CORRELATION BETWEEN EXPRESSION LEVELS OF P-STAT3 AND SURVIVIN IN BLADDER UROTHELIAL CARCINOMA TISSUES AND CLINICOPATHOLOGICAL FEATURES

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ABSTRACT

Objective: To investigate the correlation between expression levels of phosphorylated signal transducer and activator of transcription-3 (P-STAT3) and Survivin in bladder urothelial carcinoma (BUC) tissues and clinicopathological features.

Methods: During December 2010 and December 2012, the expression levels of P-STAT3 and Survivin in cancer tissues (study group) and adjacent tissues (control group) of 73 cases of BUC patients in our hospital were detected by immunohistochemical staining, and their correlations with clinicopathological features and prognosis were analysed.

Results: The positive mRNA and protein expression levels of P-STAT3 and Survivin were significantly higher in BUC tissues than those in normal bladder tissues (P<0.05). The expression level of P-STAT3 in BUC tissues was related to histological grading (P<0.05). The expression level of Survivin in BUC tissues was related to age, pathological stage, and histological grade (P<0.05). The survival time of patients with positive expression of P-STAT3 and Survivin proteins was significantly lower than those with negative expression (P<0.05). Survivin was positively correlated with P-STAT3 in BUC tissues (P<0.05).

Conclusions: P-STAT3 and Survivin were highly expressed in BUC tissues; the P-STAT3 expression level is related to histological grade and prognosis, and the Survivin expression level is related to age, pathological stage, histological grade and prognosis. Furthermore, P-STAT3 is positively correlated with Survivin in BUC tissues.

Keywords: P-STAT3, Survivin, BUC, expression level, clinicopathological features.

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Introduction

Bladder urothelial carcinoma (BUC) is a common malignant tumour of the urinary system. Due to its strong drug resistance, difficulty in radical resection, and easy recurrence and metastasis, the mortality rate of BUC is increasing year by year(1-2). Although cystectomy, urethra modification, radiotherapy and chemotherapy, among other therapies, are widely used in BUC treatment, the therapeutic effect is not satisfactory. Signal transducer and activator of transcription-3 (STAT3) is an important nuclear transcription and signal transduction factor, and phosphorylated STAT3 (p-STAT3) has the capacity to migrate to the nucleus. Moreover, it can regulate the transcriptional expression of target genes and regulate cell proliferation, apoptosis, and angiogenesis(3). The Survivin protein is an inhibitor of apoptosis and has the functions of inhibiting apoptosis, promoting cell proliferation, and participating in cell cycle regulation(4). Studies have shown that P-STAT3 and Survivin are abnormally expressed in gallbladder cancer, colon cancer and breast cancer, suggesting that they are closely related to tumourigenesis, development and prognosis. However, there are few studies on the expression of P-STAT3 and Survivin in BUC. The aim of this study was to investigate the correlation between expression levels of P-STAT3 and Survivin in BUC and the clinical features and prognosis.

Materials and methods

Clinical data

According to examinations and with the approval of the ethics committee of our hospital, cancer tissue and normal bladder tissue more than 2 cm adjacent to the cancer of 73 cases of BUC patients diagnosed in our hospital from December 2010 to
December 2012 were selected as the study group and the control group.

**Inclusion criteria:**
- Pathological examination confirmed BUC;
- Patients with detailed data;
- Did not have other malignant tumours;
- Normal electrocardiogram;
- All patients and their families signed the informed consent.

**Exclusion criteria:**
- Patient received radiotherapy and chemotherapy before surgery;
- Poor compliance, without regular follow-up;
- Abnormal renal function;
- History of bladder cancer.

Among the 73 patients, the age ranged from 28 to 82 years, with a median age of 55 years; 29 patients were <55 years old, and 44 patients were ≥55 years old; the tumour diameter was <2 cm in 16 cases, and the tumour diameter was ≥2 cm in 57 cases; lymph node metastasis occurred in 15 cases, and there was no lymph node metastasis in 58 cases; in pathological staging, 26 cases were in Ta-T1 stage, and 47 cases were in T2-T4 stage, as shown in Table 1.

**Main reagents**

Rabbit anti-human P-STAT3 polyclonal antibodies (Beijing Zhongshan Biotechnology Co., Ltd.); rabbit anti-human Survivin polyclonal antibody (Wuhan Boshide Bio-engineering Co., Ltd.); general-purpose secondary antibody (Shenzhen Huating Technology Co., Ltd.); haematoxylin (Shanghai Baoman Biotechnology Co., Ltd.); xylene (Shandong Baiqian Chemical engineering Co., Ltd.); PBS balanced salt solution (Beijing Sucolaibao Technology Co., Ltd.); DAB Colorimetric Kit (Changde Bikeman Biotechnology Co., Ltd.); Neutral gum (Shanghai Maikelin Biochemical Technology Co., Ltd.); Sodium Citrate Buffer (Beijing Kangpuhuwei Technology Co., Ltd.); Total RNA Extraction Kit (Beijing Shengdong Technology Co., Ltd.); Reverse Transcription Kit (Beijing Shengdong Technology Co., Ltd.); PCR Polymerase (Xi’an Yunhe Biotechnology Co., Ltd.); STAT3 Primer (Shanghai Xingyuan Ruimin Bioengineering Co., Ltd.); Survivin Primer (Dalian Bao Bio Company); RIPA Lysis Buffer (Shanghai Yamei Biological Technology Co., Ltd).

**Immunohistochemical staining**

Immunohistochemical staining was used to detect the expression of P-STAT3 and Survivin proteins in cancer tissue and normal bladder tissues of 73 cases of BUC patients. All specimens were fixed with formalin fixative, dehydrated, and embedded in blocks with paraffin, and the tissues were cut into 4-μm serial sections, followed by dewaxing, hydration, antigen retrieval, peroxidase blocking, and PBS rinsing. To observe the expression of P-STAT3 and Survivin, the cancer tissue and normal mucosa were incubated with P-STAT3 primary antibody (concentration 1:100) and Survivin primary antibody (concentration 1:100) at 4 °C overnight, rinsed with PBS, and then incubated with universal secondary antibody at 25 °C for 30 minutes, followed by a PBS rinse, DAB colour development, haematoxylin counterstaining, dehydration, transparency, and sealing.

Judging criteria: The results were determined by the Mattern integration method\(^{(5-6)}\). Dyeing intensity: 0 points, no colour; 1 point, light yellow; 2 points, dark yellow; and 3 points, brown-yellow. Percentage of positive cells: 0 points, no positive cells; 1 point, positive cell rate ≤25%; 2 points, positive cell rate 25-50%; and the 3 points, positive cell rate >50%. The sum of the two was judged comprehensively: ≤3 points, negative; > 3 points, positive\(^{(6-7)}\).

**RT-PCR method**

RT-PCR was used to detect the expression of STAT3 and Survivin mRNA in cancer tissue and normal bladder tissues of 73 cases of BUC patients. Total RNA was extracted from various tissues according to the total RNA extraction kit procedure. The RNA was then reverse transcribed into cDNA, and the mRNA expression levels of STAT3 and Survivin were detected by PCR.

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of cases</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;55 years old</td>
<td>29</td>
<td>39.73</td>
</tr>
<tr>
<td>≥55 years old</td>
<td>44</td>
<td>60.27</td>
</tr>
<tr>
<td><strong>Tumour diameter</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2 cm</td>
<td>16</td>
<td>21.92</td>
</tr>
<tr>
<td>≥2 cm</td>
<td>57</td>
<td>78.08</td>
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<tr>
<td><strong>Histological grading</strong></td>
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<td></td>
</tr>
<tr>
<td>Low level</td>
<td>22</td>
<td>30.14</td>
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<tr>
<td>High level</td>
<td>51</td>
<td>69.86</td>
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<tr>
<td><strong>Pathological staging</strong></td>
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<td></td>
</tr>
<tr>
<td>Ta-T1 period</td>
<td>26</td>
<td>35.62</td>
</tr>
<tr>
<td>T2-T4 period</td>
<td>47</td>
<td>64.38</td>
</tr>
<tr>
<td><strong>Lymph node metastasis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>15</td>
<td>20.55</td>
</tr>
<tr>
<td>-</td>
<td>58</td>
<td>79.45</td>
</tr>
</tbody>
</table>

Table. 1: Cases of 73 BUC patients.
Follow-up status

Follow-up was conducted by telephone and outpatient review. The follow-up result point was December 2017, with one follow-up every 3 months in the first 3 years and follow-ups every 6 months in years 4-5.

Observation indicators

Expression of P-STAT3 and Survivin in BUC tissues and normal bladder tissues; expression of STAT3 and Survivin mRNA in BUC tissues and normal bladder tissues; relationship between P-STAT3 and Survivin expression levels and clinicopathological parameters of BUC patients; correlation of P-STAT3 and Survivin expression, the relationship between the expression levels of P-STAT3 and Survivin and the prognosis of BUC patients.

Statistical methods

The data from this study were statistically analysed using SPSS23.0. The count data were analysed by the chi-square test, and the Kaplan-Meier method was used for the survival analysis. P < 0.05 indicates that the difference was statistically significant.

Results

Expression levels of P-STAT3 and Survivin proteins in BUC tissues and normal bladder tissues

Immunohistochemical staining showed that P-STAT3 protein was expressed in the nucleus and cytoplasm, with brown to dark brown granules. In 73 cases of BUC tissues, 55 cases were positive for P-STAT3 protein, with a positive expression rate of 75.34%; in 73 cases of normal bladder tissue, 4 cases were positive for P-STAT3 protein, with a positive expression rate of 5.48%. The positive expression rate of P-STAT3 protein in BUC tissues was significantly higher than that in normal bladder tissues (P<0.05), as shown in Figure 1. Survivin protein was expressed in the nucleus, with brown to dark brown granules. In 73 cases of BUC tissues, 54 cases were positive for Survivin protein, with a positive expression rate of 73.97%; Survivin was not expressed in 73 normal bladder tissues. Survivin protein was expressed in BUC. The positive expression rate of Survivin protein in BUC tissues was significantly higher than that of normal bladder tissue, and the difference was statistically significant (P<0.05), as shown in Table 2.

Expression levels of STAT3 and Survivin mRNA in BUC tissues and normal bladder tissues

The relative quantitation levels of STAT3 mRNA in BUC tissues and normal bladder tissues were 2.45±0.35 and 1.00±0.22, respectively. The relative quantitation level of STAT3 mRNA in BUC tissues was significantly higher than that in normal bladder tissues (P<0.05). The relative quantitation levels of Survivin mRNA in BUC tissues and in normal bladder tissues were 12.42±2.88 and 1.00±0.39, respectively. The relative quantitation level of Survivin mRNA in BUC tissues was significantly higher than that in normal bladder tissues (P<0.05). See Figure 2 and Table 3.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>P-STAT3</th>
<th>Survivin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>BUC tissues</td>
<td>73</td>
<td>18</td>
<td>55</td>
</tr>
<tr>
<td>Normal bladder tissue</td>
<td>73</td>
<td>69</td>
<td>4</td>
</tr>
<tr>
<td>$\chi^2$</td>
<td></td>
<td>73.981</td>
<td>85.696</td>
</tr>
<tr>
<td>$P$</td>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 2: Comparison of expression of P-STAT3 and Survivin proteins in BUC tissues and normal bladder tissues.
Relationship between the expression of P-STAT3 in BUC tissues and clinicopathological features

There were no significant differences (P>0.05) in the expression of P-STAT3 in BUC tissues between patients of different ages, tumour diameters, pathological stages, and lymph node metastasis. The expression levels of Survivin in BUC tissues were statistically significantly different in patients with different age, pathological stage and histological grade (P<0.05). See Table 5.

Correlation of P-STAT3 and Survivin expression

The expression of P-STAT3 and Survivin in BUC tissues was positively correlated (r=0.313, P=0.007), as shown in Table 6.

Correlations between P-STAT3 and Survivin expression and prognosis in patients with BUC

The survival time of patients with positive expression of P-STAT3 and Survivin protein was much lower than that of patients with negative expression, and the difference was statistically significant (P<0.05), as shown in Figure 3.
BUC is a common bladder tumour in China with a high incidence and mortality, accounting for approximately 90% of bladder cancer cases\(^1\). In the early stage of the disease, patients have mild symptoms, such as weight loss and anorexia. As the disease progresses, symptoms such as haematuria, dull pain and distant metastasis appear\(^7\)\(^8\). BUC has multiple characteristics, including easy recurrence and metastasis and a high degree of malignancy. After resection, even if there is bladder reperfusion and radiotherapy and chemotherapy, there is still a recurrence rate of 10-40%. Most patients will have stage progression or metastasis, and serious adverse reactions are common\(^6\)\(^-\)\(^10\). At present, the mechanism of BUC development is not completely clear. Therefore, it is of great significance to find a tumour marker with high sensitivity and specificity that is predictive for the development and metastasis of BUC, which is important for guiding diagnosis and treatment and improving patient prognosis.

The STAT family is a family of proteins expressed in various tissues and cells of humans and includes seven members, such as STAT1, STAT2 and STAT3. STATs are involved in cell proliferation and differentiation and promote malignant transformation of cells. The STAT3 family belongs to the STAT family, which are transcription activation factors and signal transduction factors and are also important substrates of the JAK-STAT (just another kinase-signal transducer and activator of transcription) signalling pathway. Nonactivated STAT3 is present in the cytoplasm, where it is activated by the receptor tyrosine kinase EGFR and the non-receptor tyrosine kinase Janus kinase (just another kinase, JAK) Src and then interacts with phosphorylated tyrosine residues to form a dimer in the P-STAT3 formation process. P-STAT3 is then transported to the nucleus to bind to DNA, regulating tumour cell proliferation, apoptosis and metastasis. AC Hung et al found that P-STAT3 is highly expressed in breast cancer and is associated with malignant behaviours, such as tumour proliferation, invasion and metastasis\(^11\). At the same time, T Min et al found that the activation level of P-STAT3 in gastric cancer tissues was significantly positively correlated with tumour invasion depth, differentiation degree and TNM stage. This study found that the positive expression rate of P-STAT3 protein in BUC tissues was significantly higher than that in normal bladder tissues\(^12\). The relative quantification of STAT3 mRNA in BUC tissues was significantly higher than that in normal bladder tissues, suggesting that P-STAT3 protein and STAT3 mRNA overexpression may be related to BUC. In addition, in patients with different histological grades, the expression level of P-STAT3 in BUC tissues was significantly different, and the prognosis of BUC patients with positive expression of P-STAT3 protein was poor, suggesting that the expression level of P-STAT3 is closely related to histological grade and prognosis. P-STAT3 was positively correlated with the expression of Survivin in BUC tissues, suggesting that both P-STAT3 and Survivin are involved in carcinogenesis and promote the progression of BUC. The normal STAT3 activation level in bladder tissue is relatively low, and its signal intensity is far less than that of BUC tissue; however, STAT3 is continuously activated in BUC tissues and is stably present in cells and positive after phosphorylation. Expression, in turn, affects tumour development.

Survivin is not only an important member of the family of apoptosis inhibitory proteins but is also a tumour-specific bladder cancer cell line biomarker that promotes mitosis and angiogenesis in tumour cells and is closely related to tumour cell proliferation, differentiation, invasion and metastasis\(^13\). L Zhao et al showed that the Survivin gene was expressed in a human nasopharyngeal carcinoma highly differentiated epithelial cell line (CNE-1) and a poorly differentiated epithelial cell line (CNE-2), but the expression difference was not significant\(^14\). At the same time, Z Tian et al found that the expression of the Survivin gene in nasal NK/T cell lymphoma was upregulated and closely related to the high expression of p53 but was not related to Bcl 2 protein\(^15\). This study found that the positive mRNA and protein Survivin expression rates in BUC tissues were significantly higher than those in normal bladder tissues, suggesting that Survivin
protein and mRNA overexpression may induce cell transformation and tumour formation. In addition, the expression level of Survivin in BUC tissues was significantly different with different age, pathological stage, and histological grade, with worse prognosis among BUC patients with positive expression of Survivin protein, suggesting that the expression level of Survivin is closely related to age, pathological stage, histological grade and prognosis. The expression levels of Survivin and P-STAT3 in BUC tissues were positively correlated, suggesting that both P-STAT3 and Survivin can promote cell proliferation and inhibit cell apoptosis. Survivin binds to the cell cycle regulator CDK4, which leads to CDK2/cyclin-E activation and ribosomal (Rb) phosphorylation. After phosphorylation of Rb, the cells enter the cell cycle, accelerating the G1/S phase transition, with the result that P21 is released from Survivin. The CDK4 complex then binds to mitochondrial pro-caspase-3, inhibits caspase-3 activity, prevents Cyt-c release from mitochondria, and blocks apoptosis. Therefore, Survivin protein can promote proliferation and differentiation.

In conclusion, P-STAT3 and Survivin showed high expression in BUC, and the P-STAT3 expression level was related to histological grade and prognosis. The expression level of Survivin was related to age, pathological stage, histological grade and prognosis. Finally, there was a positive correlation between p-STAT3 and Survivin expression in BUC.

References


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