Correlation of STAT3, CEA in lung adenocarcinoma cell A549

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Received: 27 June 2012 / Revised: 17 July 2012 / Accepted: 25 August 2012
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Abstract Objective: The purpose of this study was to analyze the relationship between signal transducer and activator of transcription 3 (STAT3) and carcinoembryonic antigen (CEA) in lung adenocarcinoma cell A549, and to explore the value of STAT3 on early diagnosis of lung adenocarcinoma. Methods: The expression of CEA, STAT3 mRNA and its protein in human lung adenocarcinoma cell A549 and normal human lung cells MRC-5 were tested by immunohistochemistry staining (PV) and quantitative real time fluorescent PCR. The correlation between STAT3 and CEA in human lung adenocarcinoma cell A549 was analyzed. Results: The protein and mRNA levels of STAT3, CEA in lung adenocarcinoma cell A549 were apparently higher than those in normal human lung cells MRC-5. The levels of STAT3 mRNA and its protein were positively correlated with CEA in lung adenocarcinoma cell A549. Conclusion: STAT3 have the same value in diagnosis of lung adenocarcinoma.

Key words lung adenocarcinoma; signal transducer and activator of transcription 3 (STAT3); carcinoembryonic antigen (CEA)

Lung cancer is the most common malignant tumor, where non-small cell lung cancer (NSCLC) accounts for about 80% of all lung cancer cases [1]. In NSCLC, incidence of adenocarcinoma is occult, non-specific symptoms, more difficult to be done by biopsy and easy to transfer, so the early diagnosis is difficulty in clinical work. CEA as one of tumor markers which is more commonly used on early diagnosis of NSCLC and evaluation of treatment. STAT3 is a recognized oncogene, the study have shown that blocking of JAK1-STAT3 signal transduction pathway can inhibit the growth of lung cancer [2], but if STAT3 can become a new tumor marker for clinical to improve the early diagnosis of lung adenocarcinoma, there is no aspects of clinical and experimental reports on home and abroad. This experiment analyze the relationship between of STAT3 and CEA, so as to explore the value of STAT3 on the early diagnosis of lung adenocarcinoma, find a new and ideal tumor marker for the diagnosis of lung adenocarcinoma for the clinic use. It provides a theoretical basis and experimental evidence

Materials and methods

Materials
Human lung adenocarcinoma A549 cell line, normal human lung cells MRC-5 line from the Center Laboratory of Luzhou Medical College (China). Rabbit anti-STAT3 polyclonal antibody was purchased from the Bioworld technology Co. (USA), rabbit anti-human CEA polyclonal antibody was purchased from Beijing Biosynthesis Biotechnology Co. Ltd. (China), the two-step immunohistochemical detection kit PV-6001 was purchased from Beijing Zhongshan Jinqiao Biotechnology Co. Ltd. (China), total RNA extraction kit box purchase since Tiangen Company (China), 2008 BioBRK RT Kit was purchased from Chengdu Break Biotechnology Co. Ltd. (China), Total TaKaRa RNA PCR Kit (AMV) Ver.3.0 was purchased from Takara Co. Ltd. (Japan), STAT3, CEA, GAPDH primers were synthesized by Sanggon Biotech Co. Ltd. (China).

Methods

Peroxidase Immunochemistry
Sterile coverslips was placed in 6-wells plate, human lung adenocarcinoma cell A549 and normal human lung cell MRC-5 with cell density of $2 \times 10^4$/mL were seeded on glass respectively and cells climbed pieces, 5 days afterwards the specimens were washed with PBS, 4% paraformaldehyde for 20 min, 0.5% of Triton X-100 were incu-
bated for 20 min, and the remaining operation referred to the kit instructions. Image acquisition, mean optical density (MOD) was tested by IPP6.0 image analysis software. The average of the MOD of five fields of each slice were analyzed comparatively. MOD represented the staining intensity. It could reflect the relative concentration of the positive product of expression more accurately.

**Extraction of total cellular RNA**

Human lung adenocarcinoma cell A549 and normal human lung cell MRC-5 which integrated up to about 80% respectively replaced with fresh medium 24 h before the experiment. PBS washed cells, added 1 mL of lysis buffer Trizol to cells per 10 cm², beaten several times with a sampler, the rest of the operation referred to the kit instructions. Added 1 μL of total RNA in micro-nucleic acid quantitative instrument to read the RNA samples concentration and ratio of OD260/OD280, the ratio between 1.8–2.1 could be used for subsequent experiments. Ratio less than 1.8 indicated that the residual amount of protein was too large and the sample should be re-extracted once.

**Quantitative real time fluorescent PCR**

Accessed to mRNA sequences in Genbank and designed primers by ABI primer express 3.0 software. Primers were synthesized by Sanggon Biotech Co., Ltd. (China). The STAT3 primer sequences, upstream prime: 5′-CCT GAAGCTGACCGAGTAG-3′, down stream prime: 5′-TTC CAAACTGCAATGAAATC-3′, CEA primers sequences, upstream prime: 5′-GCACCTCA GACCAATCATC AACT-3′, down stream prime: 5′-CCACTTCTCAAGGGAC AAAATACAC-3′, GAPDH primer sequences, upstream prime: 5′-GGTCATGAGTCC TTCCACGATA-3′. Reverse-transcription referred to the kit instructions, cDNA was synthesized after the establishment of SYBR Green I quantitative PCR reaction system, 95°C for 2 min, 95°C denaturation 5 s, annealing at 59.5°C 20 s, extension at 72°C for 15 s, amplified 40 cycles. After the reaction, computer analyzed the results automatically.

**Results**

**The expression of STAT3, CEA protein in human lung adenocarcinoma cell A549 and normal human lung cells MRC-5**

Positive expression signal of STAT3 in human lung adenocarcinoma cell A549 were mainly located in cytoplasm, a few in the nucleus. Positive expression signal of CEA in A549 cells were mainly located in cytoplasm and (or) cell membrane. Weak levels of expression of STAT3, CEA protein could also be found in the cytoplasm in the normal human lung cells MRC-5 (Fig. 1). The protein levels of STAT3 and CEA in A549 were significantly higher than those in MRC-5 (P < 0.01; Table 1).

**Correlation of expression of STAT3 protein and CEA protein in human lung adenocarcinoma cell A549**

After Pearson correlation analysis, we found STAT3 protein were positively correlated with CEA protein in human lung adenocarcinoma cell A549 (r = 0.583, P = 0.007, P < 0.05).

**Quantitative real time fluorescent PCR and its product identification**

The melting curve showed each gene had a single product peak (Fig. 2), no non-specific products and primer-dimers, it indicated that the specificity of the primers, the experimental results could accurately reflect initial concentration of the samples.

**Quantitative analysis of the target gene mRNA**

A certain amount of template of cDNA PCR products were prepared, diluted from 10⁴ to 10⁶ respectively, a concentration gradient of 7, and then take 2 μL of dH₂O as a zero tube. Quantitative real time fluorescent PCR amplified and established a standard curve. Amplification curve was a typical S-shaped, 10-fold serial dilutions of the standard PCR products of the Ct value as vertical axis (Y), natural logarithm of the standard PCR product concentration as the abscissa (X), it got a strict linear standard curve. Bio-Rad IQ5 analysis software analyzed and calculated standard curve (Fig. 3, Table 2). Amplification efficiency of the target gene and reference gene were similar, it ensured the accuracy and efficiency of the method.

**The expression of STAT3 mRNA and CEA mRNA in human lung adenocarcinoma cell A549 and normal human lung cells MRC-5**

The levels of STAT3 mRNA and CEA mRNA expression in human lung adenocarcinoma cell A549 were significantly higher than those in normal human lung cells MRC-5 (P < 0.01; Table 3). After Pearson correlation analysis, we found STAT3 mRNA were positively corre-