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Expression and Transport Characteristics of Urea Channel Protein B in Rat Bladder Cancer

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Abstract: The complex process of multiple genes and steps in the development of bladder tumor is definitely related to the abnormal expression of multiple tumor suppressor genes. The purpose of this study is to investigate the expression and transport characteristics of urea channel protein B in rat bladder cancer. In this study, the expression of urea channel protein B in 70 cases of bladder cancer and 30 cases of normal bladder tissue was determined by immunohistochemistry. The results of immunohistochemistry were analyzed with the sex, size, number, pathological grade and clinical stage of bladder cancer. The results showed that the expression rate of UT-B was 72.86% (51 / 70) in bladder cancer tissue and 26.67% (8 / 30) in normal bladder tissue, the difference was statistically significant ($P < 0.05$). The positive rate of UT-B was 54.17% (13/24) in low-grade bladder cancer and 82.61% (38/46) in high-grade bladder cancer, the difference was statistically significant ($P<0.05$). The positive expression rate of UT-B was 61.11% (22/36) in non-muscle invasive bladder cancer and 85.29% (29/34) in muscle invasive bladder cancer, the difference was statistically significant ($P< 0.05$). There was no significant difference in the expression rate of UT-B between the groups of gender, age, tumor size and tumor number ($P > 0.05$).

1. Introduction

Bladder Cancer is the most common malignant tumor in urological tumors in China. Incidence rate and mortality rate are the first in urologic tumors. At present, the incidence rate of bladder cancer in China has been increasing year by year, and has become a serious disease which seriously endangers the survival of the nation. The diagnosis and screening of bladder cancer by biopsy under the cystoscope is still the current "gold standard". However, nearly 30% of the patients have been in

muscular invasive bladder cancer because of its invasive examination and detection and diagnosis. There are two major categories of non myometrial invasive bladder tumor and myometrial invasive bladder tumor. In the first diagnosis, more than 70% of the patients are non myometrial invasive bladder tumor, and about 20% are myometrial invasive bladder tumor. There are obvious differences between the two types of bladder tumors in clinical treatment and prognosis, so it is very important to distinguish and make different treatment plans accurately. Therefore, we need to actively look for the differences between the two tumors at the molecular level, which will help us to accurately diagnose, reasonably treat and evaluate the prognosis of bladder tumors. Although clinicians and researchers have done a lot of research in the world, the pathogenesis of bladder cancer is still unclear. However, the complex process of multiple genes and steps in the development of bladder tumor is definitely related to the abnormal expression of multiple tumor suppressor genes. In recent years, some progress has been made in the study of tumor suppressor genes related to bladder cancer at home and abroad. Different types of tumors have a common feature in the process of occurrence and development: the disorder of cell cycle regulation mechanism leads to cell growth out of control. Cell cycle regulation includes positive and negative two-way regulation mechanism, which is regulated by various positive and negative regulatory proteins. If either side of the regulatory mechanism is abnormal, the cell growth will be out of control.

Due to the importance of urea channel protein research, many research teams began to study urea channel protein, and achieved good results. Renal epithelium expresses at least two different types of urea transporter mRNA, called UT1 and uT2, which are obtained from a single ut gene by selective splicing. Previous studies using polyclonal antibodies that did not distinguish the protein products of the two transcripts showed that the expression of urea transporter protein was limited to the descending branch of the intramedullary collecting duct and Henle ring. In order to determine the transcripts of protein expression in these two structures, Atala used UT1 - and uT2 specific primers to study the microanatomy structure by reverse transcription polymerase chain reaction. UT1 mRNA was detected only in the intramedullary collecting duct, consistent with the vasopressin regulated urea transporter. On the contrary, uT2 mRNA was expressed in the medulla of the short and long descending branches of Henle. This localization is consistent with the prediction of uT2 in the urea cycle of medulla. Therefore, combined with previous physiological studies, their data show that these transporters play a central role in the mechanism of urine concentration [1-2]. Urea transporter is a kind of membrane protein that transports urea molecules across cell membrane and plays an important role in many physiological processes. Although the crystal structure of bacterial urea channel dvut has been solved, the kinetics of urea transport in dvut is still unknown. Klein used molecular dynamics simulation, Monte Carlo method and adaptive partial pressure method to establish the equilibrium structure of dvut, calculated the change of urea free energy, determined the urea binding site of dvut, deeply understood the micro process of urea transport, studied the water permeability of dvut, including the analysis of pore water chain. This strategy can be used to study the transport behavior of other membrane proteins [3-4]. Although the current research results are relatively rich, there are still deficiencies, mainly reflected in the inability to determine the role of urea channel protein in cancer cells.

In this study, the bladder cancer tissues of rats were used for immunohistochemical examination. After surgical resection, 10% formalin was put in immediately and stored in a refrigerator at - 80 ℃. All samples detected by RT-qPCR were cut off during the operation, immediately collected and stored in liquid nitrogen, and then stored in a refrigerator of - 80 degrees. The experimental results were analyzed by spss23.0.

2. Basic Properties of Urea Channel Protein

2.1. Urea Channel Protein

UT-A is mainly distributed in the kidney, while UT-B is widely distributed, such as the lower branch of the kidney, bone marrow, red blood cells, brain, liver, heart, testis and other tissues and organs. UT-A1, UT-A3 and UT-A4 were expressed in the renal tubular epithelial cells in the muscle, and UT-A2 was expressed in the renal epithelial cells in the medullary ring industry decline. UT-A5 and UT-A6 were expressed in testicular tubular cells and colonic mucosa, respectively. Many subtypes of UT-A are also expressed in the heart, brain and liver. Compared with UT-A, UT-B is widely used. The northern tissue analysis of many human and rat tissues, such as heart, liver, brain, kidney, small intestine and prostate, found that these tissues contain the transcripts of UT-B, which are expressed in the bladder and urinary tract of dogs and rats. Because of the large expression of UT-B mRNA in stem cells and secondary neurons of midbrain, it suggests that UT-B is of great value for brain function [5].

(1)UT-A

In the mammals analyzed, the kidney contains a lot of mRNA and UT-A protein, especially UT-A1 and UT-A2. The amount of UT-A1 and UT-A2 mRNA in renal segments was analyzed with specific promoters to determine the presence of UT-A1 transcript UT-A2 mRNA in the inner and middle medullary colonies (IMCD) at the outer bottom of the short grass ring (type I) and the inner bottom of the long grass ring (type III). Stenosis was detected in the renal bone marrow, suggesting that there was apical membrane and cell proliferation of IMCD cells in UT-A1[6].

A 55kD protein was found in the muscle bone marrow. Its anti-UT-A119 amino acid antibody l194 was expressed in the fine descending branch of type I and I ring. The number of amino acids was the same as predicted by 397 amino acids and UT-A2 mRNA. In addition, the presence of UT-A2 mRNA was detected in the downstream section of Henle ring, so this protein was considered to be UT-A2 [7-8].

The antiserum of UT-A3 was detected at the carboxyl end of UT-A3, and it was found that UT-A3 existed in the cell armament of IMCD. It can be used as a cell's UTS storage tank, and has the ability to enter the plasma membrane, leading to the rapid improvement of urinary system permeability [9].

The length of UT-A5 mRNA was 1,5 KB. Recently, it was found that it was only expressed in testis. In situ hybridization was found in the cells around the spongy tube. Subcellular detection is not clear. This may be related to urine mediation in diffuse catheters, and little is known about UT-A4. The content may be very low, which requires more sensitive technical detection [10-11].

(2)UT-B

It is expressed in human heart, skeletal muscle, colon, small intestine, brain, angry gland, prostate, liver, ovary, renal pelvis and bladder. Similar distribution was found in rat tissues, but only one copy was 3.8kb. There was no mRNA expression in human erythrocytes, but its transcripts were found in mature erythrocytes of human spleen and hel cell line of erythropathy [12].

UT-B protein is particularly expressed in erythrocytes. Western blot detected 46-60kd wide band in human erythrocytes by using anti UT-B amino terminal antibody. When treated with n-glucosidase, only 36KD region was left, which indicated that UT-B only expressed one form in erythrocytes and had different degree of glucosylation. UT-B is abundant in stem cells and secondary neurons of midbrain.

2.2. Physiological Significance of UT-B Protein

(1) Concentration of urine

Urine concentration in the kidney is a complex process, in which urea protein plays an important role in urine concentration. The mechanism of urea cycle includes: 1) under the control of Angiotension and Angiotension, the cortical and outer medullary collecting tubes reabsorb water. Urine does not penetrate, resulting in a high concentration of urea in the collection tube; 2) the end of the muscle in the collection tube depends on the pressure. UT-A1 / 3 urea channel protein increases the permeability of urea, makes the concentrated urea flow into the muscle and active excretion of urea at the end of inner tube and ut-a2 urea, reduces the exhaust of pith ring, and forms the accumulation of intramedullary urea. 3) The urine taken from the ascending small vessel branch is returned to the renal marrow small vessel through the UT-B urinary tract protein of the Department. Therefore, in the urine concentration mechanism, it is very important to maintain the flow of medullary urea from the renal cortex to the concentration degree and osmotic pressure.

In addition to the direct rise of endothelial cells through the internal muscle of urine in the form of microvasculature, the permeability of the above-mentioned parts in urea is caused by urea channel protein. At present, gene targeting technology has been used to establish UT-A1AJT-A3 gene deletion models for urea channel proteins, UT-A2, UT-B and UT-A2-B * and has been applied to clinical study of kidney physiology in rats. The gene of rats shows that the spleen of UT-B nocturnal mice has decreased, the urine concentration has decreased by 50%, the water intake and urine volume have increased by about 50%, the retention of urine has been reduced by about 1/3, and the proportion of urine and urine has decreased by about 50%. The mutation of human UT-B gene also leads to the decrease of RBC urea permeability and mild urinary inconvenience. There was no significant change in other organs and functions.

(2) Water permeability

AQPl and double gene knockout mice were studied with UT-B knockout mice and aquaprin-1 mice. Compared with AQPl mice with stroke, the morphology and function of RBC in double stroke mice changed little, and the permeability of water decreased more obviously. The use of UT-B inhibitor can partially inhibit the permeability of mice by simply knocking out AQPl, but it has little effect on the permeability of WT mice, single UT-B mice and double knockout mice, indicating that UT-B is permeable in water.

2.3. Evaluation Index of Immune Function

Immunity comes from the autoimmune system, including immune organs, immune tissues, immune cells and immune factors. Immune cells and immune cells are distributed in the whole body, forming a network of mutual constraints, determining the immune function of the body, resisting bacteria and viruses, eliminating body damage and deformation, aging cells and metabolic waste, and preserving the stability of the body environment. Human immunity is divided into specific immunity and non-specific immunity, the latter is the basis of the former, and the specific immunity produced by immune substances may enhance the role of non-specific immunity. Special immunity includes cellular immunity and juice immunity. Cellular immunity is mainly achieved by T cells. T cells proliferate, differentiate and transform into T cells stimulated by antigens. When the same antigen enters the body again, t will realize that cells can directly kill the antigen, and multiple cytokines secreted directly from T cells can jointly kill and destroy cells to carry pathogens, which is called cellular immunity. Fungal immunity is the transformation of B lymphocytes into plasma cells with immune activity under the stimulation of antigens. The antibodies produced by plasma cells can be connected with the corresponding antigens, neutralized, accumulated and sat together with antigens to purify and kill pathogenic microorganisms. It seems that the number, activity and proliferation of lymphocytes are closely related to the self-defense ability of human body, which is an important indicator for evaluating human immunity.

Human lymphocytes include lymphocytes, B lymphocytes and NK cells. T cell is a complex population. According to their different immune functions, they are divided into helper T cells (th) and suppressor T cells. Inhibitory T cells, cytotoxic T cells, delayed allergic T cells and memory T cells. Cells express CD4 molecules (modulation complex 4, CD4) on their cell surface, also known as CD4 + T cells, and T lymphocytes (TC) and T lymphocyte inhibitors (TS) express CD8 on their cell surface. Modulation molecular complex (CD8) is also known as CD8 + T cells. CD3 (modulation complex 3) can be expressed in all T cells and is a common surface marker of T cells.

Clinically, flow cytometry is usually used to detect the number of lymphocyte subsets according to different lymphocyte surface indicators, and to evaluate the level of immunosuppression according to the number of lymphocytes and the ratio of CD4 + / CD8 + T cells. When the ratio of CD4 + CD8 + T cells is high, it indicates that the immune status of the body is good. When the ratio of CD4 + CD8 + T cells is low or low, it may cause various immune diseases in animals. The immune level decreased, so the number of lymphocyte subsets in the body is an indicator of the immune function of the body.

The transformation of lymphocytes means that T and B lymphocytes can be activated and transformed into lymphocytes in vitro. The transformation rate can reflect the activity and proliferation of lymphocytes. The decrease and increase of lymphocyte transformation rate indicate the increase of cellular immune level. Therefore, the lymphocyte transformation rate of spleen can be used to evaluate the cellular immune level of the body.

The immune organs closely related to fungal immunity and cellular immunity are malignant glands and spleen. The angry gland is the main organ of T cell differentiation, development and maturation. Under the unique microenvironment, the lymphoprogenitor cells infiltrating the bone marrow undergo a complex process of differentiation and development and eventually become functional CD4 + T cells and CD8. Thymic T cells are produced in local lymphoid organs and tissues, which are involved in the maintenance of adaptive immune response and immune memory. If anger develops abnormally and fails to produce functional T cells, there will be no T cell immunity.

Spleen is the hematopoietic organ of embryo. After the white bone marrow began to bleed, the spleen developed into the largest regional immune organ. Spleen is the place where T cells and B cells are formed, and it is the main place where the immune response of the body to blood-derived antigens occurs. The spleen may also synthesize and secrete some important bioactive substances. At the same time, the spleen can remove pathogens and red blood cells from the blood. Among them, white blood cells, immune complex and foreign bodies play the role of filtration, so that the blood can be purified. Therefore, immune organ index seems to be an important index to evaluate immune function from a macro perspective.

3. Experimental Materials and Methods

3.1. Experiment Preparation

100 SPF female SD rats of 10-12 weeks old with body weight of (220 ± 20) g were selected and provided by a breeding company. The experimental animals were raised in the SPF animal laboratory of the science and education building of the West Coast medical center of Huangdao. They were fed with regular diet and drinking water for 12 hours, with a humidity of 50% - 60% and a temperature of 20-24 ℃. Feed for SD rats is provided by a breeding Co., Ltd. during the whole experimental research process, SD rats are given humanitarian care according to the 3R principle of experimental animals.

Preparation of experimental sample candle block: immerse 10% formalin solution for 24 hours,

correct the surgical sample immediately, cut the obtained tissue and record it, then continue the following steps: wash it with 70% ethanol for three times, each time for 60 minutes, dehydrate: add ethanol solution according to the following proportion, 85% 95% 100% 100%, and keep the adding time at 0,5 ~ 1, 0 hour, transparent, add according to the following reasons: 50% ethanol + 50% xylene xylene xylene, keep the adding time at 0,5-1,0 hour each time, then put the material into the box filled with paraffin, and put it into water for cooling for 5-10 minutes.

3.2. Main Instruments

Inverted microscope;
Automatic high pressure steam sterilizer;
Low temperature refrigerator (- 70 ℃):
Constant temperature water bath box;
Electric blast drying oven;
Electronic balance;
Microplate Reader;
Dyy-iii2 stable voltage and current electrophoresis instrument;
Protein Mini vertical plate electrophoresis, semi dry membrane transfer;
Low temperature high speed centrifuge;
Gel imaging system;
PCR instrument;
Ultraviolet spectrophotometer.

3.3. Experimental Immunohistochemical Staining and Result Determination

5 μ Mn sections were prepared from the paraffin samples of bladder cancer after operation. Immunohistochemical SP staining was performed. The operation was carried out according to the instructions of the kit. PBS was used as the negative control instead of the first antibody, and the known positive sections of bladder cancer were used as the positive control. The results showed that the brown granules were stained with UT-B in cytoplasm or cell membrane. Five high power fields (X400) were selected from each section, and 100 tumor cells were counted under each field. The results were interpreted by the combination of staining intensity and positive area percentage. Staining intensity: 0 for no staining, 1 for light yellow, 2 for brown yellow, 3 for brown brown; 0% for positive area, 1 for 1% to 10%, 2 for 11% to 50%, 3 for 51% to 80%, 4 for > 80%. The final score is the product of staining intensity and percentage of positive area: 0 ~ 2 points are negative, 3 ~ 4 points are weak positive, 6 ~ 8 points are medium positive, 9 ~ 12 points are strong positive.

3.4. Statistical Method

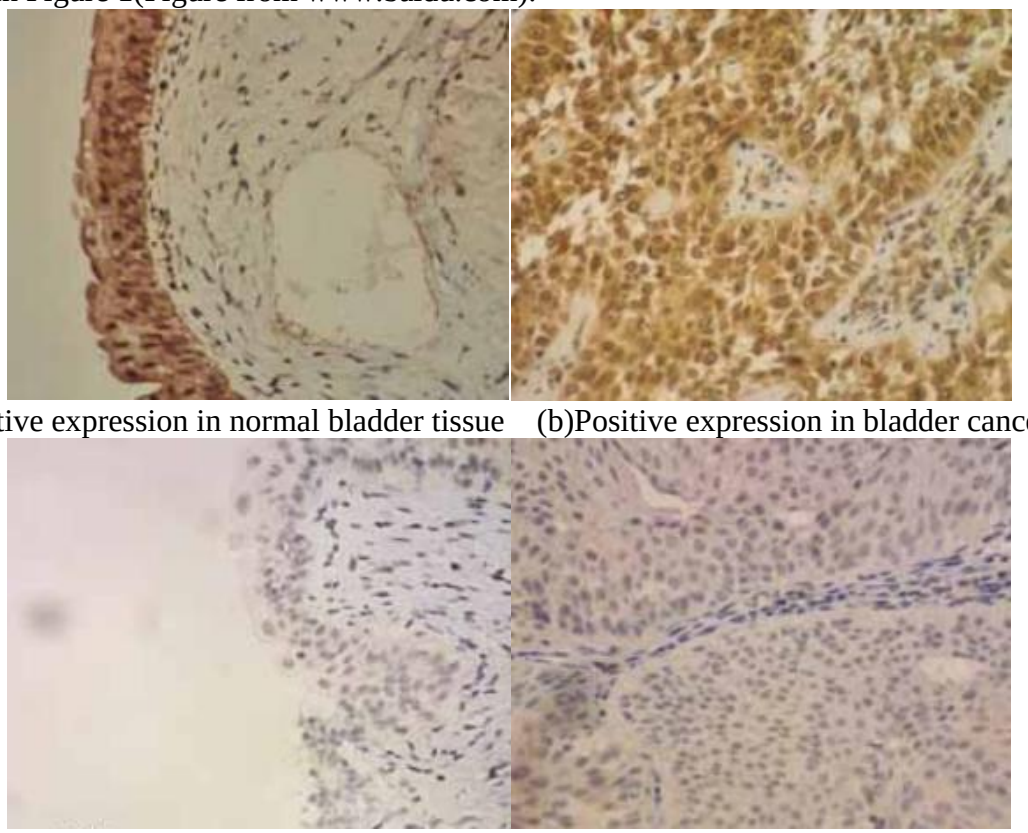
Spss19.0 statistical software for data processing, data comparison using t-test, $P < 0.05$ for the difference was statistically significant.

4. Expression of Urea Channel Protein B in Bladder Cancer of Rats

4.1. Analysis of UT-B Protein in Bladder Cancer

Quickly put the frozen bladder tumor tissue samples at - 80 ℃ into the milk bowl precooled with liquid nitrogen, add liquid nitrogen repeatedly, crush the tissue repeatedly with the milk stick until no obvious particles can be seen. Transfer the abrasives to a 1.5ml tube containing 300ul

buffer Rl (50dttsolution has been added before use) of the cracking solution, and blow the cracking solution repeatedly with a pipette until no sediment is seen. Put the solution into a 4 ℃ constant temperature centrifuge at 15000rpm for 6 minutes. Immunohistochemical method was used to detect the expression of UT-B in normal bladder tissue and bladder cancer tissue. UT-B was mainly expressed in cytoplasm, which showed that there were yellow to brown granules in the cytoplasm, as shown in Figure 1(Figure from www.baidu.com).



(a)Positive expression in normal bladder tissue (b)Positive expression in bladder cancer

(c)Negative expression in normal bladder tissue (d)Negative expression in bladder cancer

Figure 1. Expression of UT-B in normal bladder tissue and bladder cancer tissue

Most of the UT-B protein was negative expression, a few were positive expression, most of the positive expression was diffuse distribution, the cell location had two forms of cytoplasm and cell membrane, and the staining intensity was different. Among 70 cases of bladder cancer, 51 cases were positive for UT-B, the positive rate was 72.86% (51 / 70). Among 30 normal bladder tissues, 8 cases were positive for UT-B, the positive rate was 26.67% (8 / 30). The difference between groups was statistically significant ($P = 0.000 < 0.05$) (as shown in Table 1).

Table 1. Expression of UT-B in normal bladder tissue and bladder cancer tissue

UT-B						
Category	Number of cases	Positive	Negative	Positive rate(%)	X^2	*P*
Normal bladder	30	8	22	26.67		
Bladder Cancer	70	51	19	72.86	18.522	0.000

Macroscopically, the cancer tissue of rats is large, irregular in shape, densely distributed in the blood vessels, the color of incision is deep, no necrosis or a small amount of necrosis. The observation of tumor tissue fragments under microscope showed that prostate tumor cells were closely arranged, with different shapes and sizes, large nuclei and deep colors. The tumor cells nest obviously and grow vigorously. After UT-B injection, the body weight of bladder tumor mice also

changed, as shown in Figure 2.

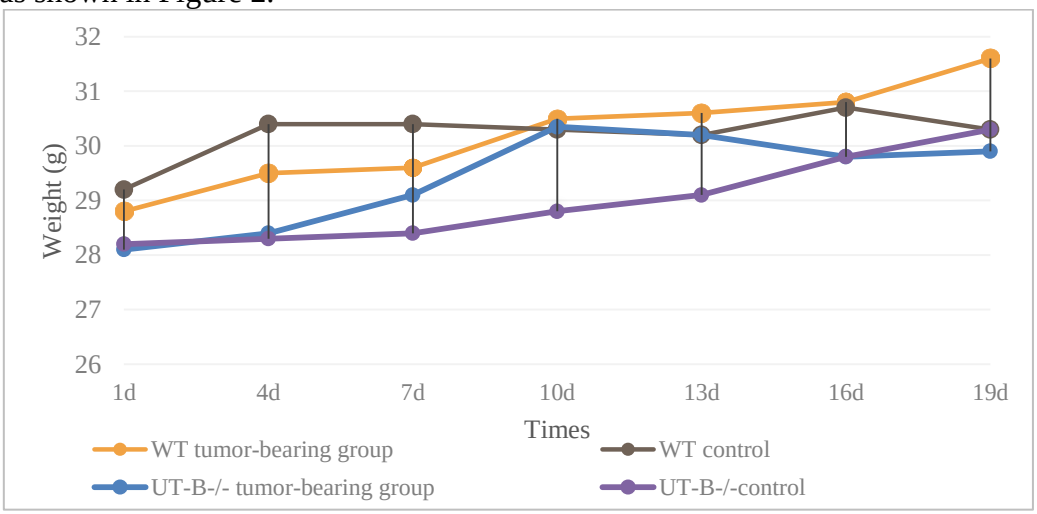


Figure 2. Dynamic changes of body weight after bladder tumor in WT and UT-B/- mice

As can be seen from Figure 2, after bladder volume, the weight of UT-B / bladder volume group increased first and then decreased slowly, with the average weight lower than the other three groups. The weight of mice in bladder tumor group increased gradually after bladder tumor, and the average weight was higher than that of other three groups: WT control group and UT-B / control group increased first and then tended to be the same; the average weight was between UT-B / bladder volume group and bladder volume group. The dynamic changes of bladder tumor weight in mice indicate that the establishment of bladder tumor model is successful. The knockout of UT-B gene enhanced the immunity of mice and played a certain role in the inhibition of tumor. UT-B gene affects the weight and size of bladder tumor transplanted in mice.

4.2. Analysis of Immunohistochemistry Results

The results of immunohistochemistry showed that UT-B was abundantly expressed in the normal bladder tissue adjacent to cancer, and decreased in the normal bladder tissue adjacent to cancer. The positive expression rate of UT-B in the normal bladder tissue adjacent to cancer was 93,8%, 53,8% and 7,1% in the low-level bladder volume group and the high-level bladder volume group, respectively (P = 0000). For each two different groups, the degree of expression is compared with each other and remains statistically important. As shown in Figure 3.

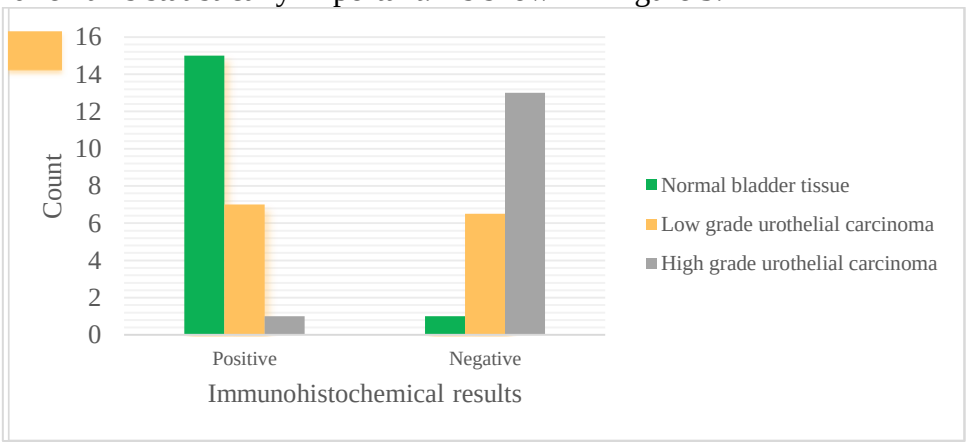


Figure 3. Results of immunohistochemistry among the three groups

The expression of UT-B in normal bladder tissue and low and advanced bladder cancer decreased with the increase of volume (r = 0723, P = 0000). The expression level of UT-B decreased with the increase of urine volume (r = 0464, P = 0026), the expression level of UT-B decreased with the increase of volume (r = 0886, P = 0000), and the expression level of UT-B decreased with the increase of volume (r = 0511, P = 0008).

The UT-B and internal reference genes were transformed into normal bladder tissues adjacent to cancer, and the low-grade urea cancer group and high-grade urea cancer group were shown in Figure 4. RT-qPCR showed that the expression level of UT-B mRNA was the highest in normal bladder tissues, while in urinary system tumors, its transcription level decreased with the increase of tumor malignancy. MRNA was the lowest in the three groups (r < 0,05). The comparison among the three groups is still statistically significant.

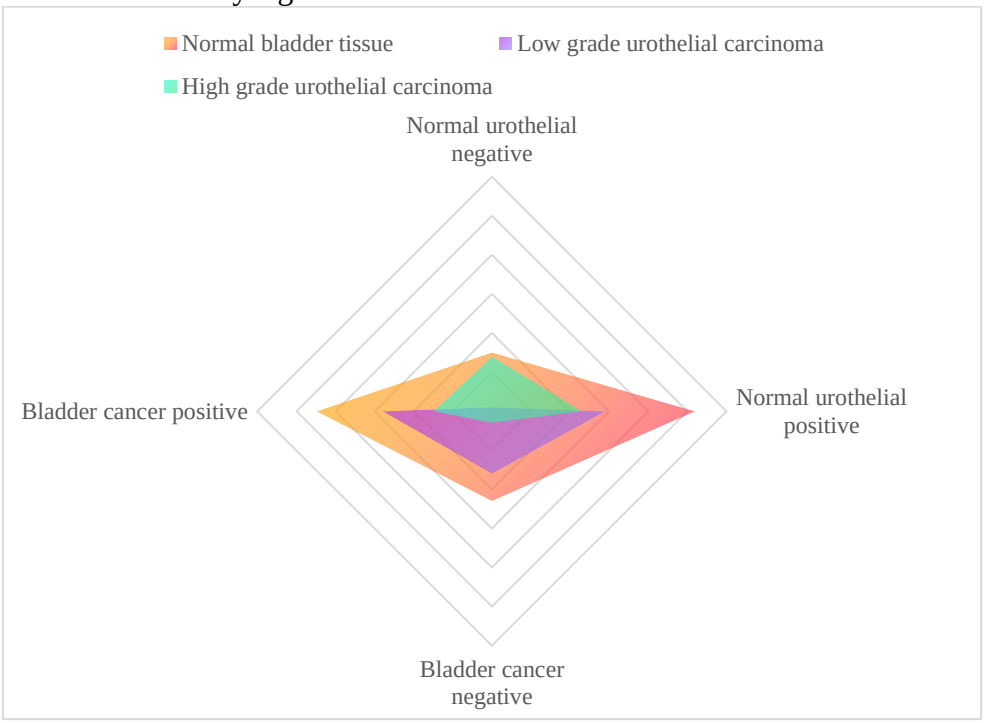


Figure 4. Levels of transcribed mRNA of UT-B and internal reference genes in bladder cancer tissues

The expression of UT-B decreased gradually in bladder cancer, paracancerous tissue and normal bladder tissue. In the animal model, the UT-B transgenic mice were exposed to the carcinogen nitrite. The UT-B transgenic mice could skip the stage of non-invasive bladder cancer and directly develop from bladder cancer in situ to invasive bladder cancer. In order to prove that UT-B plays an important role in promoting the growth and infiltration of bladder cancer, many scholars have observed the growth of bladder cancer cells by inhibiting the expression of UT-B. After silencing UT-B gene in human bladder cancer cell lines T24 and 5637 by RNA interference technology, it was found that the cycle of tumor cells was blocked in G0-G1 phase, the number of tumor cells in S phase was reduced, and the proliferation of tumor cells was effectively inhibited, and similar results were obtained in these two different bladder cancer cell lines, which fully proved that inhibiting the expression of UT-B can inhibit the growth of bladder cancer cells.

Further stratified analysis showed that the positive rate of UT-B was 54.17% (13 / 24). The positive rate of UT-B was 82.61% (38 / 46). The difference between the two groups was statistically significant (P = 0.011 < 0.05). It is suggested that the expression level of UT-B is related to the

pathological grade of the tumor, and the positive rate of UT-B expression in high-grade bladder urothelial carcinoma is higher than that in low-grade bladder urothelial carcinoma, indicating that the higher the tumor grade, the higher the expression level. The positive rate of UT-B was 61.11% (22 / 36) in 36 cases of non-muscle invasive bladder urothelial carcinoma (Ta-T1) and 85.29% (29 / 34) in 34 cases of muscle invasive bladder urothelial carcinoma (T2-T4). The results showed that the expression level of UT-B was related to the clinical stage of the tumor, and the positive rate of UT-B expression in myometrial invasive bladder urothelial carcinoma was higher than that in non myometrial invasive carcinoma, suggesting that the deeper the tumor infiltration, the higher the expression level.

5. Conclusion

A large number of data show that the harm of bladder cancer to human beings is extremely serious in the world, and it is also a huge solid tumor that researchers and medical workers need to solve urgently. At present, the treatment strategy of bladder cancer is mainly surgical resection, supplemented by chemotherapy, radiotherapy and immunotherapy, but the treatment effect is not ideal. About 50% - 70% of bladder surface cancer will recur, 10% - 15% of recurrent cases may occur, and metastasis, about 30% - 40% of invasive bladder cancer has distant metastasis. The purpose of this study was to investigate the expression and transport of urea channel protein B in rat bladder cancer, and to determine the possible role of UT-B protein in the low expression of bladder cancer.

This study found that the positive expression rate and staining intensity of UT-B protein in bladder cancer gradually decreased with the increase of tumor tissue grade. The higher the tumor tissue grade, the worse the differentiation of tumor cells, the higher the malignant degree. The results of this study suggest that the worse the differentiation of tumor tissue, the lower the expression of UT-B protein. The expression of UT-B protein in bladder cancer is closely related to the degree of tumor invasion. The positive expression rate of UT-B protein decreases with the degree of tumor invasion. Pathological grade and clinical stage of tumor are important indexes for clinical evaluation of prognosis of bladder cancer patients. Therefore, the expression of UT-B protein in bladder cancer tissue can reflect the degree of malignant progression of tumor. The malignant degree of bladder cancer patients with high expression of UT-B protein is lower, while the probability of tumor infiltration and malignant development of bladder cancer patients with low or no expression of UT-B protein may be higher, The prognosis is worse.

To sum up, we found that the decrease or absence of UT-B protein expression in bladder cancer tissue may promote the occurrence and development of bladder cancer, thus promoting the growth and metastasis of bladder cancer cells. However, due to the lack of sample size, there is no significant difference with clinical pathological parameters. All in all, this experiment can provide a theoretical basis for the later experiment, and also provide a new idea and theoretical basis for the early detection, diagnosis and treatment of bladder cancer.

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Data Availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Conflict of Interest

The author states that this article has no conflict of interest.

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