Lung Cancer (2006) **54**, 25—33

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/lungcan

Activation of the AKT and STAT3 pathways and prolonged survival by a mutant EGFR in human lung cancer cells

Hakan Akca[a,b], **Masachika Tani**[a], **Tomoyuki Hishida**[a], **Shingo Matsumoto**[a], **Jun Yokota**[a,*]

[a] *Biology Division, National Cancer Center Research Institute, Tokyo 104-0045, Japan*
[b] *Department of Biology, Faculty of Arts and Sciences, Pamukkale University, Denizli 20017, Turkey*

Received 28 March 2006; received in revised form 14 June 2006; accepted 17 June 2006

KEYWORDS
EGFR;
Mutation;
AKT;
STAT3;
Lung cancer

Summary To clarify the pathogenic and biological significance of EGFR mutations in lung cancer, we compared the status of ERBB family receptors, their downstream signal transductions and biological phenotypes between lung cancer cell lines with mutant and wild type EGFR. We initially analyzed expression and phosphorylation of ERBB family receptors and their major downstream proteins, AKT, p44/42 MAPK and STAT3, in a series of lung cancer cell lines with or without EGFR mutation. The expression levels of EGFR as well as of ERBB2 and ERBB3 proteins in cells with EGFR mutation tended to be higher than those in cells with wild type EGFR. There was no difference in stability between mutant and wild type EGFR proteins. EGF induced phosphorylation of EGFR, AKT, p44/42 MAPK and STAT3 to various extents, but the level of induction was not associated with the existence of EGFR mutation. These results implied that the activation of AKT, p44/42 MAPK and STAT3 signaling transmitted by EGFR would be critical for the growth and survival of lung cancer cells, but specific features of mutant EGFR in lung cancer cells was not discriminated by these approaches. We therefore performed transfection studies using PC-13 cells with no detectable endogenous EGFR expression. Exogenous expression of wild type and mutant EGFR (delE746-A750) in the cells revealed that only in the mutant EGFR transfected cells, EGFR itself as well as AKT and STAT3 were highly phosphorylated after 24 h of serum deprivation. The survival time of mutant EGFR transfected cells was prolonged under serum-free culture conditions, but not under standard culture conditions with 10% serum. These results suggest that cells with a mutant EGFR survive through the activation of the AKT and/or STAT3 pathways, even in low EGF microenvironments. This specific property due to EGFR mutation could be an important step of multistage lung cancer progression.
© 2006 Elsevier Ireland Ltd. All rights reserved.

Abbreviations: EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; HRG-1, heregulin-1; NSCLC, non-small cell lung cancers; RT-PCR, reverse-transcriptase polymerase chain reaction; TGF-α, transforming growth factor-alpha

* Corresponding author. Tel.: +81 3 3547 5272; fax: +81 3 3542 0807.
E-mail address: jyokota@gan2.res.ncc.go.jp (J. Yokota).

0169-5002/$ — see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.lungcan.2006.06.007

1. Introduction

Epidermal growth factor receptor (EGFR) is one of four members of the ERBB family receptor tyrosine kinases; EGFR, ERBB2 (also known as NEU, HER2 or EGFR2), ERBB3 and ERBB4. A family of ligands, including epidermal growth factor (EGF), heregulin-1 (HRG-1; also known as neuregulin-1) and transforming growth factor-alpha (TGF-α), bind the extracellular domain of specific receptors and lead to the formation of both homo- and hetero-dimers, and transduce signals into the cell. The major downstream pathways that transduce signals from the ERBB receptors are the RAS-p44/42 MAPK (also known as ERK1/2), PI3K-AKT and STAT3/5, which regulate important cellular functions, including cell proliferation, survival, migration and differentiation. Amplification and overexpression of EGFRs are frequently found in a variety of epithelial cancers, such as breast, lung, colon, ovarian, and brain tumors, and play a central role in the etiology and progression of these tumors [1,2].

Recently, it was shown that the EGFR gene is mutated in approximately 20% of non-small cell lung cancers (NSCLCs) [3], and that mutations in the tyrosine kinase domain of the EGFR gene strongly correlate with dramatic clinical response to EGFR inhibitors, such as gefitinib, in patients with NSCLC [4,5]. An increased copy number of the EGFR gene is detected in approximately 30% of NSCLC and is also associated with the sensitivity of EGFR inhibitors [6]. The mutations occur in a selective subpopulation of NSCLC patients; adenocarcinoma histology, never-smoker status, East Asian ethnicity and female gender [7,8]. It was also reported that EGFR mutations are present in atypical adenomatous hyperplasia (AAH), which is considered to be a precursor lesion of lung adenocarcinoma [9], suggesting that EGFR mutations are involved in the early stage of multistep lung cancer progression. However, the pathogenic and biological significance of EGFR mutations in the development of lung cancer is still unclear. EGFR mutations in the kinase domain are very rare in common human cancers besides NSCLCs [10]. Thirty to forty percent of glioblastomas are known to have a specific EGFR mutant, EGFRvIII, which is an in-frame deletion of exons 2—7. Thus, studies on the biological significance of EGFR alterations have been extensively performed in glioblastomas. This mutant confers enhanced tumorigenicity on glioblastoma cells through elevated proliferation and reduced apoptotic rates of the cells by activation of the PI3K/AKT pathway [11,12].

To clarify the pathogenic and biological significance of EGFR mutations in lung cancer, we took two approaches in this study. One is an analysis of the expression and phosphorylation of the ERBB family receptors and their major downstream proteins, AKT, p44/42 MAPK and STAT3, in a series of wild type and mutant EGFR expressing NSCLC cell lines. Another is a transfection study using a NSCLC cell line, PC-13, with no detectable endogenous EGFR expression, as a recipient of wild type and mutant EGFR transduction. We attempted to elucidate the differences in EGF mediated signal transductions and biological phenotypes between lung cancer cell lines with mutant and wild type EGFR.

2. Materials and methods

2.1. Cell culture

Six cell lines derived from lung adenocarcinoma, PC-9, 11-18, Ma-24, PC-3, A549 and PC-14, and two cell lines derived from lung large cell carcinoma, H1299 and PC-13, were used in this study. A549 cells were maintained in DMEM, whereas PC-9, 11-18, Ma-24, PC-3, PC-14, H1299 and PC-13 were maintained in RPMI 1640 (Sigma—Aldrich, St. Louis, MO). Media were supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) and cells were grown in 5% CO_2/95% air at 37 °C.

2.2. Real-time reverse-transcriptase polymerase chain reaction (RT-PCR) analysis

Total RNA from the cell lines was prepared as described previously [13]. Expression of ERBB family receptors, EGFR, ERBB2, ERBB3 and ERBB4, and their ligands, EGF, HRG-1, and TGF-α, was assessed by quantitative real-time RT-PCR analysis using a Quantitect Probe PCR kit (QIAGEN, Hilder, Germany) and ABI Prism 7900HT (Applied Biosystems, Foster City, CA) according to the supplier's protocols. Sets of TaqMan probe and primers were purchased from Applied Biosystems. A Pre-Developed TaqMan Assay Endogenous Control of a GAPDH kit (Applied Biosystems) was used for amplification of a GAPDH cDNA fragment. mRNA expression was expressed as a relative expression calculated by the ratio of the mRNA amount in a sample compared with that in a standard sample (cDNA from PC-9 for EGFR, ERBB2, ERBB3, EGF and TGF-α, and cDNA from PC-13 for ERBB4 and HRG-1) using *GAPDH* as an internal control.

2.3. Western blot analysis

The cells were grown to 80% confluence on the plate, washed with PBS, and cultured for an additional 24 h with serum-free medium. The cells were then treated with 100 ng/ml of recombinant human EGF (Sigma—Aldrich) for 10 min, and cell lysates were prepared in ice-cold RIPA lysis solution (10 mM Tris—HCl pH 7.5, 150 mM NaCl, 2 mM EDTA, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS). Debris was removed by centrifugation at 12,000 g for 5 min at 4 °C. Hundred micrograms of proteins were subjected to SDS-PAGE using 2—15% or 7.5% poly-acrylamide gels (Daiichi Pure Chemicals, Tokyo, Japan). Proteins were elecrotransferred to a Hybond-P PVDF membrane (Amersham-Pharmacia Biotech, Buckinghamshire, UK), and reacted with specific antibodies. Phospho-EGFR (Y1068), phospho-ERBB3 (Y1289), phospho-AKT (Ser473), phospho-p44/42 MAPK (Thr202/Thr204), and total p44/42 MAPK antibodies were obtained from Cell Signaling Technology (Beverly, MA). Phospho-STAT3 (Tyr705), as well as total EGFR, ERBB2, ERBB3, ERBB4, AKT, STAT3 antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Phospho-ERBB2 (Y1248) and α-tubulin antibodies were obtained from Upstate Biotechnology (Lake Placid, NY) and EMD Bioscience (San Diego, CA), respectively. All antibodies were used at a 1:1,000 dilution. Proteins were visualized by treatment with horseradish peroxidase-conjugated

Download English Version:

https://daneshyari.com/en/article/2144028

Download Persian Version:

https://daneshyari.com/article/2144028

Daneshyari.com