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Identification of protein expression alterations in gefitinib-resistant human lung adenocarcinoma: PCNT and mPR play key roles in the development of gefitinib-associated resistance

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ABSTRACT

Gefitinib is the first-line chemotherapeutic drug for treating non-small cell lung cancer (NSCLC), which comprises nearly 85% of all lung cancer cases worldwide. However, most patients eventually develop drug resistance after 12–18 months of treatment. Hence, investigating the drug resistance mechanism and resistance-associated biomarkers is necessary. Two lung adenocarcinoma cell lines, PC9 and gefitinib-resistant PC9/Gef, were established for examining resistance mechanisms and identifying potential therapeutic targets. Two-dimensional differential gel electrophoresis and matrix-assisted laser desorption ionization time-of-flight mass spectrometry were used for examining global protein expression changes between PC9 and PC9/Gef. The results revealed that 164 identified proteins were associated with the formation of gefitinib resistance in PC9 cells. Additional studies using RNA interference showed that progesterone receptor membrane component 1 and pericentrin proteins have major roles in gefitinib resistance. In conclusion, the proteomic approach enabled identifying of numerous proteins involved in gefitinib resistance. The results provide useful diagnostic markers and therapeutic candidates for treating gefitinib-resistant NSCLC.

Introduction

Lung cancer is a leading cause of cancer mortality. Two major types of lung cancers exist: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Approximately 15% of lung cancer cases are those of highly metastatic SCLC. This lung cancer type rapidly spreads to distant organs while it is diagnosed. By contrast, NSCLC comprises approximately 85% of all lung cancer cases and has a growth rate lower than that of SCLC (Delbaldo et al., 2002). The 3 common subtypes of NSCLC are adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. Among them, adenocarcinoma is the most common type and comprises approximately 40% of NSCLC cases (Zarogoulidis et al., 2013). In addition, in most NSCLC patients, epidermal growth factor receptor (EGFR) overexpression is observed. However, in Asian countries, approximately 30% of NSCLC patients have EGFR mutations, whereas in Western countries, approximately 15% of NSCLC patients have EGFR mutations. In NSCLC, EGFR mutations cause EGFR autophosphorylation and stimulate cell signaling through various downstream pathways, such as PI3K/Akt, Ras/Raf/MAPK, and Jak/Stat pathways involving cell proliferation, differentiation, antiapoptosis, and angiogenesis (Zarogoulidis et al., 2013; Minuti et al., 2013; Giaccone, 2004). In clinical practice, EGFR mutation-positive patients with advanced NSCLC are administered EGFR tyrosine kinase inhibitor treatment, which significantly improves clinical responses. Gefitinib, an EGFR tyrosine kinase inhibitor, is used by NSCLC patients with EGFR mutations, and it competitively blocks EGFR autophosphorylation (Giaccone, 2004). Gefitinib is currently the first-line drug for treating NSCLC patients

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with an EGFR-mutated kinase domain. Patients have a marked initial response with gefitinib, but most patients eventually develop drug resistance after 12–18 mo of treatment (Gridelli et al., 2011). Hence, investigating resistance mechanisms and therapeutic targets for gefitinib-resistant NSCLC is an urgent concern. The present study was performed for investigating in vitro gefitinib resistance mechanisms in NSCLC, increasing the understanding of the molecular processes involved, and identifying potential resistance disease markers with possible diagnostic or therapeutic applications. We used 2 lung adenocarcinoma lines, PC9 and gefitinib-resistant PC9/Gef, as a model system for investigating gefitinib resistance-associated cytosolic protein alterations by conducting quantitative proteomic analysis using 2-dimensional differential gel electrophoresis (2D-DIGE) and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Chang et al., 2014; Chou and Chan, 2014). In addition, siRNA silencing against selected identified proteins, pericentrin (PCNT) and progesterone receptor membrane component 1 (mPR), was performed for monitoring and evaluating their potencies against gefitinib resistance.

Materials and methods

Chemical and reagents

Generic chemicals were purchased from USB corporation company (Santa Clara, CA, USA), while reagents for 2D-DIGE were purchased from GE Healthcare (Uppsala, Sweden). All primary antibodies were

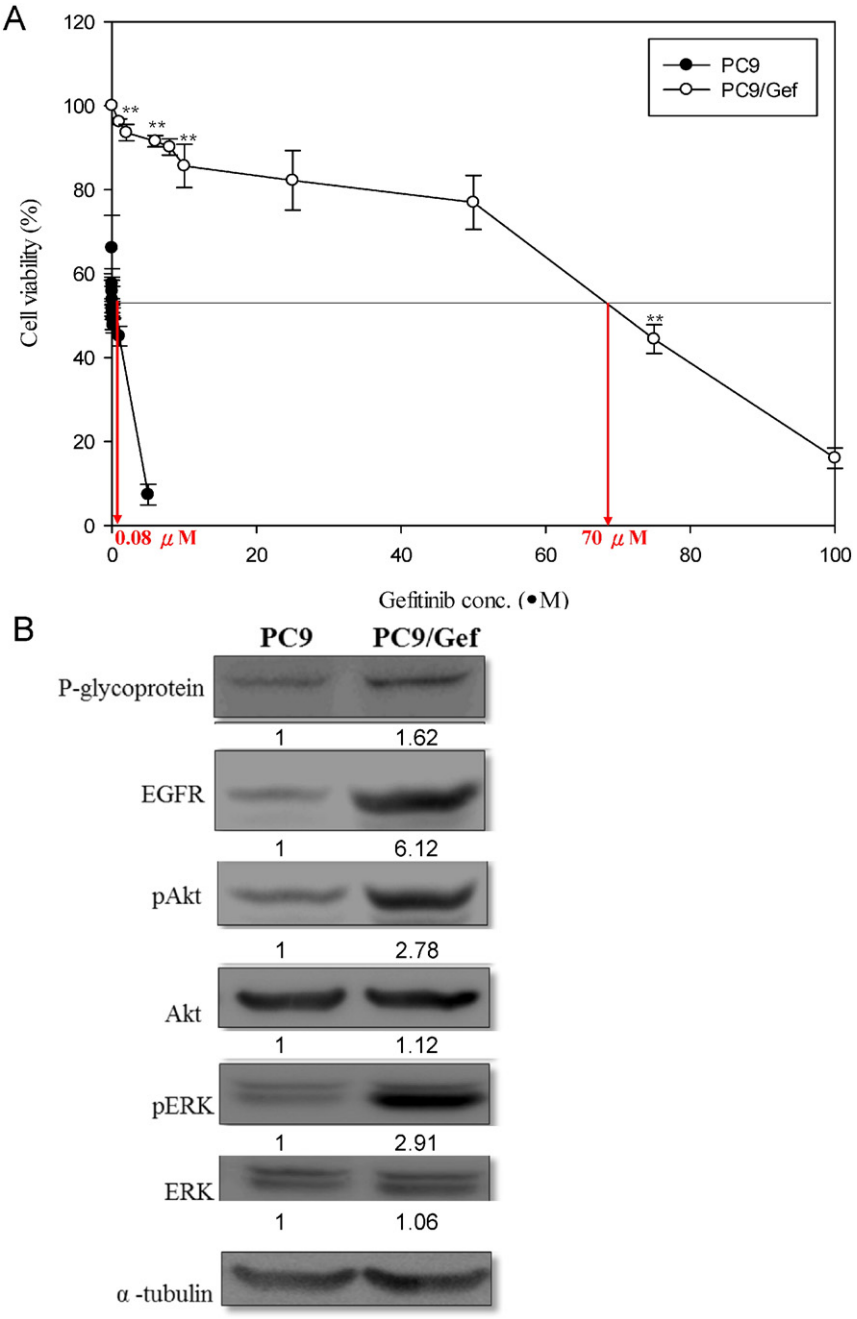

Fig. 1. Dose-dependent kinetics of gefitinib-induced loss of cell viability and increased expression and activation of receptor tyrosine kinases and downstream signaling molecules in PC9 and PC9/Gef cells. (A) PC9 and PC9/Gef cells grown overnight were treated with a range of doses of gefitinib and cell viability was determined by MTT assay. Data were represented as mean ± SD (n = 4, $^{**}p < 0.01$). (B) Immunoblotting showing the expression level of EGFR and downstream signaling proteins related with gefitinib resistance. α-Tubulin was used as loading control.