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Effects of TRPC6 on invasibility of low-differentiated prostate cancer cells

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ABSTRACT

Objective: To study the expression of TRPC6 among prostate cancer cells, establish high expression cell lines of TRPC6, and to provide potential cell mode for prostate cancer oncogenesis and development. **Methods:** Occurrence and development of prostate cancer cells, PC3, PC-3 m DU145, 22 rv1, LNCaP and normal prostate epithelial cells in the PrEC TRPC6 expression level were detected by QPCR method. Calcium phosphate transfection method was used to package retrovirus pLEGFP-N1-TRPC6 and pLEGFP-N1-vector and infect the prostate cancer cells, a stable high expression of TRPC6 prostate cancer cells. Sable cell lines of TRPC6, matrix metalloproteinase (MMP) 2, MMP9 expression was detected by QPCR and Western blot. Change of cell invasion ability was detected by Transwell. **Results:** The expression level of prostate cancer cells TRPC6 were higher than control group PrEC cells. Among TPRC6 the expression of cell line PC 3 transfer potential wre the lowest, and high transfer cell line PC-3M express was the highest. Real-time fluorescent quantitative PCR and western blot results showed that after filter, the seventh generation of cell TRPC6 protein and mRNA expression levels were higher than the control group obviously. Transwell experimental results showed that the overexpression of TRPC6 could promote the invasion ability of PC3 prostate cancer cells. **Conclusions:** TRPC6 expressed in prostate cancer cells is in disorder, and its action may be associated with the invasion and metastasis of prostate cancer cells; successful establishment of stable high expression of TRPC6 prostate cancer cells primarily confirm the invasion-trigger ability of TRPC6 on prostate cancer, and lay down the foundation for exploring the TRPC6's role in the occurrence and development of prostate cancer mechanism

1. Introduction

Prostate cancer (Pca) is one of most common malignant tumor for males, with the second highest mortality rate to lung cancer in the western countries. Pca is a kind of double stage cancer, with slow progress rate to detect in the early stage. The curative effect of combining treatment using surgery, radiation therapy and hormone is distinct[1-3]. But after a period of time after early treatment, Pca is mostly turn into a progress quickly, invasive ability strong AIPC independent on male hormones. proliferation There are studies showing some molecular targets play a role in the treatment of castration Pca (CRPC)[4]. Denosumab et al found that RANKL antibody can hobble CRPC bone metastases[5]; M Bjorkman *et al* found the high expression of PHF8 in Pca is associated with the transfer of the tumor[6]. van der Horst et al proved that the peptide hormones alpha v integrin antagonist GLPG0187 can inhibit the bone metastases of Pca *in vivo* and *in vitro*[7]. In the Pca metastasis, most of them are bone metastases[8]. TRPC1 and TRPC6 are important moleculars of TRP family regulate calcium ion channel. Fabian et al suggested that TRPC1 using calcium regulating cell migration, concluded that TRPC1 and matrix metalloproteinases (MMPs) should have close relationship in the process of tumor metastasis[9]. In the research, TRPC6 shows increased expression in high transfer Pca cell lines and decreased expression in low transfer cell lines. The effects of TRPC6 on Pca expression disorders, development and transfer capability have not been studied. We will explore the induction role of TRPC6 in increased Pca cells,

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and the correlation between TRPC6 and MMP2, MMP9 and TRPC6 regarding the influence on the Pca cells transfer for providing a new basis for the clinical treatment of Pca.

2. Materials and methods

2.1. Cells and plasmid

PrEC prostate epithelial cells were from institute of Chinese academy of medical sciences tumor cell bank tumor hospital, PC-3 M, PC3, DU145, 22 rv1, LNCaP were from ATCC cell bank, 293FT, pLEGFP-N1-TRPC6 and pLEGFP-N1-vector) retroviral packaging cells systems presented in Guangzhou Cardiac Tumor Research Institute.

2.2. Reagent

Fetal bovine serum, RPMI-1640 medium, Transwell culture plate for GIBCO products; Matrigel glue from BD company; G418 were from Sigma; TRPC6, MMP2 and MMP9 primary antibody, and secondary antibody of sheep were purchased from Abcam company; Real-time fluorescent quantitative PCR kit for self restoring genes company; Other experiments conventional reagents and consumables were from Guangzhou Langri biological technology co., LTD.

2.3. Establishment of stable cell line PC3

Virus packaging was performed by calcium phosphate transfection method, retrovirus plasmids PIK were packed respectively with ppLEGFP-N1-TRPC6, pLEGFP-N1-TRPC6-vector and the PIKs plasmid to the packaging cells of 293FT. Five hours after transfection the medium was refreshed, poison was collected 24 h after transfection. It was filtered using 0.45 μm filter and saved at -80 ℃. It was inoculated with infected cells PC3 cells, and when the fusion rate reached 70%, virus liquid was added for infection with polybrene at final concentration of 8 μg/mL. After 48 h, cells underwent passage. After adherence, they were cultured in culture medium (Sigma) containing 0.5 μg/mL G418. GFP intensity of the seventh generation of cells was observed under fluorescence microscope.

2.4. Transwell invasion experiment

Matrigel glue was dissolved overnight at 4 ℃, and was added to serum-free medium RPMI1640 1:9 to dilute Matrigel. One hundred μL diluted Matrigel was placed evenly into the bottom of the Transwell cell, and then was placed in incubator until fully cohesion; cells at logarithmic growth phase were digested and collected, washed by 1×PBS, resuspended in serum-free medium RPMI1640, and then incubated in Transwell cell at 1.0×10^4. Every pore was 200 μL, and 500 μL RPMI1640 culture medium containing 10% FBS was added as chemotactic fluid invasion; after 24 h Transwell cell was taken out, and residual Matrigel was wiped out by cotton swab. After washed by 1×PBS, the cell was fixed in methanol for 15 min, stained by hematoxylin after cleaning for 3 min. Transmembrane cells were counted under microscopy for 10 fields, and the average was calculated. The difference was analyzed by using SPSS 17.0 statistical software between the two groups of cells, $P < 0.05$ was regarded as statistically significance.

2.5. TRPC6, MMP2, MMP9 mRNA expression after infection PC3 using QPCR

Trizol was used to produce the cells lysis in logarithmic growth phase. Total RNA was extracted with chloroform, the concentration was determined by ultraviolet spectrophotometer, then they were saved at - 20 ℃. With beta Actin as the reference, expression of MMP9 and MMP2 in different cell lines was detected. Primer sequences were as Table 1.

2.6. Detection of TRPC6, MMP2, MMP9 expression using Western blot

Cells in log phase were lysized, protein concentration was determined by BCA protein concentration kit. Total 20 μg protein was determined by 9% SDS-PAGE gel, 70 eV for 4 h, followed by transmembrane, blocking and antibody incubation. It underwent horseradish peroxidase-ECL method and X-ray film exposure, to determine TRPC6, MMP2, MMP9 protein expression levels.

2.7. Statistical analysis

All statistical data were analyzed using SPSS18.0 for Windows (IBM, standard verision 18.0). Groups data were expressed by mean±SD. One-way analysis of variance was used to analyze difference among several samples. $P < 0.05$ (bilateral inspection) was regarded as statistical significance.

3. Results

3.1. Expression of TRPC6 protein and mRNA in Pca cell lines using QPCR and Western blot

QPCR and western blot showed that protein and mRNA

Table 1
Primer sequences.

Gene	Forward primer	Reverse primer
MMP2	5′CTGAGACCGCCATGTCCACT 3′	5′GCCACGAGGAACAGGCTGTA 3′
MMP9	5′ATCCGGCACCTCTATGGTCCTC 3′	5′GCACAGTAGTGGCCGTAGAAGG 3′
TRPC6	5′TCTGTGCCACAGATGTGAAG 3′	5′AGCGTGTGAGGCGGTAGTAG 3′
β-Actin	5′TGGCACCCAGCACAATGAA-3′	5′CTAAGTCATAGTCCGCCTAGAAGCA-3′