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Original article

Numb had anti-tumor effects in prostatic cancer

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ABSTRACT

Aim: The aim of this study was to explain the Numb anti-cancer effects in the prostatic cancer.
Methods: Collecting the 20 prostatic cancer patients and analyzing the correlation between Numb and Glease score. Transfection Numb into DU-145 and PC-3 cells, measuring the proliferation rate of difference groups by MTT assay, evaluating the cell apoptosis and cell cycle of difference group by Flow cytometry; measuring the invasion and migration abilities by transwell and wound healing assays. In the nude mice experiment, establish prostatic cancer nude mouse subcutaneous planting tumor model by DU-145 cells, Injection the Numb from tail vein. Evaluating the tumor volume and weight.
Results: The Numb protein expression was decreased with Glease score increasing. The proliferation rate of Numb groups were significantly decreased compared with NC groups ($P < 0.05$, *respectively*). The apoptosis and G1 phase rates of Numb groups were significantly enhanced compared with NC groups ($P < 0.05$, *respectively*). The invasion and migration abilities of Numb group cells were significantly weaken compared with NC groups ($P < 0.05$, *respectively*). In the WB assay, The relative proteins (Numb, P53, Cyclin D1, Rac1, MMP-2 and MMP-9) expression were significantly differences between NC and Numb groups ($P < 0.05$, *respectively*). In the vivo experiment, the tumor volume and weight of Numb group was significantly lighter than NC group ($P < 0.05$, *respectively*).
Conclusion: Overexpression Numb had anti-cancer effects to prostatic cancer in vitro and vivo experiments, the mechanism might be P53/Cyclin D1 and Rac1/MMP-2/-9 signaling pathway.

1. Introduction

Prostate cancer has became the second largest malignant tumor in men, and the age of onset has been younger [1], Prostate cancer has been high morbidity and mortality, which is a serious threat to men's health [2,3]. The occurrence and development of tumor has been a multi factor and multi stage process, including the inactivation of tumor suppressor genes, the activation of oncogenes and the abnormal changes of cell biological functions [4,5]. Numb gene is a newly discovered cell fate determinant, which is involved in the occurrence and development of a variety of human tumors [6,7]. NUMB is the first endogenous factor that has been found in multicellular organisms to determine the function of asymmetric cell division. The understanding of the function of NUMB began with the study of the division of the sensory organ precursor cells in Drosophila melanogaster. Compared with only one NUMB gene in Drosophila, NUMB gene in mammals by alternative splicing can encoding 4 NUMB proteins, namely as NUMB 65, NUMB 66, NUMB 71 and NUMB 72 [8]. It was found that NUMB protein was widely distributed in mature tissues and organs, and can be involved in the regulation of endocytosis, cell migration, cell attachment, cell apoptosis, cell proliferation and tumorigenesis [9].The abnormal expression of genes in cells is the molecular basis of tumorigenesis. In recent years, it has been found that NUMB protein plays an important role in tumor formation, invasion and metastasis [10–13]. However, It was limited that the correlation between Numb and prostate cancer. In this study, we firstly examined the expression of Numb protein in prostate cancer, and the correlation between the expression of Numb protein and Gleason score of prostate cancer, and by in vitro and in vivo experiments, overexpression of Numb in the mechanism of the occurrence and development of prostate cancer.

2. Material and methods

2.1. Clinical data

Collecting the prostatic cancer and adjecent normal tissues from the 20 patients, who were treated in our hospital from

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2015.12 to 2016.11. Taking one part of tissues into the formalin to fix and measuring the Numb protein expression by Immunohistochemistry (IHC). Other part of 20 patients were stored in liquid nitrogen (−80 °C) until measuring mRNA expression. All the patients were scored by Gleason score. Analysis the correlation between Numb and Gleason score of 20 patients.

2.2. Material

The DU-145 and PC-3 were purchased from ATCC, DMEM and Transfection Kit were purchased from Sigma. Other relatived material were all from Invitrogen. Numb-ORF and Mega Tran 1.0 were purchased from Origene. RPMI-1640 and 10% fetal bovine serum (FBS) (Gibco). Numb, P53, Cyclin D1, Rac1, MMP-2 and MMP-9 anti-body were all purchased from Abcam. The unde mice were purchased from Nanjing Medical University Animal Center.

3. Methods

3.1. RT-PCR

The Numb gene expression was measured by RT-PCR methods using ABI-7500. In this study, we used PPIA (peptidylprolyl isomerase A) as reference. Measuring the data by relative quantification Ct value [14]. Primer sequence: Numb:F: 5′-CTTTTACAAGAGAAGGATCATTCCG-3′; R: 5′-CAACGACTATCT-TATCTGTTTCAGC-3′. PPIA: F: 5′-ATGGTCAAGGGGAGGTGT-3′; R: 5′-TCTGCTGTCTTTGGGACCTTGTC-3′.

3.2. Cell culture and grouping

The DU-145 and PC-3 cells were cultured by RPMI-1640 contained 10% FBS at 37 °C. Taking the logarithmic phase cells to do experiments. The DU-145 and PC-3 cells were also divided into 2 groups: Normal Control group (NC) and Numb transfection group (Numb). After transfection 48 h, collecting the cell to experiment.

3.3. Cell proliferation assay

The DU-145 and PC-3 cells were treated by above packet processing, after 48 h, adding 20 μL MTT (5 mg/mL), Continue to cultivate for 4 h, The supernatant of each hole were removed, and adding 150 μL DMSO to every hole, oscillating the plate on table for 10 min. Measuring the optical density (OD) value of every holes at 570 nm.

3.4. Flow cytometry

Collecting the cells digested by trypsin; Centrifugation at 1000 *g* speed for 5 min, removing supermatant, the cells were washed by PBS for 2 times, Suspension fixation by 75% ethanol, fasten at 4 °C overnight; Centrifugal fixation, adding 100 mL Propidium iodide staining which concentration was 50 mg/mL, Incugating at room temperature for 30 min, avoid light, detecting the cell apoptosis and cell cycle by flow cytometry.

3.5. Transwell invasion experiment

The DU-145 and PC-3 cells were treated by every groups for 48 h (NC groups were treated with nothing; Numb groups were transfected the Numb), The cells were digested by trypsin, adjusting the cell number to 1×10^6 cells/mL, 200 μL cell suspension was inoculated into Transwell cell, adding 600 μL complete medium in the lower chamber, then, placed in the 37 °C incubator to culture 24 h, removing the Transwell chamber, With 0.2% crystal violet staining, the number of cells in the 5 high field of vision was counted under the light microscope, and the average value was calculated.

3.6. Wound healing testing

DU-145 and PC-3 cells were inoculated in each group (3×10^6/hole) in a culture plate, containing 10%FBS RPMI 1640 medium. When the cell growth area reached 90%, the serum-free RPMI 1640 culture medium was used to make the cells starved for 24 h. With a cell scraper in the central hole on the cell as a straight width of about 0.8 mm with scratches, serum-free medium will scrape cells washed clean. At 0 h after scratching, at the same time, the light was taken under the microscope of 24 h and 48 h, and the distance between the cells was measured at the same time as the distance between the scratches of the 5 data points, and the average value was calculated to calculate the wound healing rate.

3.7. WB assay

Extraction of proteins in cells, on the sample, electrophoresis and transfer film, adding Numb, P53, Cyclin D1, Rac1, MMP-2 and MMP-9 antibody diluted for 1:500 and GAPDH antibody diluted for 1:2500, incubation for 4 °C overnight. After washing, Adding horseradish peroxidase labeled IgG (1:2500) was incubated at 37 °C for 1 h, Using GAPDH as control, the relative expression of each protein was analyzed by chemiluminescence method.

3.8. Nude mouse tumorigenicity assay

Using DU-145 as a tumor cell. DU-145 was digested by trypsin centrifuged to remove medium plus PBS suspension into 1×10^7 cells/mL cell suspension inoculation in nude mice back, and at the same time the Numb lentiviral vector from nude mice injected into the tail vein. After 2 weeks, the nude mice were killed and the tumor tissues were taken out to measure the tumor volume and weight.

3.9. Statistical analysis and methods

All experiments were performed by at least three independent repeats. Data were present ed as means ± SEM. Statistical analysis was done using SPSS 11.0 software (SPSS, USA). Unpaired two-tail student's *t*-test was used to analyze differences between two groups of data. One-way *ANOVA* with post-hoc test was used to analyze differences between three or more groups of data. *P* values of <0.05 were considered as significantly different.

4. Results

4.1. Clinical analysis

Depending on the Gleason score and Numb protein expression by IHC, we found that the Numb protein expression was reduced with Gleason level increasing (Shown in Fig. 1A–D). The Numb gene expression of cancer tissues were significantly reduced compare with adiacent normal tissue ($P < 0.05$, shown in Fig. 1E). There were negative correlation between Gleason score and Numb protein expression ($r = -3.0274$,shown in Fig. 1F).

4.2. Cell proliferation in difference groups of two cells

In the DU-145 and PC-3 cells experiments, the proliferation rate of Numb overexpression were significantly reduced compared with NC group (*80.49 ± 6.43 vs 109.65 ± 5.63, shown in* Fig. 2A; *79.75 ± 4.84 vs 108.79 ± 6.35, shown in* Fig. 2B; *$P < 0.05$, respectively*).