EFFECTS OF RAB27 ON THE PROLIFERATION AND INVASION OF BLADDER CANCER CELLS BY REGULATING NF-KB AND FAK SIGNALING PATHWAY ACTIVITY AND CYCLIN

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ABSTRACT

Objective*: To study the effects of Rab27 on the proliferation and invasion of bladder cancer cells by regulating the activity of nuclear transcription factor-κB (NF-κB) and focal adhesion kinase (FAK) signalling pathways and cyclin.*

Methods: *Cancer tissues from 12 bladder cancer patients who underwent surgical treatment in our hospital from April 2017 to February 2019 were selected. At the same time, normal tissues adjacent to the cancer were selected for comparison. Immunohistochemistry was used to test the expression levels of Rab27 in bladder cancer tissue and normal tissue adjacent to the cancer. Cells from the human bladder cancer cell line BIU-87 were transfected with the Rab27 overexpression plasmid, and cells from human bladder cancer cell line 5637 were transfected with the Rab27-specific siRNA; a negative control group was prepared. The expression levels of Rab27 in BIU-87 and 5637 cells were determined by Western blotting and RT-PCR. Cell proliferation was detected by CCK-8. Transwell cells were used to determine the change in invasion ability of BIU-87 and 5637 cells. The expression levels of apoptosis-related proteins Caspase-3, poly (ADP-ribose) polymerase (PARP), Bcl-2, p-IκB, p-p65 and p-FAK, and cyclins CyclinE and CyclinD1 were tested by Western blot.*

Results: *The expression level of Rab27 in bladder cancer tissue was significantly higher than that in normal tissues adjacent to the cancer. In BIU-87 cells in the RAB27 overexpression group, the expression levels of Rab27, apoptosis-related proteins, p-IκB, p-p65, p-FAK, CyclinE and CyclinD1 were significantly increased compared to the control group, and proliferation and invasion ability were significantly enhanced (P<0.05). In 5637 cells in the siRab27 group, the expression levels of Rab27, apoptosis-related proteins and p-IκB, p-p65, p-FAK, CyclinE and CyclinD1 were significantly decreased compared to the control group, and cell proliferation and invasion ability were significantly reduced (P<0.05).*

Conclusion: *Rab27 may upregulate the expression of CyclinE and CyclinD1 by activating NF-κB and FAK signalling pathway activities to promote the proliferation and invasion of bladder cancer cells.*

Keywords: *Rab27, NF-κB, FAK signalling pathway activity, cyclin, bladder cancer, cell proliferation, invasion.*

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Introduction

Bladder cancer is the most common malignant tumour occurring in the urinary system; of these tumours, 90% are urothelium carcinomas. The incidence and mortality of bladder cancer in developing countries are significantly higher than in developed countries[(1)]. With social and economic development, people's diets have become less healthy, causing the incidence of bladder cancer to increase year by year, which seriously affects people's quality of life and health. At present, surgical resection is an important treatment for bladder cancer. Because early clinical symptoms of bladder cancer are not obvious, most patients are in the middle and advanced stages when diagnosed. Proliferation, invasion and metastasis of tumour cells are important reasons for the unsatisfactory treatment or recurrence of patients with advanced bladder cancer[(2)]. Rab27 is a member of the Ras superfamily. It is mainly responsible for the secretion of intracellular materials and has two isomers, Rab27A and Rab27B. Studies have found

that Rab27 is highly expressed in tumour tissues of patients with liver cancer and can significantly promote growth and metastasis of breast cancer and melanoma[3]. In addition, Rab27 can modify the environment in an exocrine-dependent and non-dependent manner, thereby providing conditions for promoting tumour growth and metastasis.

Rab27 may play a role as an oncogene in tumorigenesis and development, but its role and molecular mechanism in bladder cancer cells are not clear[4]. This study explores the role and mechanism of Rab27 in bladder cancer cells.

Materials and methods

Experimental materials

Bladder cancer tissues and normal adjacent tissues from 12 bladder cancer patients who underwent surgical resection in our hospital.

Experimental cells

Human bladder cancer cell lines BIU-87 and 5637 (Shanghai Hongshun Biotechnology Co., Ltd.).

Main instruments and reagents

Flow cytometer (Beijing Keyu Xingye Technology Development Co., Ltd., model: BD FACSCanto II); Microtome (Henan Zhengzhou South North Instrument Equipment Co., Ltd., model: YD-202); Low-temperature high-speed centrifuge (Guangzhou Jidi Instrument Co., Ltd. , Model: JIDI-4D-WS); low-temperature refrigerator (Aipu Instrument Equipment Co., Ltd., model: AP-40-395LA); biological microscope (Beijing Jinghao Yongcheng Trading Co., Ltd., model: S600T); foetal bovine serum (Shanghai Hengyuan Biotechnology Co., Ltd.); CyclinE cyclin E antibody (Wuhan Purity Biotechnology Co., Ltd.); CyclinD1 antibody (Shanghai Kemin Biotechnology Co., Ltd.); rabbit anti-mouse NF-κB polyclonal antibody (Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.); FAK antibody (Shanghai Xinyu Biological Engineering Co., Ltd.).

Methods and observation indicators

Immunohistochemistry was used to determine the expression level of Rab27 in bladder cancer tissues and normal tissues adjacent to the cancer.

Cell culture

The human bladder cancer cell lines BIU-87 and 5637 were cultured in DMEM cell culture medium with 15% foetal bovine serum and conditions of 5% CO_2, 37°C. The colour of the culture solution and growth of the cells were observed daily, and the medium was replaced in time. When the cells were adherent and grew to the logarithmic growth phase, culture medium was removed and 1 ml of 0.25% trypsin was added to make a cell suspension, which was placed in the incubator for 4 min and morphological changes observed with a microscope.

Cell transfection

0.25% trypsin was added to the cells to prepare a cell suspension. BIU-87 and 5637 cell suspensions in logarithmic growth phase were seeded in 6-well plates at 100,000 cells/well, with 3 holes in each group. Rab27 overexpression plasmid was transfected in BIU-87 cells, Rab27 specific siRNA was transfected in 5637 cells, and a negative control group was prepared. The expression levels of Rab27 in BIU-87 and 5637 cells were determined by Western blotting and RT-PCR.

Cell proliferation

A CCK-8 test was used to determine the proliferation of BIU-87 and 5637 cells. The configured cell suspension was seeded in a 96-well plate, 10 μL of CCK-8 solution was added to each group, and the plate was cultured for 2 h, after which absorbance at 450 nm was measured.

Invasion ability

A Transwell chamber was used to measure the change in invasion ability of BIU-87 and 5637 cells; 200 μL of cell suspension was added to the upper chamber of the Transwell chamber, 800 μL of 20% foetal bovine serum was added to the lower chamber, and the suspension was then incubated in a constant temperature incubator for 24 h. The Transwell chamber was then removed and washed 3 times with phosphate buffer. Cells above the basement membrane were gently wiped off with a cotton swab and fixed in paraformaldehyde solution for 20 min. Cells that migrated to the lower layer of the microporous membrane were observed with a microscope and the number was counted.

Apoptosis

The expression levels of apoptosis-related proteins Caspase-3, poly (ADP-ribose) polymerase (PARP) and Bcl-2 were determined by Western blotting. The expression levels of p-IκB, p-p65, p-FAK, CyclinE and CyclinD1 were also determined by Western blotting.

Statistical methods

SPSS 20.0 software was used for statistical data analysis. Measurement data were compared using one-way ANOVA and LSD-t test. Western blotting was used to detect the expression of Rab27 in bladder cancer tissues and normal tissues adjacent to cancer. The expression levels of Rab27 in BIU-87 and 5637 cells were determined by Western blotting and RT-PCR. Cell proliferation was detected by the CCK-8 test. Transwell assays were used to determine the changes in invasion ability of BIU-87 and 5637 cells. The expression levels of apoptosis-related proteins Caspase-3, PARP, Bcl-2, p-IκB, p-p65, p-FAK, CyclinE and CyclinD1 were determined by Western blotting. Results with P<0.05 were considered statistically significant.

Results

Expression of Rab27 in bladder cancer tissues and normal tissues adjacent to cancer

The expression of Rab27 in bladder cancer tissues was significantly higher than that in normal tissues adjacent to the cancer. See Figure 1.

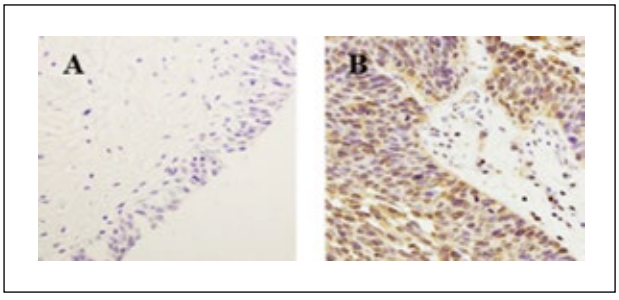

Figure 1: Expression level of Rab27 in bladder cancer tissues and normal tissues adjacent to cancer.
A: Normal tissue adjacent to cancer; B: Bladder cancer tissue.

Changes of Rab27 expression in bladder cancer cells in each group

In BIU-87 cells, compared with the control group, Rab27 expression in the Rab27 overexpression group was significantly increased (P<0.001); in 5637 cells, compared with the control group, Rab27 expression in the siRab27 group was significantly reduced (P<0.05). See Figure 2, Table 1.

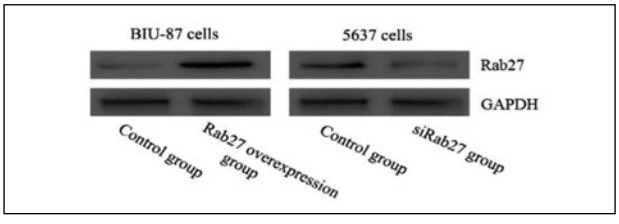

Figure 2: Changes of Rab27 expression levels in bladder cancer cells in each group.

Groups	Rab27 expression levels in BIU-87 cells
Control group	0.12±0.02
Rab27 overexpression group	38.75±2.67
t	25.059
P	<0.001

Groups	Rab27 expression levels in 5637 cells (%)
Control group	100.00±0.00
siRab27 group	30.66±4.18
t	28.732
P	

Table 1: Changes of Rab27 expression levels in bladder cancer cells in each group ($\bar{x}\pm s$).

Changes in proliferation ability of bladder cancer cells in each group

In BIU-87 cells, compared with the control group, the cell proliferation ability of the Rab27 overexpression group was significantly increased after 24 h (P<0.05); in 5637 cells, compared with the control group, the cell proliferation of the siRab27 group was significantly reduced (P<0.05) after 24 h. See Table 2.

Groups	Proliferation ability of BIU-87 cells		
	0 h	24 h	48 h
Control group	0.21±0.01	0.45±0.02	0.98±0.02
Rab27 overexpression group	0.20±0.01	0.59±0.03	1.52±0.05
t	1.225	6.725	17.368
P	0.288	0.003	<0.001

Groups	Proliferation ability of 5637 cells		
	0 h	24 h	48 h
Control group	0.24±0.01	0.63±0.05	1.76±0.11
siRab27 group	0.23±0.03	0.51±0.02	1.25±0.04
t	0.548	3.860	7.547
P	0.613	0.018	0.002

Table 2: Changes in the proliferation ability of bladder cancer cells in each group ($\bar{x}\pm s$).

Changes in invasion ability of bladder cancer cells in each group

According to Transwell experiments, in BIU-87 cells, compared with the control group, the cell invasion ability of the Rab27 overexpression group was significantly enhanced (P<0.05); in 5637 cells, the cell invasion ability of the siRab27 group was significantly reduced compared with the control group (P<0.05). See Figure 3.

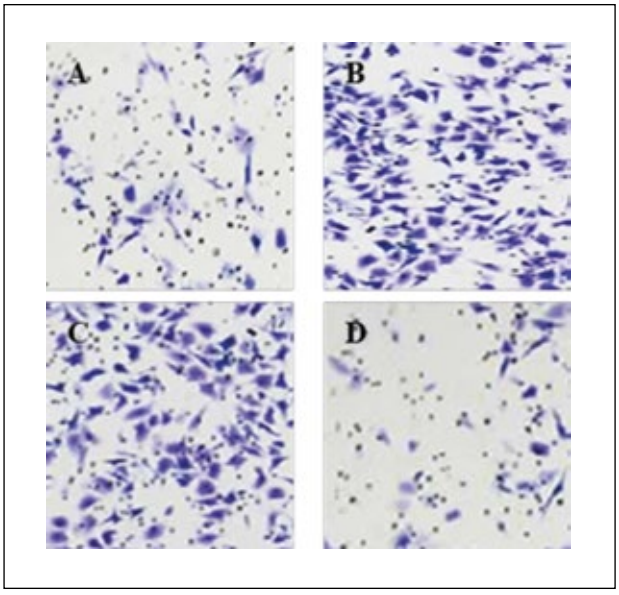

Figure 3: Invasion ability of bladder cancer cells in each group.
A: BIU-87 cell of control group; B: Rab27 overexpression group of BIU-87 cells; C: 5637 cell of control group; D: siRab27 group of 5637 cells.

Changes in expression of apoptosis-related proteins in bladder cancer cells in each group

Western blot analysis showed that in BIU-87 cells, compared with the control group, the expression levels of Caspase-3, PARP and Bcl-2 in the Rab27 overexpression group were significantly increased (P<0.05); in 5637 cells, compared with the control group, the expression levels of Caspase-3, PARP and Bcl-2 in the siRab27 group were significantly reduced (P<0.05). See Figure 4.

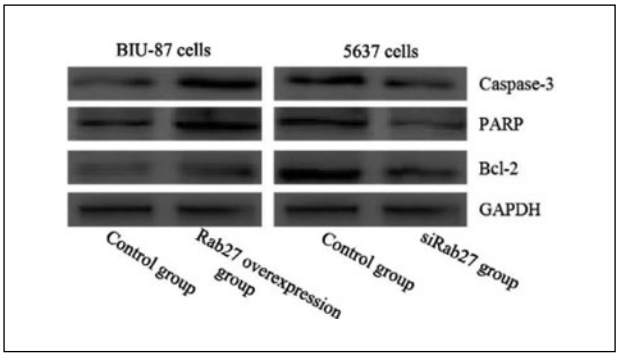

Figure 4: Changes in expression of apoptosis-related proteins in bladder cancer cells in each group.

Expression of p-IκB, p-p65, p-FAK, CyclinE and CyclinD1 in bladder cancer cells in each group

Western blot analysis showed that in BIU-87 cells, compared with the control group, the expression levels of p-IκB, p-p65, p-FAK, CyclinE and CyclinD1 in the Rab27 overexpression group were significantly increased (P<0.05); in 5637 cells, compared with the control group, the expression levels of p-IκB, p-p65, p-FAK, CyclinE and CyclinD1 in the siRab27 group were significantly reduced (P <0.05). See Figure 5.

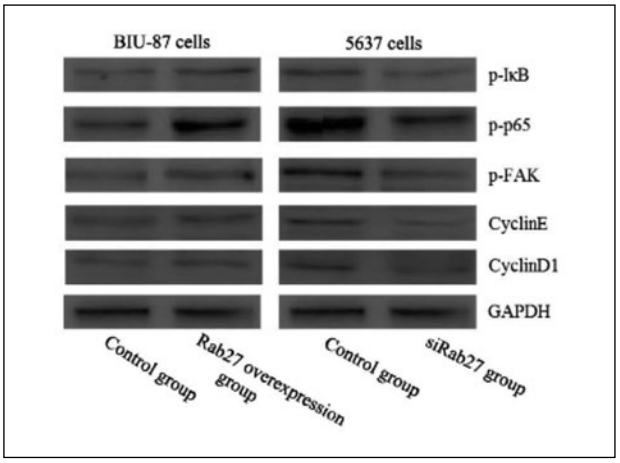

Figure 5: Expression of p-IκB, p-p65, p-FAK, CyclinE and CyclinD1 in bladder cancer cells in each group.

Discussion

Bladder cancer is the most common malignant tumour in the urological system and is also the fifth most common type of tumour in the world. According to Hanahan and Weinberg, tumour cells have ten defining characteristics, including unlimited proliferation, vigorous blood vessel formation, and abnormal energy metabolism[5]. The pathogenesis and metastasis mechanisms are mainly histone chemical modifications under the action of related enzymes, causing abnormal gene expression and other key biological behaviour abnormalities, which leads to unlimited proliferation of tumour cells and promotes tumour cell metastasis. High expressions of HRAS and FGFR3 may activate the MAPK/PI3K pathway, thereby causing abnormal growth of urothelial tumour cells[6]. Bladder cancer is a heterogeneous disease with no typical clinical symptoms in the early stages, with the result that most patients are in the middle to advanced stages when diagnosed. Surgery is the main treatment for bladder cancer, but the recurrence rate is high and the prognosis is poor. Analysis of the pathogenesis of bladder cancer and the molecular mechanism of invasion is critical to improving diagnosis and treatment, reducing recurrence rate and mortality and improving the prognosis of patients.

The Rab protein is a member of the small GTPase protein superfamily, which is mainly involved in vesicle formation, vesicle formation transport and membrane fusion that together regulate cell mem-

brane transport[7]. Some studies have found that the Rab protein family is closely associated with tumours and can regulate material transfer and signal translocation in tumour cells. These processes play an important role in tumour cell growth, invasion and metastasis[8]. As a member of the Rab family, Rab27 plays an important role in the occurrence and development of tumours[9]. Jiang et al.[10] showed that Rab27 can enhance the activity of glioma cells, promote cell proliferation and invasion till apoptosis. In this study, the expression level of Rab27 in bladder cancer cells was significantly higher than that of normal tissues adjacent to the cancer, which can be used as an important molecular marker for the diagnosis of bladder cancer.

Studies have suggested that tumours are a disease with abnormal cell proliferation and differentiation. The proliferation, invasion and migration of cancer cells are important factors affecting the poor prognosis of bladder cancer patients[11]. This study found that Rab27 overexpression can promote cell proliferation, invasion, and cell apoptosis, suggesting that Rab27 plays an important role in the occurrence and development of bladder cancer as an oncogene. Nuclear transcription factor kappa-B (NF-κB) is an important nuclear transcription factor that can be found in the cytoplasm of almost all cell types as a homo- or heterodimer. NF-κB is closely associated with the activation of immune cells, stress response, and the occurrence and development of apoptosis[12]. Studies have found that NF-κB can inhibit the activation of Caspase-3 directly and promote tumour apoptosis[13]. Focal adhesion kinase (FAK) is a widely expressed cytosolic protein tyrosine kinase that is mainly involved in integrin-mediated signalling. Studies have shown that FAK activation is involved in the invasion and metastasis of bladder cancer[14]. The results of this group of studies found that Rab27 overexpression can activate NF-κB and FAK signalling pathways.

The cell cycle is the most basic process of cell proliferation. There are a series of cycle regulation proteins in the cell that ensure the orderly progress of the cell cycle[15]. Cell cycle regulation is a crucial process in the occurrence and development of malignant tumours. It has been reported that tumour cell differentiation, senescence and apoptosis can be regulated by the cell cycle[16]. CyclinE and CyclinD1 can bind to CDK4/CDK6 to activate the cyclin-dependent kinase phosphatase activity, promote Rb phosphorylation, promote the transcription of genes related to DNA synthesis in cells, and then shift the cell cycle into S issue[17]. This study showed that Rab27 overexpression can significantly promote the expression of CyclinE and CyclinD1.

In summary, Rab27 may upregulate the expression of cyclins CyclinE and CyclinD1 by activating NF-κB and FAK signalling pathway activities to promote the proliferation and invasion of bladder cancer cells.

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