Effect of Exogenous Expression of VEGF165b on the Invasiveness of Human Bladder Cancer T24 Cells

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Keywords: Bladder neoplasms, Vascular endothelial growth factor 165b, Tumor invasion.

Abstract. Objective To observe the effects of exogenous expression of VEGF165b on the viability, migration rate and invasion ability of human bladder cancer T24 cells, and to explore the influence of VEGF165b in the biological constructed. The T24 cells were divided into T24 cells group (control), pcDNA3.0 group (transfected by pcDNA3.0 plasmid) and pcDNA-VEGF165b group (transfected by pcDNA-VEGF165b plasmid). The viability of T24 cell was detected by MTT assay; Western blotting method was used to determine the expressions of VEGF165b, matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) proteins. Transwell assay was used to detect the rate of cell migration and invasive ability. Results Compared with T24 cells group, the expression levels of VEGF165b, MMP-2 and MMP-9 proteins in the T24 cell in pcDNA3.0 group had no significant difference (P>0.05). Compared with T24 cells group or pcDNA3.0 group, the expression level of VEGF165b protein in the T24 cells in pcDNA-VEGF165b group was significantly increased (P<0.05). The cell viabilities of T24 cells had no significant difference between three groups (P>0.05). Compared with T24 cells group (migration rate 100%), the migration rate of the T24 cells in pcDNA3.0 group (97.2±7.8%) had no significant difference (P>0.05), but the migration rate in pcDNA-VEGF165b group was decreased significantly (63.5±10.4%) (P<0.05). Compared with T24 cells group (70.8±8.5), the number of transferred T24 cells in pcDNA3.0 GROUP (66.3±11.2) had no significant difference (P>0.05), but in pcDNA-VEGF165b group (23.5±7.3) it was decreased significantly (P<0.05). Conclusion Exogenous expression of VEGF165b in the T24 cells has no significant effect on the proliferation of T24 cells, but it can reduce the migration and invasion abilities significantly.
Introduction

Bladder transitional cell carcinoma is the most common urogenital malignancy in China. Most patients need close follow-up and observation after operation for many years. Bladder transitional cell carcinoma, including almost all the tumors had vascular endothelial growth factor (vascular endothelial growth factor, VEGF) increased the expression level of mRNA of confirmed that the angiogenesis and the expression of VEGF in bladder transitional cell cancer occurrence, development and recurrence plays an important role\cite{1}. VEGF because of different mRNA splicing can form multiple isoforms, one of the most important VEGF165 through autocrine manner to stimulate tumor cells progress to the malignant phenotype of VEGF mutant VEGF165b and the newly discovered VEGF165 could antagonize, with anti angiogenesis and tumor suppression function\cite{2}, so VEGF165b as a potential the anti-tumor therapeutic target of attention, but there is no about the effects of VEGF165b on migration and invasion potential of bladder cancer cell research reports. In this study, human bladder cancer T24 cells as a model, using molecular cloning and transfection technique, the effect of exogenous expression of VEGF165b on T24 cell survival rate, migration rate and invasiveness, explore its mechanism, to provide a new theoretical basis and experimental basis for the clinical treatment of bladder cancer.

Materials and Methods

Main reagents
Fetal bovine serum RPMI 1640 medium was purchased from American Gibco company; PCR primers were synthesized by Takara Biotechnology (Dalian) Co., Ltd; DNAmarker, AMV reverse transcriptase, restriction endonuclease and Plasmid Extraction Kit was purchased from Takara Biotechnology (Dalian) Co., Ltd.; 2 x Taqmaster was purchased from Tiangen biochemical Technology (Beijing) Co., Ltd., 2000; TRIzol reagent liposome was purchased from American Invitrogen company; antibody was purchased from Abcam company; two, anti Marker protein was purchased from American Thermo company; Matrigel and Transwell chamber (diameter 8 m) was purchased from BD company

Cell line and Culture Method.
Human bladder cancer T24 cells were purchased from the Shanghai cell of the Chinese Academy of Sciences. T24 cell culture was carried out in RPMI 1640 culture medium containing 10% fetal bovine serum (37°C, 5%CO₂, 0.25% trypsin).

Construction of VEGF165b expression vector, the total RNA of T24 cells was extracted. According to the literature primers were designed to clone the full-length VEGF165b, connected to the expression vector pcDNA3.0 (Invitrogen), sequencing, VEGF165b expression vector was successfully constructed (referred to as PcDNAVEGF165b)\cite{3}.

Cell transfection, the growth and fusion rate of T24 cells reached 90%, and pcDNA VEGF165b was transfected into T24 cells according to liposome 2000 instructions. 48h collection of cell reserve after transfection.

Determination of cell survival rate, the growth and fusion rate of T24 cells reached 90%, and pcDNA VEGF165b was transfected into T24 cells according to liposome 2000 instructions. After transfection, 48h was collected and the cells were collected. The survival
rate of 1.5 cells was measured by MTT method. The logarithmic growth phase cells were seeded on 96 Kong Banzhong (5 x 103 holes / 48h). After routine culture, the cells were transfected with pcDNA3.0 and pcDNA VEGF165b72h. The T24 cells in the control group were not treated. Before the end of the experiment, 4H was added to MTT solution (5g·L⁻¹) 10 μL to cultivate 4H before the end of the experiment. Discard the culture medium, each hole joined DMSO150 L, room temperature oscillation 10min; with automatic ELISA (570nm) determination of absorbance (A) value of each hole, and calculate the cell survival rate. Cell survival rate = A value of experimental group / control group A value * 100%.

The level of cells were collected after centrifugation and added RIPA buffer 4 °C pre cooling of the expression of VEGF165b, MMP 2 and MMP 9 protein in T24 cells was detected in western blotting method (containing protease inhibitor) 500μL, total protein extraction, protein concentration was determined by Bioford method. Total protein samples 40μg polyacrylamide gel electrophoresis after transfer membrane, PVDF membrane after removing the closure, an anti 4 DEG C overnight incubation, two anti incubated at room temperature for 1 ~ 2H, DAB color film, Tanon and 1600figuregel camera records; imaging system analysis with A value, the ratio of said to the gene and reference gene A.

Cell mobility and Invasiveness test, The T24 cells were added in Transwell cells (diameter 8μm, 1 x 10⁶ cells), cells were stained with crystal violet 0.1% 24h after culture, 10min was extracted with 10% acetic acid, with automatic ELISA (600nm) determination of A value calculation, cell migration rate. Matrigel (3.6g·L⁻¹) coated Transwell chambers (diameter 8 m), T24 cells were added in Transwell cells (1 x 10⁵ cells), cells were stained with 0.1% crystal violet culture 48h. Under light microscope, 8 non repeated fields were taken under 200 times. Counting the number of penetrating cells in the bottom membrane of each Transwell field through each field of view, and the invasive ability of cells in every 200 fold field of view.

Statistical analysis, Statistical analysis was carried out by SPSS13.0 statistical analysis software. The cell survival rate, the mobility, the number of membrane cells and the protein expression level were all expressed in $\bar{x} \pm s$, and the single factor analysis of variance was used in the group.

Results

VEGF165b T24 cells expression construct VEGF165b expression vector was transfected into pcDNA VEGF165b (pcDNA - VEGF165b), by double enzyme digestion confirmed the expression vector was constructed successfully (Figure 2).

Westernblotting results: T24 cells only showed low expression of VEGF165b, Transfection of pcDNA3.0 expression vector of T24 cells had no obvious effect on the expression level of VEGF165b (P > 0.05), and pcDNA VEGF165b72h after transfection, expression of T24 cells VEGF165b protein levels were significantly increased, compared with the T24 group was significant (P < 0.05)

The survival rate of T24 cells in each group, T24 cell group, pcDNA3.0 group and pcDNA VEGF165b group cell survival rate (100%, 97.2% ± 5.3% and 102.4% ± 2.1%) 22 showed no significant difference (P >0.05).
The mobility and invasiveness of T24 cells in each group. With the T24 cell group (100%) compared with pcDNA3.0 group, the cell migration rate (97.2% ± 7.8%) no significant changes (P >0.05), and PcDNAVEGF165b group cell migration rate (63.5% ± 10.4%) was significantly lower (P<0.05). Cell invasion (each 200 times from the perspective of transmembrane cell number) results: T24 cells and cells (70.8±8.5) compared with pcDNA3.0 group, cell penetrating cell number (66.3±11.2) there was no statistically significant difference (P >0.05), The number of cell transmembrane cells in pcDNA VEGF165b group (23.5 ± 7.3) was significantly decreased (P<0.05).

The expression level of MMP 2 and MMP 9 protein in T24 cells of each group was Westernblotting.

Results: T24 cells in all groups expressed MMP 2 and MMP 9. Compared with T24 cells group, pcDNA3.0 cells of MMP - 2 and MMP - 9 protein expression levels were not significantly changed (P >0.05), and pcDNA VEGF165b group 72h cells MMP - 2 and MMP - 9 protein expression levels were decreased (P<0.05).

Discussion

In a variety of tumors, VEGF expression can be used as an independent prognostic indicator for survival and recurrence of patients[4]. After the transcription of the VEGF gene, 2 types of angiogenic and antiangiogenic subtypes are formed due to the different mRNA shear forms. In the subtype of angiogenesis, VEGF165 and VEGF121 play a major role [5]. Vascular endothelial factor receptor (VEGFR) protein is widely expressed in many kinds of human tumors, including bladder cancer, glioma, lung cancer and prostate cancer, and plays a key role in tumor cell migration and invasion[6]. VEGF165 binds with VEGFR to activate its downstream signaling pathway and stimulate cancer cells to progress towards malignant phenotype. As an inhibitor of tumor angiogenesis, VEGF165b can antagonize VEGF165 and bind to VEGFR, resulting in inhibition of downstream signal transduction pathway [7]. So far, there is no report about the effects of VEGF165b on migration and invasion of bladder cancer cells, so the author of this paper for the exogenous expression of VEGF165b on T24 cell survival, migration rate and invasiveness were studied.

Study[8] showed that the expression of VEGF165b in different tissues was different, the expression of VEGF165b was found in the bladder tissue. VEGF165b is widely distributed in many kinds of tumor tissues, and is also likely to play a key role in the occurrence and development of bladder transitional cell carcinoma. The results of this study showed that T24 cells only low expression of VEGF165b, this study constructed PcDNAVEGF165b expression vector and transfected into T24 cells. The expression of VEGF165b protein significantly increased expression of VEGF165b inhibited the migration and invasion of T24 cells. At the same time, the results of this study show that: the expression of VEGF165b increased the survival rate of T24 cells had no obvious effect, the possible reasons were that in T24 cells VEGFR and its downstream signal pathway on cell proliferation does not play a major role. Matrix metalloproteinases (MMPs) are highly homologous to the zinc dependent endopeptidases that degrade the extracellular matrix and basement membrane, promote tumor
invasion. Studies have confirmed: there is a positive feedback regulation of VEGF and MMP 9, in a variety of tumors found in the study of mutual regulation relationship between VEGF and MMPs, and is thought to play an important role of VEGF MMP signaling pathway in tumor growth, invasion and angiogenesis. The results of this study showed that the expression level of VEGF165b increased inhibited the expression of MMP-2 and MMP-9, MMP-2 and MMP-9 that may play a key role in inhibiting T24 cell invasiveness in VEGF165b. However, further studies are needed to elucidate the signaling pathways that connect MMP 2, MMP - 9 and VEGF165b. In conclusion, VEGF165b can inhibit the migration and invasion of T24 cells, down regulate the expression of MMP-2 and MMP-9 is one of the important reasons that VEGF165b can be applied to the research of anti-tumor therapy in bladder cancer.

Acknowledgement

This study was the outcome of the “establishment and experimental study of the mouse deep vein thrombosis model” of Jilin University.

References


