EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND BASIC FIBROBLAST GROWTH FACTOR IN HUMAN NON-SMALL CELL LUNG CANCER AND THEIR CLINICAL SIGNIFICANCE

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ABSTRACT

Objective: To explore the expression of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) in non-small cell lung cancer (NSCLC) and their clinical significance.

Methods: The expression of VEGF and bFGF was examined at the protein levels by immunohistochemical staining in 96 NSCLC patients, and in 36 of which at the mRNA levels by reverse transcription-PCR analysis.

Results: VEGF mRNAs were expressed predominately as its secretory forms (VEGF121 and VEGF165) in NSCLC tissues. The positive ratios of VEGF121 and VEGF165 were 69.5% (25 of 36) and 41.7% (15 of 36) respectively. The positive ratio of bFGF was 52.8% (19 of 36) in the same tumor specimens. The positive ratios of VEGF and bFGF at protein levels were 55.55% (20 of 36) and 58.33% (21 of 36) respectively. A significant positive correlation was observed between VEGF and bFGF expression in NSCLC tissues (P=0.002). No significant interrelationship was observed between VEGF and bFGF expression and clinical data (age, sex, histological subtype differentiation, p-stage, metastasis and survival) was found. Conclusions: VEGF and bFGF may play an important role in angiogenesis and act in a synergistic manner in NSCLC.

Key word: Non-small cell lung cancer, Vascular endothelial growth factor (VEGF), Basic fibroblast growth factor (bFGF)

Materials and Methods

Patients and Tumor Samples

Specimens from 96 patients (59 males and 37 females) with non-small cell lung cancer untreated with chemotherapy were obtained from biopsies of metastatic mass outside the thorax or primary tumors from surgery. The tissues were immediately frozen...
and stored in liquid nitrogen for use. The median age of the patients was 58.6 years (range 29-78). All the specimens were processed routinely for histologic examination. The histologic classification of the carcinomas was based on the WHO criteria (WHO, 1982) and comprised 40 squamous cell carcinomas, 45 adenocarcinomas, 7 aden-squamous mistype carcinomas and 4 large cell carcinomas. The TNM (Tumor-node-metastasis) stage was: 6 in stage I cases, 17 cases in stage II, in stage IIIa 25 cases, 20 cases in stage IIIb and 28 cases in stage IV. The median follow-up duration was 18 months. The survival times were determined from the day of surgery or chemotherapy.

RNA Extraction and RT-PCR

All samples were frozen in liquid nitrogen or stored at -80°C prior to use. 50mg to 100mg of each resected NSCLC tissue in 36 cases were used for total RNA isolation using TRLzol reagent (Life Technologies, Inc.), according to the manufacturers instructions. First-strand cDNA was synthesized by priming 1ug of total RNA with oligo(dT)-Adaptor primer in a 20ul reverse transcription mixture containing 4ul of 25mmol MgCl2; 2ul of 10×RNA PCR buffer; 2ul of dNTP mix containing 10mmol each deoxynucleotid triphosphate base; 20U of RNase inhibitor and 1ul of AMV Reverse transcriptase (5U) (TakaRa RNA PCR kit, TaKaRa Biotech). RT mixture was reverse-transcribed at 42°C for 60min, then the cDNA was incubated at 99°C for 5min to inactivate the reverse transcription and stored at -20°C.

The polymerase chain reaction amplification was performed using the synthesized cDNA as template DNA. Each amplification mixture (20ul) contained 2ul of 10×PCR buffer; 1.6ul of 25mmol/L MgCl2; 0.6ul of 10mmol/L each sense and antisense primer; 2ul of RT products; 2ul of 10mmol/L dNTPs; 0.5ul of Taq DNA polymerase (TakaRa RNA PCR kit, TaKaRa Biotech) and 10.7ul of ddH2O. Amplification was performed in a thermal cycler (Sigma CO, USA) under the following condition: predenaturation at 95°C for 5min, then 1min at 94°C, 1.5min(last 2 cycles, 2min) at 58°C, and 2min(last 2 cycles, 5min) at 72°C for VEGF; 40 seconds(last 3 cycles, 1.5min) at 94°C, 1.3min(last 3 cycles, 2min) at 48°C and 1.2min(last 3 cycles, 2min) at 72°C for bFGF; 30 seconds(last 3 cycles, 1.5min) at 94°C, 1min at 55 and 1min at 72°C for β-actin. The final cycle was followed with an extension at 72°C for 10min. The cycle number were 32 rounds(VEGF), 28 rounds(bFGF) and 30 rounds(β-actin) respectively. The PCR products were electrophoresed on 2.0% agarose gel and visualized with ethidium bromide under UV light. RT-PCR of β-actin was used as a internal control for integrity of mRNA and for the polymerase reaction.

Immunohistochemistry

Monoclonal antibody against VEGF(1:50) and polyclonal antibody against bFGF(1:100) were the products of Santa Cruz Company. The samples were stained by SP method. Negative controls were carried out by substituting the primary antibody with PBS. The evaluation of VEGF and bFGF expression was established according to both staining intensity and percentage of positive cell[3].

Statistical Analysis

The X² and Fisher’s exact probability tests were used to evaluate the significant association of VEGF, bFGF expression and clinicopathological parameters. Survival curves were obtained by the Kaplan-Meier method, and the differences between the curves were measured using the log-rank test. Multivariate analysis was performed using COX proportional hazard model. The criterion for statistical significance was P<0.05. Statistical analyses were performed using the SPSS software (SPSS, Inc, Chicago, IL).

RESULTS

Expression of VEGF and bFGF in NSCLC

Two kinds of amplified product, VEGF121 (408bp) and VEGF165(541bp) were detected in NSCLC samples by the RT-PCR analysis (Figure 1), and the positive rates of VEGF121 and VEGF165 mRNA were 69.5%(25of 36) and 41.7%(15 of 36) respectively. The positive rate of VEGF121 and VEGF165 mRNA were 70.6%(12 of 17) and 52.9%(9 of 17) in adenocarcinoma; 50%(8 of 16) and 31.3%(5 of 16) in squamous cell carcinoma and 100.0%(3 of 3) and 33.3%(1 of 3) in adeno-squamous mixed carcinoma according to the pathological type. The immunohistochemical results showed that the expressions of VEGF and bFGF protein were mainly identified in the cytoplasm of cancer cells (Figure 3, 4). The positive ratios of VEGF and bFGF protein expression were 55.6%(20 of 36) and 58.3%(21 of 36), respectively. In nine