CORRELATION OF YKL-40 EXPRESSION LEVEL WITH CELL MIGRATION AND INVASION ABILITY IN HUMAN PROSTATE CANCER DU145 AND PC3 CELL LINES

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ABSTRACT

Objective: *To investigate the correlation between the expression levels of human cartilage glycoprotein 39 (YKL-40) and cell migration and invasion abilities in human prostate cancer DU145 and PC3 cell lines.*

Methods: *Prostate cancer tissue and paracancerous tissue were collected from 58 patients in our hospital from May 2016 to January 2018. The expression of YKL-40 in prostate cancer tissues and paracancerous tissues was detected by immunohistochemistry. Human prostate cancer DU145 and PC3 cell lines were collected, and the invasion and migration abilities and YKL-40 mRNA expression of DU145 and PC3 cell lines were detected by Transwell chamber and Western blot methods, respectively. PC3 cells were randomly divided into a PC3-WT group (wild-type PC3 cells), a PC3-control group (empty vector), a PC3-YKL-40 group (up-regulation of YKL-40 expression), and a PC3-YKL-40-KD group (down-regulation YKL-40 expression) DU145 cells were randomly divided into a DU-145-WT group (wild-type DU-145 cells), a DU-145-control group (empty vector), a DU-145-KD-YKL-40 group (up-regulation of YKL-40 expression), and a DU-145-KD group (down-regulation of YKL-40 expression). Cell invasion and migration abilities of each group were analysed and compared.*

Results: *The high expression rate of YKL-40 was 72.41% (42/58) in prostate cancer tissue and 10.34% (6/58) in paracancerous tissues, with statistical significance between the groups ($p<.05$). Compared with PC3 cells, DU145 cell invasion and migration abilities, YKL-40 protein and mRNA expression levels were significantly increased ($p<.05$). The cell invasion and migration abilities of the PC3-YKL-40 group were significantly higher than that of the PC3-WT group, and the cell invasion and migration abilities of the PC3-YKL-40 group were significantly higher than that of the PC3-YKL-40-KD group ($p < .05$). Compared with the PC3-control group and the PC3-YKL-40-KD group, the cell invasion and migration abilities of the PC3-WT group had no significant difference ($p>.05$). The cell invasion and migration abilities of the DU-145-KD group were significantly lower than those of the DU-145-WT group ($p<.05$). The Cell invasion and migration abilities of the DU-145-KD group were significantly lower than those of the DU-145-KD-YKL-40 group ($p<.05$). Compared with DU-145-control group and DU-145-KD-YKL-40 group, the cell invasion and migration abilities of the DU-145-WT group had no significant difference ($p>.05$).*

Conclusion: *The expression of YKL-40 is significantly increased in prostate cancer, and the expression level of YKL-40 in prostate cancer DU145 and PC3 cell lines is positively correlated with cell migration and invasion abilities.*

Keywords: *Prostate cancer, DU145 cells, PC3 cells, YKL-40, migration, invasion.*

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Introduction

Prostate cancer originates from the prostate epithelium and is a common malignant tumour of the reproductive system in males. The incidence of prostate cancer in western countries is extremely high, and it is the most common malignant tumour among males in the United States(1). The incidence of prostate cancer in China is slightly lower than that in Western countries, but the incidence is still increasing yearly. Once prostate cancer is diagnosed, most patients are in an advanced stage(2). Genetic factors, diet and lifestyle can increase the risk of prostate cancer, and patients in the early stages have no obvious symptoms. With tumour progression, however, progressive dysuria, bone metastasis and other

symptoms can further develop and seriously affect the safety of patients' life[3]. It has been reported that more than 70% of the deaths of prostate cancer patients are attributed to tumour tissue invasion and metastasis, which poses a serious threat to the patient's prognosis and survival time[4]. Surgery or hormone therapy is currently the most effective method for treating prostate cancer. Although these methods can delay the progress in patients with advanced or metastatic disease, the survival time of patients after endocrine therapy is only about one year[5].

Therefore, it is necessary to reveal the pathogenesis of prostate cancer and further explore its targets, which is beneficial to alleviate clinical symptoms and improve the prognosis of patients.

Human chondroprotein 39 (YKL-40) is highly expressed in many malignant diseases, and it is closely related to the invasion and metastasis of malignant tumours. Therefore, it can be used as an important reference index for assessing the condition and judging prognosis[6].

The purpose of this study is to investigate the correlation between the expression level of YKL-40 and cell migration and invasion abilities by detecting the expression level of YKL-40 in human prostate cancer DU145 and PC3 cell lines.

Materials and methods

Study subjects

Prostate cancer tissue and paracancerous tissue resected by our hospital from 58 patients from May 2016 to January 2018 were collected.

The enrolment criteria were:

• The paracancerous tissue was more than 5 cm away from the prostate cancer tissue;

• All patients had complete clinical-pathological data;

• No treatment had been performed before surgery;

• All had signed informed consent.

The study was approved by the medical ethics committee in our hospital.

Each tissue sample was divided into two parts, one was stored in liquid nitrogen for protein detection, and the other was fixed in paraformaldehyde for immunohistochemical detection.

Human prostate cancer DU145 and PC3 cell lines (National Experimental Cell Resource Sharing Platform) were selected for routine culture. After resuscitation, passage and other processes, they were placed in liquid nitrogen waiting for detection.

Main reagents and instruments

Reagents

YKL-40 immunohistochemical polyclonal antibody was purchased from Abcam.

HRP-labelled anti-rabbit IgG antibody was purchased from Wuhan Saiweier Biotechnology Co., Ltd. Citrate antigen repair solution was purchased from Shanghai Sanly Biotechnology Co., Ltd. Bovine serum albumin was purchased from Chongqing Preco Biotechnology Co., Ltd. DAB reagent was purchased from Wuhan Saiweier Biotechnology Co., Ltd.

Formalin was purchased from Shenzhen Advanced Medical Services Co., Ltd. Hematoxylin was purchased from Shenzhen Kang Chuyuan Co., Ltd. Eosin dyestuff was purchased from Biogot Biotechnology Co., Ltd.

Instruments

High-speed low-temperature centrifuge was purchased from Jinan Xinbeixi Technology Co., Ltd. Paraffin automatic embedding machine was purchased from Shenyang Hengsong Technology Co., Ltd. Paraffin microtome was purchased from Shanghai Jumu Medical Equipment Co., Ltd. Low-speed horizontal shaker was purchased from Jiangsu Tianling Instrument Co., Ltd. An ordinary optical microscope was purchased from Beijing Oubotong Optical Technology Co., Ltd. Micropipettes were purchased from Zhengzhou Laipu Biotechnology Co., Ltd. A -80oC ultra-low temperature refrigerator was purchased from Hangzhou Aipu Instrument Equipment Co., Ltd. A thermostatic water bath box was purchased from Beijing Changfeng Instrument Co., Ltd. The magnetic stirrer was purchased from Weihai Dingda Chemical Machinery Co., Ltd.

Methods

• The expression of YKL-40 in paracancerous tissues and prostate cancer tissues was detected by immunohistochemistry.

• The invasion and migration abilities of PC3 cells and DU145 cells were analysed and compared using transwell chamber.

• The expression of YKL-40mRNA in PC3 cells and DU145 cells was detected and analysed by real-time quantitative PCR.

• The expression of YKL-40 protein in PC3 cells and DU145 cells was detected by Western blot analysis.

• PC3 cells were randomly divided into a PC3-

WT group (wild-type PC3 cells), a PC3-control group (empty vector), a PC3-YKL-40 group (up-regulation of YKL-40 expression), and a PC3-YKL-40-KD group (down-regulation of YKL-40 expression).

DU145 cells were randomly divided into a DU-145-WT group (wild-type DU-145 cells), a DU-145-control group (empty vector), a DU-145-KD-YKL-40 group (up-regulation of YKL-40 expression) and a DU-145-KD group (down-regulates YKL-40 expression). The cell invasion and migration abilities of each group were analysed and compared.

Statistical methods

SPSS 22.0 was used for statistical analysis. The expression of YKL-40 in paracancerous tissues and prostate cancer tissues were expressed rates and tested with χ^2; the invasion and migration abilities of PC3 cells and DU145 cells and the expression of YKL-40 mRNA were expressed as ($\bar{x}\pm s$) and with a t-test; $p<.05$ was considered statistically significant.

Results

Expression of YKL-40 in paracancerous and prostate cancer tissue

The high expression rate of YKL-40 was 72.41% (42/58) in prostate cancer tissue and 10.34% (6/58) in paracancerous tissue. The statistical difference between the groups was significant ($p<.05$). See Figure 1.

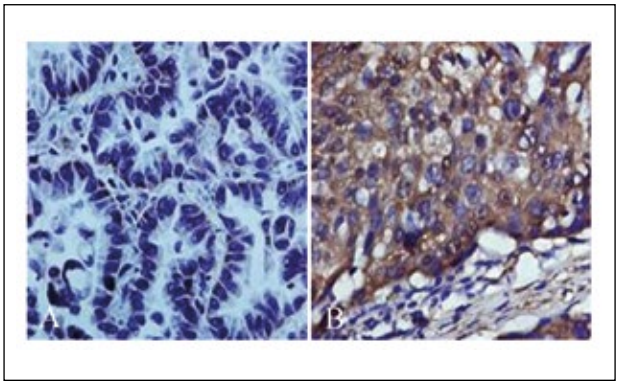

Figure 1: Expression of YKL-40 in (A) paracancerous tissue and (B) prostate cancer tissue.

Relationship between the expression of YKL-40 and invasion and migration of prostate cancer cells

Compared with PC3 cells, invasion and migration abilities of DU145 cells, YKL-40 protein, and mRNA expression levels were significantly increased ($p<.05$). See Figures 2 and 3 and Table 1.

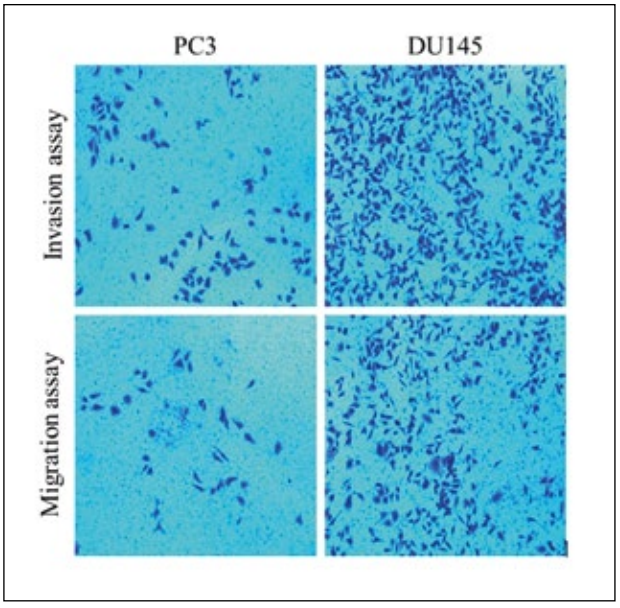

Figure 2: Comparison of invasion and migration abilities of PC3 cells and DU145 cells.

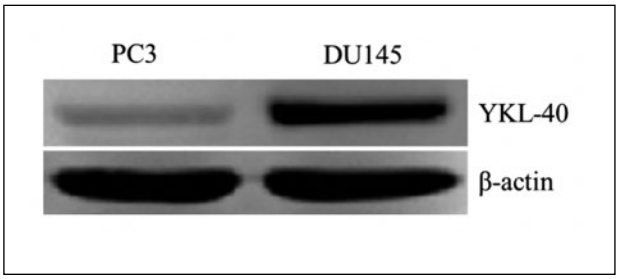

Figure 3: Comparison of expression levels of YKL-40 protein in PC3 cells and DU145 cells.

Group	Invasion ability	Migration ability	Expression of YKL-40 mRNA
PC3 cells	452.56 ±88.63	236.23±46.45	1.39±0.38
DU145 cells	5536.89±156.85	3743.82±159.73	4.05±0.91
t	12.840	16.605	8.530
p	< .001	< .001	< .001

Table 1: Comparison of Invasion Ability, Migration Ability and Expression of YKL-40 mRNA in PC3 Cells and DU145 Cells ($\bar{x}\pm s$).

Effect of up- or down-regulation of YKL-40 expression in PC3 cells and DU145 cells on tumour cell invasion and migration

The cell invasion and migration abilities of the PC3-YKL-40 group were significantly higher than that of the PC3-WT group. The cell invasion and migration abilities of the PC3-YKL-40 group were significantly higher than that of the PC3-YKL-40-KD group ($p<.05$). Compared with the PC3-control group and the PC3-YKL-40-KD group, the cell invasion and migration abilities of the PC3-WT group had no significant difference ($p>.05$). See Table 2.

Group	Invasion ability	Migration ability
PC3-WT group	423.56±99.28	205.43±56.89
PC3-control group	412.36±115.89	229.46±125.78
PC3-YKL-40 group	1327.65±548.23[ab]	823.45±289.65[ab]
PC3-YKL-40-KD group	428.83±165.89	214.36±175.37

Table 2: Effect of Up- or Down-regulation of YKL-40 expression in PC3 cells and DU145 cells on tumour cell invasion and migration ($\bar{x}\pm s$).
Note: a means p<.05 compared with the PC3-WT group; b means p<.05 compared with the PC3-YKL-40-KD group.

The cell invasion and migration abilities of the DU-145-KD group were significantly lower than those of the DU-145-WT group (p<.05). The cell invasion and migration abilities of the DU-145-KD group were significantly lower than those of the DU-145-KD-YKL-40 group (p<.05). Compared with the DU-145-control group and the DU-145-KD-YKL-40 group, the cell invasion and migration abilities of DU-145-WT were not significantly different (p>.05). See Table 3.

Group	Invasion ability	Migration ability
DU-145-WT group	5863.56±127.91	3802.49±189.65
DU-145-control group	5568.31±99.56	3659.87±269.82
DU-145-KD-YKL-40 group	5447.12±73.87	3561.43±202.56
DU-145-KD group	1523.68±112.48[cd]	856.28±303.65[cd]

Table 3: Effect of Up- or Down-regulation of YKL-40 expression in PC3 cells and DU145 cells on tumour cell invasion and migration ($\bar{x}\pm s$).
Note: c means p<.05 compared with the PC3-WT group; d means p<.05 compared with the DU-145-KD-YKL-40 group.

Discussion

Prostate cancer has a high degree of malignancy, and its invasion and migration abilities are very important biological characteristics which seriously threaten the prognosis of patients with prostate cancer. Various prostate cancer treatment methods can improve a patient's condition to a certain extent, but they have not reached the expected goal, and the one-year survival rate of patients is low[7]. Therefore, early detection, early diagnosis and timely and effective treatment are the keys for controlling the disease, improving the quality of life of patients and prolonging their survival time.

It is worth noting that there is a highly expressed secreted protein-YKL-40[8-9] in many malignant tumours. Clinical studies have shown that serum levels of YKL-40 in patients with neurologically related tumours, breast cancer, and other diseases are significantly higher than those in the general population[10]. Retrospective studies have shown that YKL-40 was highly expressed in the serum of about 13 malignant tumour patients, and its expression level was closely related to the patients' prognosis, survival time, tolerance, migration ability, etc. This was especially the case for bone metastasis or visceral metastasis patients; their serum levels of YKL-40 were significantly higher than those of patients with skin or lymph node metastases[11]. Therefore, YKL-40 is expected to be an important diagnostic indicator for evaluating the metastasis or prognosis of patients with lung adenocarcinoma. Some studies have shown that YKL-40 can cooperate with insulin-like growth factor to activate extracellular signal regulation through mitogen-activated protein kinase (MAPK) or phosphatidylinositol 3-kinase (PI3K) pathways to activate extracellular signal-regulated kinases 1 and 2 (ERK1/2) and serine-threonine kinase (serine-threonine kinase, AKT), further promoting cell proliferation and fibrosis[12-13]. Some scholars have up-regulated the expression of YKL-40 in human stellate cells, causing the cell to acquire tumour-like cell invasion, in addition, obtaining radiation and low serum tolerance. This suggests that YKL-40 can have cytoprotective effects[14-15].

In this study, the expression levels of YKL-40 in prostate cancer and paracancerous tissue were detected by immunohistochemistry. The results showed that the high expression rate of YKL-40 was 72.41% (42/58) in prostate cancer tissue and 10.34% (6/58) in paracancerous tissue, and the difference between the two groups was statistically significant(p<.05). This suggests that YKL-40 plays an important role in the development of prostate cancer. Next, we explored prostate cancer PC3 cells and DU145 cells and the results showed that compared with PC3 cells, DU145 cell invasion and migration abilities, YKL-40 protein and mRNA expression levels were significantly increased (p<.05). The cell invasion and migration abilities of the PC3-YKL-40 group were significantly higher than that of the PC3-WT group. The cell invasion and migration abilities of the PC3-YKL-40 group were significantly higher than that of the PC3-YKL-40-KD group (p<.05). The cell invasion and migration abilities of the DU-145-KD group were significantly lower than those of the DU-145-WT group (p<.05). The cell invasion and migration abilities of the DU-145-KD group were significantly lower than those of the DU-145-KD-YKL-40 group (p<.05).

This suggests that the expression of YKL-40 in prostate cancer cells is positively correlated with cell migration and invasion abilities.

In conclusion, the expression of YKL-40 is significantly increased in prostate cancer, and the expression level of YKL-40 in prostate cancer DU145 and PC3 cell lines is positively correlated with cell migration and invasion abilities. Thus, YKL-40 can be used as a reference index for judging prostate cancer metastasis.

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