Differential expression of βig-h3 in human hepatoma cell lines*

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【Abstract】 **Objective**: To observe the differential expression of TGF-β-induced gene human clone 3 (βig-h3) in human hepatoma cell lines. **Methods**: Human hepatoma cells HHCC, 7721, T7721 constructed by stably transfecting HAb18G/CD147 cDNA into 7721 and normal human liver cell QZG were cultured as previously. RT-PCR and western blot were used to investigate the differential expression of βig-h3 in human hepatoma cell lines and normal liver cell. **Results**: The results of RT-PCR suggested that the expression of βig-h3 mRNA in human hepatoma cells was higher than that in normal human liver cell QZG ($P<0.01$), and its expression level in human hepatoma cells in turn was HHCC>T7721>7721. Moreover, the similar results of βig-h3 protein expression were testified by western blot. **Conclusion**: This study demonstrates that the expression of βig-h3 in hepatoma cell lines is higher than that in normal liver cell QZG, which provides a sound basis for exploring the function of βig-h3 in processes of adhesion and metastasis of human hepatoma cells.

【Key words】 βig-h3; hepatoma; HAb18G/CD147; adhesion

TGF-β-induced gene human clone 3 (βig-h3) was initially identified by differential screening of a cDNA library produced from A549 human lung adenocarcinoma cells treated with TGF-β1. It encoded a 68 ku secretory protein that contained an amino terminal signal peptide, four internal FASI repeat domains and an RGD (Arg-Gly-Asp) motif, which was a recognition sequence for some integrins[1]. Further functional analysis had revealed that βig-h3 was secreted into extracellular matrix (ECM) and might act as an extracellular attachment protein, involving in cell growth, wound healing and morphogenesis, tumorigenesis[2].

Our previously study showed that the expression of βig-h3 mRNA in human hepatoma cell T7721 was up-regulated significantly, 4.4-fold as that in 7721 by microarray. However, the expression of TGF-β1 was not changed obviously, suggesting that it was HAb18G/CD147 that stimulated the up-regulation of βig-h3 mRNA in T7721. This study detected the expression of βig-h3 in human hepatoma cell lines T7721, 7721, HHCC and normal human liver cell QZG in order to investigate the mechanism of βig-h3 in the adhesive and metastatic process of hepatoma cells.

MATERIALS AND METHODS

Materials RPMI1640, fetal bovine serum (FBS), Geneticin (G418 sulfate) were purchased from Gibco BRL, Life Technologies (Gaithersburg, Mass.). TRIZOL for total RNA isolation was from Gibco BRL, Life Technologies. First Strand cDNA Synthesis kit-ReverTra Ace-a-™ was from TOYOBO CO., LTD. PCR amplification kit was purchased from TaKaRa Biotechnology (Dalian) CO., LTD. Affinity purified goat polyclonal antibody against a peptide mapping near the carboxy terminus of βig-h3 of human origin was obtained from Santa Cruz Biotechnology, Inc. And HRP-conjugated affinity purified anti-goat IgG (rabbit) was purchased from Rockland, Inc. Other reagents were gained from Sigma (St. Louis, Mo.). The human hepatoma cell lines 7721, HHCC and the human normal liver cell line

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QZG was obtained from the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. T7721 which was stably transfected with HAb18G/CD147 cDNA was constructed and conserved by our laboratory.

Cell culture Human hepatoma cells HHCC, 7721, T7721 and normal liver cell QZG were cultured with RPMI1640 medium supplemented with 10% FBS, 1% penicillin/streptomycin, and 2% L-glutamin at 37℃ in a humidified atmosphere of 5% CO_2. G418 was added into the medium for T7721 cells at the final concentration of 400 μg/ml.

RT-PCR Human βig-h3 cDNA sequences were identified from human genomic sequence in GenBank. Primers were designed by Primer 3.0 software and synthesized by Shanghai Bioasia Technology CO., LTD. The forward primer was 5′-CAT TGA GAA CAG CTG CAT CG-3′, the reverse primer was 5′-AGT CTG CTC CGT TCT CTT GG-3′, and the size of PCR product was 255 bp. β-actin served as internal reference housekeeping gene to assess the status of sample mRNA. The forward and reverse primers for β-actin were 5′-CCA AGG CCA ACC GCG AGA AGA TGA C-3′and 5′-AGG GTA CAT GGT GGT GCC GCC AGA C-3′, with the size of PCR product being 592 bp.

Total RNA was isolated from HHCC, 7721, T7721 and QZG cells using TRIZOL Reagent according to the manufacture's instructions. RNA concentration was determined spectrophotometrically at 260 nm by Lambda 25 UV/vis Spectrometer (Perkin Elmer, Inc.) followed by resolving RNA (2 μg) on 1% agarose/formaldehyde gels. Then the total RNA was reverse transcribed into the first strand cDNA using First Strand cDNA Synthesis kit-ReverTra Ace-a-™. Amplification of cDNA templates was performed in a 50 μl reaction volume consisting of 10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 200 μmol/L deoxynucleotide triphosphates, 0.2 μmol/L forward and reverse primers, 1 μl cDNA template from the reverse transcribed-product, and 1U TaKaRa Taq™ DNA polymerase with the following cycling parameters: (1) initial denaturation, 94℃, 3 min; (2) denaturation, 94℃, 45 s; annealing, 57℃, 45 s; extension, 72℃, 45 s (32 cycles); (3) final extension, 72℃, 10 min. The PCR products were resolved on 1% agarose gel electrophoresis and analyzed by image analysis system.

Western blot Western blot was performed as described previously[3]. Briefly, human hepatoma cells HHCC, 7721, T7721 and normal human liver cell QZG were suspended in serum-free medium at density of 5×10^5/ml, and 2 ml of the cell suspension was seeded into 6-well plate, respectively. After cultured for 48 h, the conditioned medium was collected and the protein was concentrated using Amicon® Ultra-4 centrifugal filter devices (Millipore) because of the low basal expression level of βig-h3. BCA Protein Assay Kit (Pierce Biotechnology, Inc.) was employed to determine the total protein density, and equal amounts of proteins were separated on 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE), electransferred to polyvinylidene fluoride (PVDF) microporous membrane (Millipore). After blocked with 5% non-fat milk, the membrane was incubated for 2 h at room temperature in the presence of polyclonal goat anti-human βig-h3 (1 ∶ 500) in PBS followed by incubation with monoclonal HRP-conjugated rabbit anti-goat IgG (1 ∶ 10 000) for 1 h at room temperature. The membrane was developed by using enhanced chemiluminescence (ECL; Amersham Biosciences).

Statistical analysis Data were expressed as *mean* ± *SD*. Comparison between groups were done by one-way ANOVA.

RESULTS

Quality of total RNA There were clear bands of total RNA by agarose gel electrophoresis, the ratio of 28S to 18S was 1 ∶ 1 or more (Fig 1).

Results of RT-PCR The results of RT-PCR showed that there were special fragments in T7721, 7721, HHCC and QZG (Fig 2). Through semi-quantitative analysis, the standard ratios of βig-h3 mRNA/β-actin mRNA were shown in the