pathway alternation (escape from EGFR signaling regulation via recruiting an alternate signaling pathway) [9]. In addition, tumor microenvironmental cues and tumor heterogeneity can cause intrinsic resistance to EGFR TKI [10].

Moreover, even in patients who initially have a partial or complete response to an EGFR TKI, acquired resistance may ultimately occur. The mechanisms underlying development of acquired resistance of NSCLC to first-generation EGFR TKIs include *EGFR* T790M secondary mutation (present in ~60% cases of acquired resistance), *MET* amplification (5%–10%), *PIK3CA* mutation (~5%), *BRAF* mutation (~1%), and small-cell cancer transformation (~5%) [11]; in another approximately 20% to 25% of cases of acquired resistance, the underlying mechanisms remain unclear.

Many NSCLC patients, whose tumor initially responds to a first-generation EGFR TKI but develops resistance due to secondary *EGFR* T790M mutation, benefit from treatment with a third-generation EGFR TKI, such as osimertinib; however, a significant percentage of patients with acquired resistance due to *EGFR* T790M mutation do not respond to a third-generation EGFR TKI [12,13]. Novel insights into the mechanisms of resistance to EGFR TKIs are critical for developing new therapeutic strategies for improving the outcome of NSCLC patients with advanced disease.

Hypoxia-inducible factor-1 (HIF-1), a master regulator of response to tumor hypoxia, is a heterodimer consisting of an oxygen-sensitive alpha subunit (HIF-1α) and a constitutively expressed beta subunit (HIF-1β) [14–18]. The level of HIF-1α is increased dramatically in hypoxic tumor microenvironments because of decreased ubiquitination and degradation of HIF-1α protein associated with tumor hypoxia [19,20]. The level of HIF-1α is also upregulated by aberrant cell signaling through increased expression [21–25]. We previously showed that downregulation of HIF-1α through inhibiting EGFR downstream cell signaling is required for the antiproliferative effects of the anti-EGFR antibody cetuximab in head and neck cancer, colorectal cancer, and NSCLC models [26–36].

Activator protein-1 (AP-1) is a transcription factor that regulates