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Expression and Clinical Significance of SATB1 Gene in Urothelial Carcinoma of Bladder

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Abstract: Urothelial carcinoma of bladder is a highly malignant urological tumor, and its occurrence and development are regulated by multiple genes. This article selected 40 cases of bladder urethral epithelial cell carcinoma confirmed by pathology in the urology department of the hospital from 2020 to 2021. Mutations in the SATB1 (Special AT-rich Binding protein 1) gene can cause changes in thousands of genes, which have a significant impact on the development and metastasis of cancer. Moreover, the incremental regulation of SATB1 is likely to be a key gene cluster switch leading to cancer metastasis. This article used SP immunohistochemistry to detect the expression of SATB1 gene in bladder urothelial carcinoma cells and verify its function, in order to lay a foundation for future research and clinical application. The positive rate of SATB1 protein was 56.3%, and the negative rate of SATB1 protein was 43.7%.

1. Introduction

Urothelial carcinoma of bladder is one of the most common causes of death in urinary system tumors. Its incidence rate is as high as 85-90%, which seriously threatens human life and health. The incidence rate of urothelial carcinoma of bladder is increasing year by year. At present, only about 30% of urothelial carcinoma of bladder patients in clinical practice have local or distal recurrence and metastasis, with a median survival rate of less than 1 year, poor prognosis, and limited treatment options. Therefore, a deep understanding of the malignancy and pathogenesis of urothelial carcinoma of bladder, as well as the search for molecular markers that can effectively predict its prognosis, has become a hot topic in renal cell carcinoma research. Finding and utilizing new molecular markers combined with conventional tumor diagnosis methods, clinical pathological features, and prognostic indicators is of great significance for guiding clinical doctors to develop personalized treatment strategies, achieving early prevention, diagnosis, and treatment of urothelial carcinoma of bladder, and improving the prognosis of urothelial carcinoma of bladder.

So far, there have been few reports on the role of SATB1 in renal cell carcinoma, and the molecular mechanism of whether SATB1 affects the high invasiveness and metastasis of renal cell

carcinoma cells by regulating EMT is also unclear. Yang Tao planed to use the urothelial carcinoma of bladder cell line Beclin1 as the entry point to detect the expression level of miR-34a in gemcitabine resistant cell lines. He used flow cytometry to measure cell apoptosis [1]. Ji Bozhong analyzed the clinical and pathological characteristics of incidental prostate cancer in radical resection of the bladder and prostate, and explored the reasons for the impact on the detection rate of urothelial carcinoma of bladder cells. He studied the clinical data, pathological characteristics and immunophenotype of 7 male patients with bladder cancer who underwent radical cystoprostatectomy and followed them [2]. Wang Zihua established an enhanced CT (Computed Tomography) imaging omics model for preoperative diagnosis of bladder urothelial carcinoma. Using logistic regression, he constructed an imaging omics prediction model to plot the working characteristic curve of the subjects and calculate the area under the curve, thereby evaluating the effectiveness of the model for histological grading of BUC [3]. Their study did not consider the relationship between urothelial carcinoma of bladder and the SATB1 gene.

This article aimed to analyze the expression of SATB1 in bladder epithelial cell carcinoma using methods such as immunohistochemistry and reverse transcription polymerase chain reaction (RT-PCR). The expression of SATB1 in bladder epithelial cell carcinoma tissue was also preliminarily analyzed, and the role of SATB1 in bladder epithelial cell carcinoma was further verified in vitro experiments, providing theoretical basis for subsequent research and clinical application. This provided important experimental and theoretical basis for the subsequent expression and clinical prognosis evaluation of SATB1 in urothelial carcinoma of bladder.

2. Urothelial Carcinoma of Bladder and SATB1 Gene

2.1. Urothelial Carcinoma of Bladder

Urothelial carcinoma of bladder is the most common type of urogenital system tumor. The prognosis of urothelial carcinoma of bladder is poor. Currently, surgery is still the main treatment in clinical practice, and it is not sensitive to radiation or chemotherapy. Infiltration and metastasis are important factors determining the prognosis and efficacy of patients with urothelial carcinoma of bladder, and are also a hot topic in current research on urothelial carcinoma of bladder [4-5]. The infiltration and metastasis of tumors are closely related to molecular mechanisms such as decreased cell adhesion, breakthrough of the basement membrane into the bloodstream, evasion of immune surveillance, and distant metastasis. Among them, they are closely related to various signaling pathways, cytokines, and other factors, which are important factors affecting the prognosis of tumor patients. At the same time as a tumor undergoes metastasis, specific changes in tumor cells also occur, resulting in the ability to metastasize to a distant location. The occurrence, development, and metastasis of tumors are important links in their occurrence and development. The progression of tumor cells from early growth to late metastasis is a complex multi-level process. Early research focused on genetic mutations, but such mutations were not yet sufficient to cause tumor metastasis. This genetic or acquired change can cause infiltration and metastasis of tumor cells. The circular structure of chromosomes is the foundation of gene expression. In recent years, research has found that the occurrence of tumors is closely related to changes in chromatin structure, and the occurrence of tumor gene mutations is closely related to it. The research results indicate that the expression level of proteins related to chromosome structure has a significant impact on the mutation frequency of oncogenic genes [6].

2.2. SATB1 Gene

The invasion and metastasis of cancer are determined by multiple levels of transcriptional

regulation. Currently, research on cancer metastasis mainly focuses on three levels, namely DNA (Deoxyribonic Acid), chromatin, nucleus, etc. [7-8], and there is a close relationship between the three levels. The nuclear matrix is a network like structure in eukaryotes, which is the site of DNA replication and closely related to chromatin, thus playing a very important role in regulating gene expression. SATB1, as an important nuclear interstitial binding protein, interacts with and interacts with the nuclear interstitium and interstitium.

The occurrence of cancer is caused by abnormal changes in one or more oncogenic or tumor suppressor genes, which can affect cell growth, proliferation, differentiation, apoptosis, and ultimately lead to malignant progression of cancer [9]. The transcription regulation pathways involved in SATB1 are diverse and can be summarized into three levels: the first is the regulation of SATB1 at the transcription and translation levels; The second is the interaction between proteins, which includes the formation of their own dimers. The third is to study the posttranslational regulation mechanism of SATB1 protein in cells. This article further explored the regulatory mechanisms of SATB1 at the transcription and translation levels, with a focus on the latter two aspects. SATB1 is a type of gene with a wide range of functions, which can promote gene transcription while also inhibiting gene transcription.

Tumor metastasis is the main factor leading to patient death, but its molecular mechanism is still unclear [10-11]. In a nude mouse tumor model, knocking out SATB1 can inhibit the proliferation and migration of liver cancer cells with high metastasis rates [12]. However, overexpression of SATB1 in low invasive cancer cell lines can significantly enhance the invasion of breast cancer cells [13-14].

2.3. Research Materials

SATB1 is widely expressed in the human thymus and is a newly discovered gene transcription regulatory factor that plays an important role in T cell activation and epigenetic regulation. SATB1 is highly expressed in human highly invasive renal cell carcinoma cell lines, and knocking out SATB1 can significantly inhibit the invasion and proliferation of highly invasive renal cell carcinoma cell lines [15]. Based on the previous work, this article aimed to detect the expression of SATB1 in renal cell carcinoma and adjacent tissues, and analyze its correlation with clinical pathological indicators of renal cell carcinoma [16-17].

This article selected 40 cases of bladder and urethral epithelial cell carcinoma tissue confirmed by pathology in the urology department of the hospital from 2020 to 2021. All patients have not received preoperative chemotherapy or radiation therapy [18]. Bladder cancer samples were put into a refrigerator at -80℃ for RT-PCR detection [19-20].

Among 40 samples of bladder cancer, 20 were male and 20 were female. According to the diagnosis results of WH02004, there were 15 cases of low grade and 25 cases of high grade [21]. Using the UICC (Union International ControleCancer) method, patients were divided into Ta to T1 stages, and 30 patients were divided into T2 to T4 stages. Among them, 10 patients underwent preoperative radiation, intraoperative frozen sections, or postoperative follow-up, and 30 patients did not experience lymph node metastasis.

UICC TNM classification: 5 cases Ta~T1, 10 cases T2~T4. 14 patients were followed up after surgery, of which 21 patients did not experience lymph node metastasis after surgery.

3. Experimental Methods for Expression in Urothelial Carcinoma of Bladder

3.1. Immunohistochemical Testing

Expression of SATB1 in 40 cases of bladder cancer mucosa.

3.1.1. Organizational Production

(1) After taking bladder cancer specimens, they were immersed in 10% neutral formalin solution for 12 hours at room temperature [22-23].

(2) Cleaning: After wrapping with sterile sandcloth, the specimen was placed in a glass container, fixed with surgical yarn in the container, and then rinsed with water for 10 hours. After taking bladder slices, they were placed in a glass container and then soaked in physiological saline. They were changed every 30 minutes for 6 hours.

(3) Dehydration: It is necessary to remove water from the tissue and the water contained in the tissue. The cancerous tissue was sequentially placed in a 70%, 80%, 95%, and 100% ethanol jar, which was replaced and dehydrated from low to high, and soaked in different concentrations of solution for 30 minutes. The slices of the bladder were wrapped in a sterile gauze and then dehydrated using the same method from low to high alcohol.

(4) Penetrating wax: The tissue used for penetrating wax is transparent. The mucosa of bladder cancer cells was put into a glass bottle of xylene aqueous solution, and the tissue block can be seen to be translucent or completely transparent with the naked eye. It usually takes 30 minutes to recover.

(5) Wax immersion method: Different tissues were placed in a melted wax solution, and after multiple washes, the residual wax solution in the wax solution can be completely removed. Afterwards, the dissolved wax was poured into a disposable embedding box, and the tissue was quickly picked up with wet tweezers. It was placed flat at the bottom of the embedding box, and when the paraffin was about to solidify, it was soaked in water for 30 minutes. After the paraffin has completely solidified, the slices can be taken out.

(6) Slicing: The slice was gently clamped with ophthalmic forceps. The front of the slice was placed in the exhibition box, unfolded, and then the slice was picked up. During the pasting process, the operator held one end of the clean glass sheet with his left hand to throw it into the water and stick the glass sheet onto it. Then, with the help of the right hand, the operator pushed the glass sheet onto it. After pasting the slices, they were cooled and dried slightly in air, and then they can be baked. After baking, they were sealed and then applied to immunohistochemical testing.

3.1.2. Immunohistochemical Two-step Detection

Expression of SATB1.

(1) Dewaxing and hydration: Before coloring, all wax flakes must be dewaxed with xylene. After preheating the slices in an incubator (which can melt the paraffin on the slices), they were immediately placed in xylene for dewaxing. A tertiary dewaxing agent was used for dewaxing. For tissues that have not been completely dewaxed, after staining, the tissues and cells became blurred, and even formed a map. After dewaxing, it was washed with anhydrous alcohol to remove the xylene component.

(2) Removal: Due to the presence of hydrogen peroxide in the sample causing serious background pollution, it is necessary to remove hydrogen peroxide. Hydrogen peroxide was added to the thin film to inactivate it, and then incubated at room temperature for 10 minutes to inactivate it. After that, it was rinsed with PBS solution for 2 minutes and 3 times.

(3) Antigen repair: After certain tissue cells are fixed by formaldehyde, their sensitivity to immunohistochemical markers decreases, so it is necessary to repair or expose certain antigens before staining. Antigen repair using microwave method: A container containing citrate buffer (pH6.0) was placed into a pathological section to stabilize at around 100℃ for 2 to 5 minutes. In the first 5 minutes, the citrate buffer was observed for any decrease in steam. If so, distilled water can be added appropriately, and the second 5 minutes can be continued.

(4) This project planed to use formalin as the embedding technique for frozen paraffin. By co precipitation with frozen samples and embedding them with non immune animal serum, non specific antigens can be removed and background interference can be reduced. After removing the PBS solution, 50ul of sheep serum was added to the slices and incubated at room temperature for 10 minutes.

(5) After removing the serum and slicing, appropriate dilution of Satb1 primary antibody was added, with a dilution ratio of 1:100. Rinse for 2 minutes and 3 times.

(6) Diantibody working solution A was dropped and incubated at 37℃ for 20 minutes, and rinsed with PBS (Phosphate Buffered Saline) for 2 minutes, 3 times.

(7) Diantibody working solution B was dropped into 37℃ and incubated for 20 minutes, then rinsed with PBS for 2 minutes, 3 times.

(8) After removing the PBS solution, the sample was rinsed with double distilled water to terminate the color reaction.

(9) After washing with clean water, the sample was re stained with hematoxylin, and then washed with PBS solution to darken the staining color. After dehydration and drying, it was sealed with neutral resin. At the same time, PBS was used instead of primary antibody as the blank control group (other methods were consistent with it), and the photos were taken under a microscope.

3.1.3. Measurement and Selection of SATB1 Expression in Bladder Cancer

Using a Japanese color image recording input device and combined with a university image analysis system, slices of different parts were performed under the same staining conditions. Five high magnification (x400 times) fields of view were taken from each slice, with 100 cells per slice. One observer was selected from each of the two and observed under a microscope to count the number of positive cells. When SATB1 appears as a brown granular substance in the cytoplasm, it can be considered positive. The positive rate was calculated and the average value greater than or equal to 25% was taken as positive.

MRNA expression status

Extraction of Total RNA from Cells:

Step 1: Plant cell lines of human renal tubular epithelial cells in different culture bottles at a rate of 5x106/ml, and add them to the corresponding culture medium for in vitro culture.

Step 2: Centrifuge the cell suspension and collect it, add 1mL of Trizol, repeatedly blow or vigorously shake to fully dissolve the cells, transfer to a 1.5mL new EP tube, and let it sit for 5 minutes;

Step 3: Add 0.2mL of chloroform to the EP test tube mentioned above, with the lid tightly closed;

Step 4: Set the conditions to 4 ℃, 12000rcf, and centrifuge for 10 minutes;

Step 5: Take a new clean EP test tube, transfer the aqueous phase from it to the test tube, and centrifuge at 12000rcf (4℃) for 10 minutes;

Step 6: After removing the supernatant, colloidal precipitates can be seen in the EP tube. Add 1 milliliter of 75% alcohol to the tube for cleaning, centrifuge at low temperature for 5 minutes at 4℃ and a rotation speed of 7500rcf;

Step 7: Take out the supernatant and dry the precipitated RNA naturally at room temperature for 10-12 minutes. Add 50ul of DEPC water to the bottom of the EP test tube and let it stand at 55℃ for 10 minutes;

Step 8: Dilute the above RNA with TE (Tris EDTA) solution, measure its content and purity using UV method, and then store at -80℃.

(4) Result evaluation

Using RT PCR method, amplification was carried out according to the reaction conditions and

system described; a standard curve was obtained, and the Ct value was measured.

The Ct value is a pre-set reaction threshold using a negative control as a reference. When the fluorescence signal values collected in each PCR reaction tube reach this threshold, the corresponding minimum number of cycles is the CT value. The SATB1 mRNA (Ribonucleic Acid) of all cell lines was standardized as follows:

$$\Delta_{Ct}\text{Numerical value} = \Delta_{Ct}\,\text{SATB1} - \Delta_{Ct}\text{GAPDH} \quad (1)$$

Next, the values of SATB1 mRNA in the tested sample and negative control were calculated separately, with the Formula (2).

$$\text{SATB1}_{\text{relative}} = 2\,(\Delta\text{Ct}_{\text{To be tested}} - \Delta\text{Ct}_{\text{Negative control}}) \quad (2)$$

3.2. Statistical Analysis

This article used SPSS16.0 for data processing, and the experimental results were in good agreement with the experimental results. The results showed that the difference in measurement data between the two groups was measured using Student's t-test with independent samples. Significant differences are indicated by $P<0.05$.

4. Exploration Results of Urothelial Carcinoma of Bladder

The positive and negative expression rates in urothelial carcinoma of bladder tissue are shown in Figure 1. The positive expression rate was 55.3%, and the negative expression rate was 44.7%.

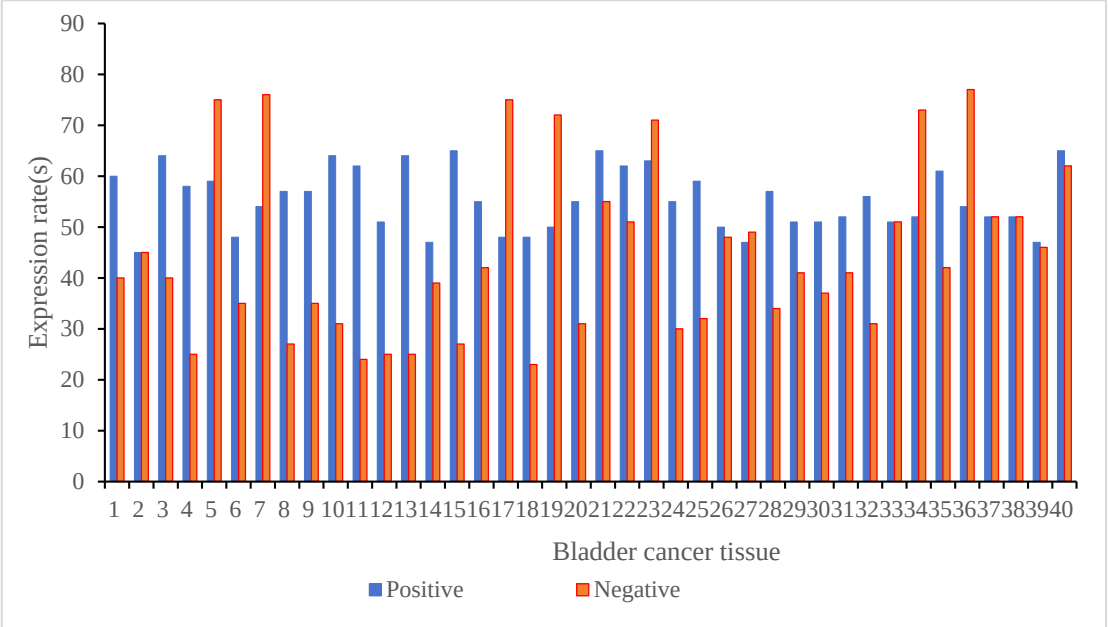

Figure 1: Positive and negative expression rate in bladder cancer tissue

The probability of positive and negative metastasis of SATB1 expression in urothelial carcinoma of bladder tissue is shown in Figure 2. The probability of positive metastasis was 66.6%, and the probability of negative metastasis was 33.4%.

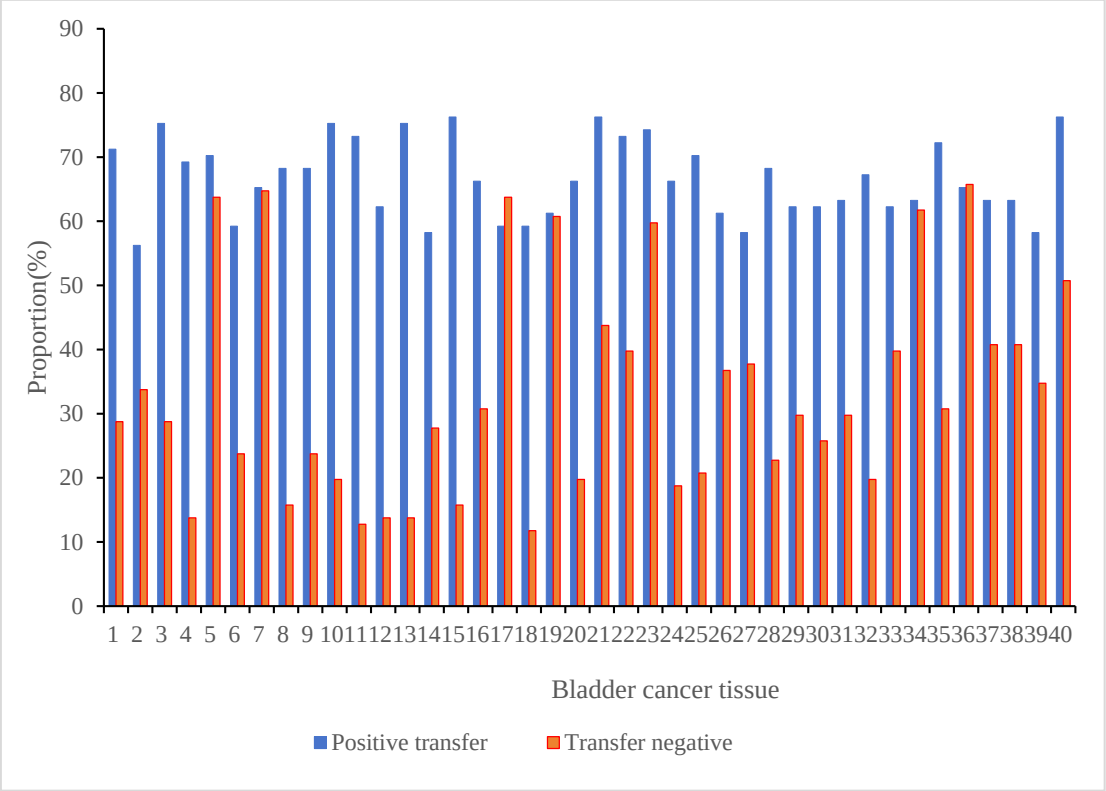

Figure 2: Probability of positive and negative metastasis of SATB1 expression in urothelial carcinoma of bladder tissue

The correlation between the expression of SATB1 mRNA and clinical pathological parameters in urothelial carcinoma of bladder tissue is shown in Table 1. The relative expression level of low-level SATB1 mRNA was 0.143 ± 0.069, while the relative expression level of high-level SATB1 mRNA was 0.021 ± 0.028. The Ta-T1 SATB1 mRNA content in clinical staging was 0.144 ± 0.063, and the T2-T4 SATB1 mRNA content was 0.243 ± 0.026.

Table 1: Correlation between the expression of SATB1 mRNA and clinical pathological parameters in urothelial carcinoma of bladder tissue

Clinical parameters	Grouping	SATB1 mRNA	P
Pathological grading	Low level	0.143±0.069	0.551
	High level	0.021±0.028	0.022
Clinical stages	Ta-T1	0.144±0.063	0.125
	T2-T4	0.243±0.026	0.324
Transfer situation	Transfer negative	0.155±0.053	0.124
	Positive transfer	0.118±0.066	0.442

The proportion of positive and negative SATB1 protein in urothelial carcinoma of bladder tissue is shown in Figure 3. The positive rate of SATB1 protein was 56.3%, and the negative rate of SATB1 protein was 43.7%.

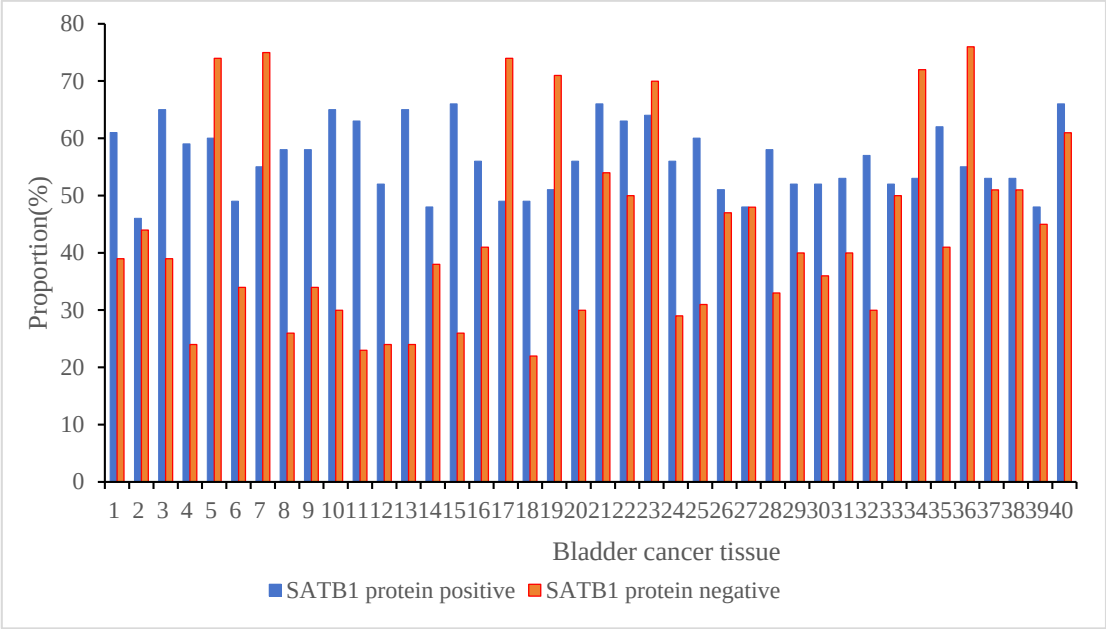

Figure 3: Proportion of positive and negative SATB1 protein in urothelial carcinoma of bladder tissue

5. Conclusion

In this article, SATB1 was highly expressed in urothelial carcinoma of bladder and in metastatic urothelial carcinoma of bladder, suggesting that SATB1 may be involved in the occurrence, development, and metastasis of bladder epithelial carcinoma. This article provided a new idea for the early diagnosis and treatment of bladder cancer. However, whether and how the SATB1 gene is associated with these genes involved in the occurrence and development of urothelial carcinoma of bladder, and how to use the SATB1 gene for early diagnosis of urothelial carcinoma of bladder and participate in the formulation of its treatment plan, all require further research.

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