Change of the EGFR expression and downstream signal pathway in A549 cell treated with ZD1839

Xu, Chongrui; Guo, Ailin; Lin, Jiayin; Zhou, Qing; Wang, Zheng; Wang, Kun; Yang, Suqing; Wu, Yilong
Guangdong Provincial People’s Hospital, Guangzhou, China

Object: Discuss the change of the EGFR expression and downstream signal pathway in A549 cell treated with ZD1839.

Methods: The inhibition of the A549 cell treated with ZD1839 was measured by MTT assay and Real-Time PCR was used to evaluate the expression of EGFR and downstream signal pathway.

Results: A549 cell was inhibited by ZD1839 in vitro. After treated with ZD1839, the expression of EGFR was 1.10 fold compared with the cell without ZD1839 and Ras gene was 1.09 fold. The expression of MAPK was 52.1 percent of the cell without ZD1839, PI3K was 16.4 percent and Akt was 25.3 percent. ZD1839 didn’t affect the expression of EGFR and Ras in A549 cell but down regulated the expression of MAPK, PI3K and Akt.

Conclusion: ZD1839 inhibited A549 cell by inhibiting the tyrosine kinase activity of EGFR and the PI3K/Akt, MAPK signal pathway.

Expression and significance of RUNX3 in human lung cancer

Yanada, Masashi1 Shimada, Junichi1 Ito, Kazuhiro1 Terauchi, Kunihiko1 Shimomura, Masanori1 Yaoi, Takeshi1 Fushiki, Shinji1
1 Dept. of Cardiovascular and Thoracic Surgery, Kyoto Prefectural University of Medicine, Kyoto, Japan 2 Dept. of Pathology and Applied Neurobiology, Kyoto Prefectural University of Medicine, Kyoto, Japan

Background: Lung cancer is the leading cause of cancer related deaths throughout the world. A better understanding of the molecular pathogenesis of lung cancers is needed in order to achieve a preventive or therapeutic breakthrough for reducing the number of deaths. Runx-related transcription factor 3 (RUNX3) has recently been shown to be down-regulated in human cancer tissues, including lung cancer. However, the clinical value of that finding is largely unknown. We investigated the associations of RUNX3 expression in lung cancer tissues with clinical characteristics and tumor recurrence.

Materials and Methods: The expression of RUNX3 in lung cancer cell lines was examined using a quantitative real-time reverse transcriptase-polymerase chain reaction (RT-PCR) method. We also determined whether the expression of RUNX3 in the cell lines was associated with hemizygous deletion of the locus and methylation status in the exon 1. Further, we investigated the methylation status of RUNX3 using a methylation specific PCR (MSP) technique and studied hemizygous deletion using bicolor fluorescence in situ hybridization (FISH). In addition, primary lung tissues were obtained from Kyoto Prefectural University of Medicine, Japan. Among the 56 cases confirmed as primary lung carcinomas, 40 were adenocarcinomas, 15 were squamous cell carcinomas, and 1 case was large cell carcinoma. The intratumoral expression level of RUNX3 mRNA was determined and compared with that in adjacent non-tumor lung tissue using quantitative real-time RT-PCR in the 56 cases of non-small cell lung cancer. From those results, the relationship between the expression level of RUNX3 and clinicopathological factors was examined.

Results: RUNX3 gene expression was reduced or disappeared in all cell lines examined (P < 0.001). Eight of 15 lung cancer cell lines revealed methylated bands of RUNX3, whereas 7 showed unmethylated bands. Further, hemizygous deletion of RUNX3 was observed in 8 of the cell lines by bicolor FISH. RUNX3/GAPDH mRNA levels were significantly different between tumor tissues from the lung cancer specimens and adjacent non-malignant lung tissues (P < 0.001). No significant differences in RUNX3/GAPDH mRNA levels were found related to age, gender, lymph node metastasis or tumor recurrence in the non-small cell lung cancer cases.

Conclusions: The present clinical and experimental data suggest that the comprehensive study of RUNX3 using quantitative real-time RT-PCR, MSP, and FISH would be beneficial for understanding the pathogenetic mechanisms of human lung cancer at the molecular level.

Growth regulation via insulin-like growth factor binding protein-4 and -2 in lung epithelial cells and cancers

Yazawa, Takuya1 Sato, Hanako1 Shimoyamada, Hiroaki1 Okudela, Koji1 Kitamura, Hitoshi1
1 Dept. of Pathobiology, Yokohama City University Graduate School of Medicine, Yokohama, Japan 2 Dept. of Anatomy, St. Marianna University, Kawasaki, Japan

Gain-of-function point mutation in K-ras affects early events in pulmonary bronchioloalveolar carcinoma and continuous K-Ras activation accelerates growth of lung epithelial cells. We investigated altered mRNA expression on K-Ras activation in human lung epithelial cells and found that two kinds of growth-modulating molecules, insulin-like growth factor binding protein (IGFBP)-4 and -2 were significantly upregulated by mutated K-ras transgene. Lung cancer cell growth was also accelerated by mutated K-ras gene transduction, whereas IGFBP-4/-2 induction was weaker compared with mutated K-Ras-expressing lung epithelial cells. To understand the differences in IGFBP-4/-2 inducibility via K-Ras-activated signaling between nonneoplastic lung epithelia and lung carcinoma, we addressed the mechanisms of IGFBP-4/-2 transcriptional activation. Our results revealed that Egr-1, which is induced on activation of Ras-MAPK signaling, is crucial for transactivation of IGFBP-4/-2. Furthermore, IGFBP-4 and IGFBP-2 promoters were often hypermethylated in lung carcinoma, yielding low basal expression/weak induction of IGFBP-4/-2. These findings suggest that continuous K-Ras activation accelerates cell growth and evokes a feedback system through IGFBP-4/-2 to prevent excessive growth. Moreover, this growth regulation is disrupted in lung cancers because of promoter hypermethylation of IGFBP-4/-2 genes.
introduced into cancer cells, in which the gene is inactivated, to restore the cell’s normal gene functions. Such introduction of TSGs in the cancer cells generally induces apoptosis and/or cell cycle arrest. Gene therapy using various known and newly identified TSGs as therapeutic genes have reached clinical trials for treatment of several cancer types. However, as different types of cancers have different tumor suppressor deficiencies, the strategy must be customized to each type. Thus, the aim of this study is to identify and characterize novel TSG candidate(s) specific SCLC, which eventually can be tested for use in cancer gene therapy.

By a global gene expression analysis (Affymetrix) on a large panel of SCLC cell lines, corresponding tumor xenografts, and transformed mouse cell lines, we have identified four genes, whose expression is markedly downregulated in cancer and transformed mouse cell lines compared to normal cells and tissues. This was further verified by semi-quantitative RT-PCR. To determine whether they possess tumor suppressing activity we are currently investigating the effect of overexpression of each of these genes on cell proliferation, migration and cell cycle arrest by transiently transfecting selected SCLC and transformed mouse cell lines. If any demonstrate tumor suppressive activity, they will be chosen as potential candidates for the development of gene therapy for patients with SCLC. An update will be presented.

P2-155

BSTB: Tumor and Cell Biology Posters, Tue, Sept 4

STI-571 inhibits the growth of non-small cell lung cancer cell and intensifies the cisplatin effect in vitro

Zhou, Caicun; Su, Chunxia; Su, Bo; Zhao, Yingmin

Dept. Medical Oncology, Shanghai Pulmonary Hospital, Tongji University, Shanghai, China

Background: STI571 Gleevec, Imatinib(c) is the first cancer-related signal transduction inhibitor which selectively suppresses the activity of c-KIT, ABL and platelet-derived growth factor receptor(PDGFR).

Methods: We conducted MTT assay to explore the growth inhibitory effect of STI571 alone or with cisplatin on lung cancer cells, cell cycle analysis to confirm the cell distribution, immunocytochemistry to test the expression of PDGFRs, c-KIT in the non-small cell lung cancer cell lines immunohistochemistry to test the expression of PDGFRs and c-KIT.

Results: A549-resistant cell lines showed marked inhibition of cell growth after treatment with STI571 and combination with cisplatin, but not A549-sensitive cells. Cell cycle analysis by flow cytometry revealed that STI571 treatment increased the fraction of A549-resistant cells in S and G2/M phases, respectively, indicating induction of S and G2/M transition arrest. A549 cell lines highly express PDGFRs, A549-resistant cell lines also express c-KIT.

Conclusion: These results provide a potential therapeutic selection for non-small cell lung cancer. STI571 as a single agent or in combination with cisplatin is a potential new targeted strategy.