Difference of EGFR-Binding Proteins between Wild Type and Mutant EGFR

Tsuyoki Kadofuku¹, Tomoko Kanome¹, Kazuto Nishio³, Toshio Kuroki⁴, and Tohru Ohmori¹,²

¹Institute of Molecular Oncology, Showa University, ²First Department of Internal Medicine, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan, ³Department of Genome Biology, Kinki University School of Medicine, 377-2 Osakasayama, Osaka 589-8511, Japan, ⁴Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan

Summary. Non-small cell lung cancer cells expressed mutant EGFR are more sensitive to gefitinib (Iressa) than that expressed wild type EGFR. To elucidate the mechanism of the hypersensitivity to gefitinib in the mutant EGFR, we explored the difference of EGFR-binding proteins between wild type and a 15 bp deletion mutant EGFR using respective stable transfectant cells. EGFR-binding proteins in respective transfectant cells were collected by co-precipitation with polyclonal anti-EGFR and the co-precipitate proteins were separated by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). Both co-precipitate proteins showed a similar 2D-PAGE pattern, however, several proteins differed between both transfectant cells. Among these proteins, one protein, detected to high concentration in mutant EGFR transfectant cells, was identified as heat shock protein 70 (HSP 70) by peptide mass fingerprinting. Much binding of HSP 70 to mutant EGFR was also confirmed by Western blotting. There was no significant difference of HSP 70 protein expression between both transfectant cells. These results suggest that the difference of HSP70 binding to EGFR may modify the EGFR-downstream signaling and influence the sensitivity to gefitinib.

Key words. Gefitinib, non-small cell lung cancer cells, EGFR-binding proteins, heat shock protein 70, two-dimensional polyacrylamide gel electrophoresis, LC-MS/MS

1 Introduction

Epidermal growth factor receptor (EGFR), a 170-kDa membrane glycoprotein with tyrosine kinase activity, plays central roles in cell proliferation, survival, migration, differentiation, and angiogenesis.
EGFR-mediated signaling is thought to play an important role in the progression of epithelial neoplasm. Increased EGFR expression has been reported in a wide variety of human tumors. Gefitinib (Iressa) is an orally active EGFR-tyrosine kinase inhibitor that block signal transduction pathways in cancer cell proliferation, survival and other host-dependent process that promote cancer growth. Gefitinib has demonstrated antitumor efficacy in patients with relapsed or recurrent non-small cell lung cancer (NSCLC) and has received approval for the treatment of advanced NSCLC (Herbst 2003, Fukuoka et al. 2003, Kris et al. 2003). A possible paradigm for determining response to gefitinib has been reported that in-frame mutation of EGFR are well-corrected to the hyper-responsiveness to gefitinib in patients with NSCLC (Lynch et al. 2004, Paez et al. 2004). These mutations were small, in-frame deletion or substitutions clustered around the ATP-binding site in exons 18, 19, and 21 of EGFR.

We previously identified a 15-bp in-frame deletion in exon 19 of EGFR (2411-2425) in a gefitinib-hypersensitive NSCLC cell line, PC-9. The PC-9 cell line was hypersensitive to gefitinib (IC\textsubscript{40} =53.0±8.1 nM) as compared with another NSCLC cell line, PC-14, which expresses wild type EGFR (IC\textsubscript{40}=47.0±9.1 \mu M). These observations suggest that this deletion mutant might be an active mutant EGFR that may correlate with tumor responsiveness to gefitinib. In the present study, to elucidate the mechanism of the hypersensitivity to gefitinib in the mutant EGFR, we explored the difference of EGFR-binding proteins between wild type EGFR and a 15 bp deletion mutant EGFR using respective stable transfectant cells.

2 Materials and methods

2.1 Transfectant cells

Wild type EGFR stable transfectant cells (293_pEGFR) and a 15 bp deletion mutant EGFR stable transfectant cells (293_pA15) were kindly provided by Dr. Fukumoto (Shien Laboratory, National Cancer Center) and maintained in RPMI 1640 medium containing 10% fetal calf serum (FCS) at 5% CO\textsubscript{2}. 293_pA15 cell is about 1000-fold sensitive to gefitinib as compared to 293_pEGFR cell.

2.2 EGFR-stimulation and sample preparation

To eliminate the effect of FCS, cells were starved by 0.1% FCS contained medium for overnight, then exposed to 10 ng/ml of TGF\textalpha for 1 h. After the exposure, cells were washed 3 times in 50 ml of cold PBS, lysed with