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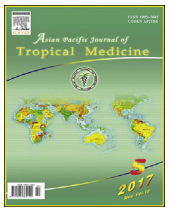

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Matrine inhibits bladder cancer cell growth and invasion *in vitro* through PI3K/AKT signaling pathway: An experimental study

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ABSTRACT

Objective: To study the inhibitory effect of matrine on bladder cancer cell growth and invasion *in vitro* through PI3K/AKT signaling pathway.
Methods: Human T24 bladder cancer cell lines were cultured and treated with different doses of matrine (0.25 mg/mL, 0.5 mg/mL and 1.0 mg/mL) as well as 20 μmol/L PI3K inhibitor LY294002 for 24 h, and the cell proliferation activity, the number of invasive cells as well as the expression of p-PI3K, p-AKT, proliferation genes and invasion genes were determined.
Results: Different doses of matrine could decrease the cell viability value, the number of invasive cells as well as the expression of p-PI3K, p-AKT, MMP2 and MMP9, and increase the expression of p16, p21 and p27 in dose-dependent manner; p16, p21 and p27 expression in cells of 20 μmol/L LY29002 group were significantly higher than those of 0 μmol/L LY29002 group while MMP2 and MMP9 expression were significantly lower than those of 0 μmol/L LY29002 group ($P < 0.05$).
Conclusions: Matrine can inhibit bladder cancer cell proliferation and invasion *in vitro* and regulate the expression of cell cycle-inhibiting molecules and invasion-related genes through PI3K/AKT signaling pathway.

1. Introduction

Bladder cancer is the most common malignant tumor of urinary system, transurethral resection is the major means for its clinical treatment, and it can effectively remove the tumor lesions [1,2]. However, bladder cancer cells are highly invasive, postoperative recurrence rate is high in patients with invasive bladder cancer, and the effect is not ideal after postoperative intravesical perfusion chemotherapy is used together to reduce long-term recurrence rate [3,4]. Matrine is one of the alkaloids extracted from *Sophora alopecuroides*; it has extensive antitumor activity and studies have confirmed that matrine has significant inhibitory effect on the *in vitro* growth of lung cancer [5], gastric cancer [6] and liver cancer [7], but there is no specific report on the regulating effect of matrine on bladder cancer cell growth and invasion *in vitro*. Phosphoinositide 3-kinase (PI3K)/serine–threonine kinase AKT is the important signaling pathway in the body, and it can regulate the expression of a variety of proliferation and invasion genes and thus regulate the cell growth and invasion. In the following study, the inhibitory effect of matrine on bladder cancer cell growth and invasion *in vitro* through PI3K/AKT signaling pathway was analyzed.

2. Materials and methods

2.1. Experimental materials

Human bladder cancer cell lines T24 were bought from Shanghai Institute of Cell Biology of Chinese Academy of Sciences, the DMEM, fetal bovine serum and trypsin for cell culture were purchased from Gibco Company, matrine was bought from Ningxia Zijinghua Pharmaceutical Co., LTD., PI3K inhibitor LY294002 was purchased from Sigma Company, cell viability detection kits (MTS kits) were purchased from Promega Company, protein lysis buffer was bought from Shanghai

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Beyotime Company, and the MMP2, MMP9, p16, p21, p27, p-PI3K, p-AKT, PI3K and AKT monoclonal antibodies were bought from CST Company.

2.2. Experimental methods

2.2.1. Cell culture and treatment methods

T24 cells were recovered and then cultured with DMEM containing 10% fetal bovine serum, and the incubator conditions were 5%CO_2 and saturated humidity 37 °C; when cell density reached about 90%, the cells were digested and sub-cultured by trypsin; the sub-cultured cells in logarithmic phase were collected, and matrine treatment methods were as follows: matrine was added in the culture medium to make the final drug concentration reach 0.25 mg/mL, 0.5 mg/mL and 1.0 mg/mL, and the cells treated without matrine were used as the control group (0 mg/mL); PI3K inhibitor treatment methods were as follows: LY294002 was added in the culture medium to make the final drug concentration reach 20 μmol/L, and the cells treated without LY294002 were used as the control group (0 μmol/L).

2.2.2. Cell viability and invasion detection methods

To detect the cell viability, the digested and sub-cultured cells were inoculated in 96-well cell plate, the cell density was 1×10^4/well, 20 μL test fluid in the MTS kits was added in the culture medium 24 h after matrine treatment, the cells continued to be incubated for 4 h in the incubator, then the cell plate was taken out and fully oscillated, and then the absorbance value at 450 nm wave length was determined in microplate reader and used as the cell viability value. To detect cell invasion, the cells were inoculated in Transwell chambers, the cell density was 1×10^4/well, the filter membrane at the bottom of the Transwell chambers was removed 24 h after matrine treatment for DAPI staining, then three random visual fields were observed under the fluorescent microscope, and the number of invasive cells was counted.

2.2.3. Protein expression detection methods

To detect protein expression in cells, the digested and sub-cultured cells were inoculated in 12-well cell plate, the cell density 5×10^5/well, the culture medium was abandoned 24 h after matrine or LY294002 treatment, the cells were kept, added in 60 μL protein lysis buffer and then fully broken with scraper, the cell suspension after lysis were centrifuged in 4 °C centrifuge for 20 min at 12 000 r/min, the upper protein samples were separated and added in loading buffer for high-temperature denaturation, then the polyacrylamide gel was configured, and the denatured protein samples were added in it for vertical electrophoresis and horizontal electrophoretic transfer and then closed with 5% skim milk to incubate p-PI3K, p-AKT, PI3K, AKT, p16, p21, p27, MMP2, MMP9 and β-actin antibodies over night respectively; the second antibodies were incubated on the next day, development was performed, the protein bands were obtained and scanned with Image J software to get the gray value of different proteins, β-actin was used as reference to calculate the expression of p16, p21, p27, MMP2 and MMP9, and PI3K and AKT were used as reference to calculate the expression of p-PI3K and p-AKT.

2.2.4. Statistical methods

SPSS20.0 software was used to input and analyze data, measurement data analysis between two groups was by *t* test, measurement data analysis among four groups was by variance analysis, pair-wise comparison was by LSD-t TEST and $P < 0.05$ indicated statistical significance in differences.

3. Results

3.1. Regulatory effect of matrine on cell proliferation activity and number of invasive cells

After different doses of matrine treatment, analysis of the cell proliferation activity and the number of invasive cells was as follows: matrine could decrease the cell viability value and the number of invasive cells in dose-dependent manner; the cell proliferation activity and the number of invasive cells of 0.25 mg/mL, 0.5 mg/mL and 1.0 mg/mL matrine group were significantly lower than those of 0 mg/mL matrine group ($P < 0.05$); the cell proliferation activity and the number of invasive cells of 0.5 mg/mL and 1.0 mg/mL matrine group were significantly lower than those of 0.25 mg/mL matrine group ($P < 0.05$); the cell proliferation activity and the number of invasive cells of 1.0 mg/mL matrine group were significantly lower than those of 0.5 mg/mL matrine group ($P < 0.05$). Differences in pair-wise comparison of the cell proliferation activity and the number of invasive cells were statistically significant among different doses of matrine treatment groups ($P < 0.05$) (Table 1).

3.2. Regulatory effect of matrine on p-PI3K and p-AKT expression in cells

After different doses of matrine treatment, analysis of the cell p-PI3K and p-AKT expression in cells was as follows: matrine could decrease the p-PI3K and p-AKT expression in dose-dependent manner; p-PI3K and p-AKT expression in cells of 0.25 mg/mL, 0.5 mg/mL and 1.0 mg/mL matrine group were significantly lower than those of 0 mg/mL matrine group ($P < 0.05$); p-PI3K and p-AKT expression in cells of 0.5 mg/mL and 1.0 mg/mL matrine group were significantly lower than those of 0.25 mg/mL matrine group ($P < 0.05$); p-PI3K and p-AKT expression in cells of 1.0 mg/mL matrine group were significantly lower than those of 0.5 mg/mL matrine group ($P < 0.05$). Differences in pair-wise comparison of p-PI3K and p-AKT expression in cells were statistically significant among different doses of matrine treatment groups ($P < 0.05$) (Table 1).

3.3. Regulatory effect of matrine on proliferation and invasion gene expression in cells

Twenty-four hours after different doses of matrine treatment, analysis of proliferation and invasion gene expression in cells was as follows: matrine could increase the p16, p21 and p27 expression while decrease MMP2 and MMP9 expression in dose-dependent manner; p16, p21 and p27 expression in cells of 0.25 mg/mL, 0.5 mg/mL and 1.0 mg/mL matrine group were significantly higher than those of 0 mg/mL matrine group while MMP2 and MMP9 expression were significantly lower than those of 0 mg/mL matrine group ($P < 0.05$); p16, p21 and p27 expression in cells of 0.5 mg/mL and 1.0 mg/mL matrine group were significantly higher than those of 0.25 mg/mL matrine group while MMP2 and MMP9 expression were significantly lower than those of 0.25 mg/mL matrine group ($P < 0.05$); p16,

Table 1

Regulatory effect of different doses (mg/mL) of matrine on cell proliferation activity, the number of invasive cells and p-PI3K and p-AKT expression in cells (repeated batches = 5; mean ± SD).

Matrine dose	Cell viability value	Number of invasive cells	p-PI3K/PI3K	p-AKT/AKT
0.00	1.06 ± 0.14	35.86 ± 5.67	1.00 ± 0.14	1.00 ± 0.18
0.25	0.77 ± 0.09[a]	24.21 ± 3.09[a]	0.78 ± 0.08[a]	0.72 ± 0.09[a]
0.50	0.56 ± 0.07[ab]	17.41 ± 2.21[ab]	0.52 ± 0.06[ab]	0.49 ± 0.05[ab]
1.00	0.38 ± 0.05[abc]	11.36 ± 1.37[abc]	0.28 ± 0.04[abc]	0.30 ± 0.04[abc]

[a]$P < 0.05$, compared with 0 mg/mL matrine group; [b]$P < 0.05$, compared with 0.25 mg/mL matrine group; [c]$P < 0.05$: compared with 0.5 mg/mL matrine group.

Table 2

Regulatory effect of different doses (mg/mL) of matrine on proliferation and invasion gene expression in cells (repeated batches = 5; mean ± SD).

Matrine dose	p16	p21	p27	MMP2	MMP9
0.00	1.00 ± 0.14	1.00 ± 0.12	1.00 ± 0.09	1.00 ± 0.08	1.00 ± 0.13
0.25	1.77 ± 0.19[a]	1.62 ± 0.18[a]	1.65 ± 0.19[a]	0.75 ± 0.10[a]	0.69 ± 0.09[a]
0.50	2.18 ± 0.27[ab]	2.28 ± 0.35[ab]	2.35 ± 0.24[ab]	0.51 ± 0.07[ab]	0.51 ± 0.06[ab]
1.00	2.90 ± 0.44[abc]	3.25 ± 0.47[abc]	3.52 ± 0.53[abc]	0.33 ± 0.05[abc]	0.28 ± 0.04[abc]

[a]$P < 0.05$, compared with 0 mg/mL matrine group; [b]$P < 0.05$, compared with 0.25 mg/mL matrine group; [c]$P < 0.05$: compared with 0.5 mg/mL matrine group.

Table 3

Regulatory effect of PI3K inhibitor on proliferation and invasion gene expression in cells (repeated batches = 5; mean ± SD).

LY29002 dose (μmol/L)	p16	p21	p27	MMP2	MMP9
0	1.00 ± 0.12	1.00 ± 0.07	1.00 ± 0.13	1.00 ± 0.14	1.00 ± 0.09
20	2.12 ± 0.25	2.38 ± 0.34	2.08 ± 0.26	0.53 ± 0.07	0.31 ± 0.04
T	11.275	13.205	10.982	9.481	17.851
P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

p21 and p27 expression in cells of 1.0 mg/mL matrine group were significantly higher than those of 0.5 mg/mL matrine group while MMP2 and MMP9 expression were significantly lower than those of 0.5 mg/mL matrine group ($P < 0.05$). Differences in pair-wise comparison of p16, p21, p27, MMP2 and MMP9 expression in cells were statistically significant among different doses of matrine treatment groups ($P < 0.05$) (Table 2).

3.4. Regulatory effect of PI3K inhibitor on proliferation and invasion gene expression in cells

Twenty-four after PI3K inhibitor treatment, analysis of proliferation and invasion gene expression in cells was as follows: p16, p21 and p27 expression in cells of 20 μmol/L LY29002 group were significantly higher than those of 0 μmol/L LY29002 group while MMP2 and MMP9 expression were significantly lower than those of 0 μmol/L LY29002 group ($P < 0.05$). Differences in p16, p21, p27, MMP2 and MMP9 expression in cells were statistically significant between 0 μmol and 20 μmol LY29002 group ($P < 0.05$) (Table 3).

4. Discussion

Bladder cancer has the characteristic of invasive growth, and intravesical perfusion chemotherapy is needed after transurethral resection to achieve the goal of killing cancer cells and preventing tumor recurrence. Epirubicin and pirarubicin are the common chemotherapeutics for intravesical perfusion chemotherapy, they have certain killing effect on the cancer cells, but drug resistance easily occurs during perfusion chemotherapy and influences the effect of chemotherapy [8,9]. Matrine is a new alkaloid with extensive antitumor activity discovered in recent years, and it is extracted from the *S. alopecuroides*. Studies have shown that matrine can inhibit the *in vitro* growth of lung cancer [5], gastric cancer [6], liver cancer [7] and other malignant tumor cells, but there is no report about the effect of matrine on bladder cancer proliferation and invasion. In the study, in order to define the inhibitory effect of matrine on bladder cancer cell growth and invasion *in vitro*, the cell proliferation activity and the number of invasive cells were analyzed, and the results showed that matrine could decrease the cell proliferation activity and the number of invasive cells in dose-dependent manner. This means that matrine has obvious inhibitory effect on bladder cancer cell growth and invasion *in vitro*.

After confirming the role that matrine played during bladder cancer proliferation and invasion, the molecular mechanism of matrine to adjust bladder cancer proliferation and invasion was further analyzed. PI3K/AKT signaling pathway is an important pathway that regulates cell proliferation, invasion and other biological behaviors in the body [10,11]. PI3K, as a signaling protein with the catalytic activity within cells, will be activated under the action of extracellular cytokines, drugs, stress and other factors, then phosphorylates PIP2 into PIP3 and promotes AKT activation so as to regulate the expression of a variety of cell proliferation and invasion-related genes [12–14]. In order to determine whether matrine regulated bladder cancer cell proliferation and invasion by PI3K/AKT signaling

pathways, the activation of PI3K/AKT pathway in cells was analyzed in the study. PI3K and AKT are activated by means of phosphorylation, the detection of p-PI3K and p-AKT expression can reflect the activation of PI3K/AKT signaling pathway, and analysis of the results in the study showed that different doses of matrine could decrease the p-PI3K and p-AKT expression in dose-dependent manner. This means that matrine can inhibit the activation of PI3K/AKT pathway in bladder cancer cells.

The activation of PI3K/AKT signaling pathway can on the one hand, directly start the expression of MMP2, MMP9 and other invasion-related genes, and promote cells invasion [15,16], and on the other hand, can also block the AMP-activated protein kinase activity, inhibit the expression of a variety of tumor suppressor genes and cell cycle-inhibiting molecules and promote cell proliferation [17]. p16, p21 and p27 are the cell cycle-inhibiting molecules that play an important role in the progression of malignant tumors, and they can be combined with a variety of cyclin and cyclin-related protein kinase to hinder the process of cell cycle and thereby inhibit cell proliferation [18]; MMP2 and MMP9 are the important members of the matrix metalloproteinase family that can degrade type Ⅳ collagen, laminin, elastin and various other components in the extracellular matrix and basement membrane, and the MMP2 and MMP9 secreted by cancer cells can promote cells to break through the limit of basement membrane and invade the nearby tissue [19,20]. In the study, analysis of the expression of above proliferation and invasion-related genes showed that different dose of matrine could increase p16, p21 and p27 expression while decrease MMP2 and MMP9 expression in dose-dependent manner. This means that matrine can promote the expression of tumor suppressor genes p16, p21 and p27 as well as inhibit the expression of invasion genes MMP2 and MMP9 in bladder cancer cells, and the changes in the expression of above tumor suppressor genes and invasion genes can inhibit the proliferation and invasion of bladder cancer cells.

The research results show that matrine can inhibit the activation of PI3K/AKT signaling pathway and the expression of MMP2 and MMP9 in bladder cancer cells, and promote the expression of p16, p21 and p27. Related studies have confirmed that PI3K/AKT signaling pathway can target and regulate the expression of invasion genes MMP2 and MMP9 as well as the cell cycle-inhibitory molecules p16, p21 and p27 in colorectal cancer and breast cancer [16,17]. In order to further clarify whether the PI3K/AKT signaling pathway targeted and regulate the above invasion genes and cell cycle-inhibitory molecules in bladder cancer cells, the PI3K-specific inhibitor LY29002 was used in the study to treat bladder cancer cells, and analysis of the expression of invasion genes and cell cycle-inhibitory molecules showed that p16, p21 and p27 expression in cells of 20 μmol/L LY29002 group were significantly higher than those of 0 μmol/L LY29002 group while MMP2 and MMP9 expression were significantly lower than those of 0 μmol/L LY29002 group. It means that targeted inhibition of PI3K can increase the expression of cell cycle-inhibitory molecules p16, p21 and p27, and inhibit the expression of invasion genes MMP2 and MMP9.

To sum up, it is believed that matrine can inhibit bladder cancer cell proliferation and invasion *in vitro* through PI3K/AKT signaling pathway, increase the expression of cell cycle-inhibiting molecules p16, p21 and p27, and inhibit the expression of invasion genes MMP2 and MMP9.

Conflict of interest statement

We declare that we have conflict of interest.

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