Correlation of apoptosis inducer TWEAK and extracellular matrix protein Del-1 with pancreatic cancer malignancy

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OBJECTIVE: To study the correlation of apoptosis inducer TWEAK and extracellular matrix protein Del-1 with pancreatic cancer malignancy. METHODS: A total of 54 patients with pancreatic cancer who received radical resection and 32 patients with pancreatic trauma who received partial resection in Tianyou Hospital Affiliated to Wuhan University of Science & Technology between May 2014 and March 2017 were selected. Proper amount of pancreatic cancer tissue and normal pancreatic tissue were collected respectively, RNA was extracted and synthesized into cDNA by reverse transcription, and then fluorescence quantitative PCR kits were used to determine TWEAK, Del-1, Survivin, USP9X, DPF2, Stathmin, CD44v9, GPSM2, MMP9, Snail and Vimentin mRNA expression. RESULTS: TWEAK, Del-1, Survivin, USP9X, DPF2, Stathmin, CD44v9, GPSM2, MMP9, Snail and Vimentin mRNA expression in pancreatic cancer tissue were significantly higher than those in normal pancreatic tissue; Survivin, USP9X, DPF2 and Stathmin mRNA expression in pancreatic cancer tissue with high TWEAK expression were significantly higher than those in pancreatic cancer tissue with low TWEAK expression; CD44v9, GPSM2, MMP9, Snail and Vimentin mRNA expression in pancreatic cancer tissue with high Del-1 expression were significantly higher than those in pancreatic cancer tissue with low Del-1 expression. Conclusion: The high expression of TWEAK and Del-1 in pancreatic cancer tissue can promote the proliferation and invasion of pancreatic cancer cells respectively.

1. Introduction

Pancreatic carcinoma is one of the most common malignant tumors of digestive system. Pancreatic ductal adenocarcinoma is the most important pathological type with extremely high malignancy and poor prognosis. Proliferation and invasion are the most important and prominent malignant biological behaviors of pancreatic cancer cells, which are closely related to local infiltration of tumor and local recurrence after surgical resection[1,2]. The regulatory mechanism of abnormal cell proliferation and invasion in pancreatic cancer is not clear at present. TWEAK (TNF-like weak inducer of apoptosis) is a new member of the tumor necrosis factor ligand superfamily, which can be combined with the receptor Fn14 to mediate inflammatory response and cell proliferation via the downstream NF-kB signaling pathway[3]. Del-1 (developmental endothelial locus-1) is a kind of extracellular matrix protein, and the RGD motif in protein structure can activate integrin receptor αvβ3 to mediate cell migration and invasion[4]. In the following studies, we analyzed the TWEAK and Del-1 expression in pancreatic cancer tissue and their correlation with pancreatic cancer malignancy.

2. Research subjects and methods

2.1 General information of research subjects

A total of 54 patients with pancreatic cancer who received radical resection and 32 patients with pancreatic trauma who received partial resection in Tianyou Hospital Affiliated to Wuhan University of Science & Technology between May 2014 and March 2017 were selected as the research subjects. Patients with pancreatic
cancer had not received chemoradiotherapy before surgery, and the tissue properties were confirmed by postoperative pathological examination; patients with pancreatic trauma had a clear history of trauma and preoperative imaging tests were consistent with pancreatic trauma characteristics. Patients with pancreatic cancer included 33 men and 21 women that were 42-59 years old; patients with pancreatic trauma included 19 men and 13 women that were 38-56 years old. There was no significant difference in the general data of patients with pancreatic cancer and patients with pancreatic trauma ($P>0.05$).

### 2.2 Research methods

#### 2.2.1 Tissue sample collection

Proper amount of pancreatic cancer tissue was taken after radical operation for pancreatic cancer; appropriate amount of normal pancreatic tissue was taken after the pancreatic trauma operation. Pancreatic cancer tissue and normal pancreatic tissue were washed with saline for several times and then placed in a -80 °C cryogenic refrigerator.

#### 2.2.2 Gene expression detection

Appropriate amount of pancreatic cancer tissue and normal pancreatic tissue were taken, and RNA extraction kit from Takara was used to separate the total RNA in the tissue; the obtained RNA sample was dissolved with DEPC water and then synthesized into cDNA by reverse transcription with reverse transcription kit from Takara; the cDNA samples after reverse transcription were amplified with fluorescent quantitative PCR kit from Takara company, gene primer was synthesized by Shanghai Sangon Company, the cycle threshold was read after amplification curve was obtained, and GAPDH cycle threshold was used as reference to calculate the mRNA expression of TWEAK, Del-1, Survivin, USP9X, DPF2, Stathmin, CD44v9, GPSM2, MMP9, Snail and Vimentin.

### 2.3 Statistical methods

SPSS 21.0 software was used to input and count data, the median of TWEAK and Del-1 in pancreatic cancer tissue were calculated, the pancreatic cancer tissue with expression $< \text{median}$ was judged as lower TWEAK and Del-1 expression, and the pancreatic cancer tissue with expression $> \text{median}$ was judged as higher TWEAK and Del-1 expression. Comparison between two groups was by t test and $P<0.05$ indicated statistical significance in differences.

### 3. Results

#### 3.1 TWEAK and Del-1 expression

TWEAK and Del-1 mRNA expression in pancreatic cancer tissue were (2.55±0.41) and (2.94±0.47) respectively; TWEAK and Del-1 mRNA expression in normal pancreatic tissue were (1.05±0.12) and (1.02±0.16) respectively. Analysis of TWEAK and Del-1 mRNA expression in pancreatic cancer tissue and normal pancreatic tissue was as follows: TWEAK and Del-1 mRNA expression in pancreatic cancer tissue were significantly higher than those in normal pancreatic tissue, and the differences were statistically significant in TWEAK and Del-1 mRNA expression in pancreatic cancer tissue and normal pancreatic tissue ($P<0.05$).

#### 3.2 Apoptosis-related gene expression and the correlation with TWEAK

Analysis of apoptosis-related genes Survivin, USP9X, DPF2 and Stathmin expression in pancreatic cancer tissue and normal pancreatic tissue was as follows: Survivin, Survivin, USP9X, DPF2 and Stathmin mRNA expression in pancreatic cancer tissue were significantly higher than those in normal pancreatic tissue. Differences were statistically significant in Survivin, USP9X, DPF2 and Stathmin mRNA expression in pancreatic cancer tissue and normal pancreatic tissue ($P<0.05$). Analysis of apoptosis-related genes Survivin, USP9X, DPF2 and Stathmin expression in pancreatic cancer tissue with different TWEAK expression was as follows: Survivin, USP9X, DPF2 and Stathmin mRNA expression in pancreatic cancer tissue with high TWEAK expression were significantly higher than those in pancreatic cancer tissue with low TWEAK expression. Differences were statistically significant in Survivin, USP9X, DPF2 and Stathmin mRNA expression in pancreatic cancer tissue with different TWEAK expression ($P<0.05$).

**Table 1.** Apoptosis-related gene expression in pancreatic cancer tissue and normal pancreatic tissue.

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>n</th>
<th>Survivin</th>
<th>USP9X</th>
<th>DPF2</th>
<th>Stathmin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic cancer</td>
<td>54</td>
<td>2.87±0.44</td>
<td>2.29±0.39</td>
<td>3.16±0.47</td>
<td>3.35±0.52</td>
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<tr>
<td>Normal pancreas</td>
<td>32</td>
<td>1.05±0.18</td>
<td>1.02±0.11</td>
<td>0.97±0.14</td>
<td>0.95±0.13</td>
</tr>
<tr>
<td>T</td>
<td>17.598</td>
<td>12.485</td>
<td>22.139</td>
<td>24.586</td>
<td></td>
</tr>
<tr>
<td>$P$</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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<td></td>
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</tbody>
</table>

**Table 2.** Apoptosis-related gene expression in pancreatic cancer tissue with different TWEAK expression.

<table>
<thead>
<tr>
<th>TWEAK</th>
<th>n</th>
<th>Survivin</th>
<th>USP9X</th>
<th>DPF2</th>
<th>Stathmin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low expression</td>
<td>27</td>
<td>1.82±0.27</td>
<td>1.51±0.23</td>
<td>1.94±0.28</td>
<td>2.05±0.34</td>
</tr>
<tr>
<td>High expression</td>
<td>27</td>
<td>3.94±0.62</td>
<td>2.83±0.46</td>
<td>4.33±0.61</td>
<td>4.61±0.62</td>
</tr>
<tr>
<td>T</td>
<td>13.495</td>
<td>8.968</td>
<td>14.208</td>
<td>12.136</td>
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</tr>
<tr>
<td>$P$</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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<td></td>
</tr>
</tbody>
</table>
motifs, and the activation of the motif on integrin receptor αvβ3 can promote the cell adhesion[7]. In the study, analysis of the TWEAK and Del-1 expression in pancreatic cancer tissue and normal pancreatic tissue showed that TWEAK and Del-1 mRNA expression in pancreatic cancer tissue were significantly higher than those in normal pancreatic tissue. This means that the high expression of apoptosis regulation gene TWEAK and invasion regulation gene Del-1 are closely related to the occurrence of pancreatic cancer, and it can be speculated based on the analysis of biological function of genes that the highly expressed TWEAK and Del-1 in pancreatic cancer tissue can affect cancer cell proliferation and invasion to participate in the development and change of the disease.

The main biological effect of TWEAK is to influence cell proliferation and apoptosis, and the abnormal cell proliferation in pancreatic cancer process is related to the high expression of various anti-apoptotic genes. Survivin, USP9X, DPF2 and Stathmin are several known pancreatic cancer-associated anti-apoptotic genes. The anti-apoptotic effect of Survivin can be played by direct antagonism to the apoptotic process mediated by Caspase-3, and also played by increasing the activity of CDK2 and CDK4 and accelerating the process of cell cycle[8]; USP9X is a kind of deubiquitinating enzyme, which regulates the ubiquitination process to increase the stability of various anti-apoptotic proteins, and thus enhance the anti-apoptotic effect of corresponding proteins[9]; DPF2 is involved in the composition of the SWI/SNF complex and can regulate the interaction between the complex and RelB/p52, and DPF2 knockdown can weaken its anti-apoptotic effect and induce apoptosis of pancreatic cancer cells[10]; Stathmin is a kind of microtubule-associated protein, which regulates the phosphorylation and dephosphorylation processes of a variety of molecules in the cell cycle process, and can accelerate the cell cycle, stabilize microtubule structure and prevent apoptosis[11,12]. In the study, analysis of the anti-apoptosis-related gene expression in pancreatic cancer tissue and normal pancreas tissue showed that Survivin, USP9X, DPF2 and Stathmin mRNA expression in pancreatic cancer tissue were significantly higher than those in normal pancreatic tissue. This indicates that the increased expression of anti-apoptotic genes is closely related to the occurrence of pancreatic cancer. Further analysis of the correlation between TWEAK and antiapoptotic genes in pancreatic cancer tissue showed that Survivin, USP9X, DPF2 and Stathmin mRNA expression in pancreatic cancer tissue with high TWEAK expression were significantly higher than those in pancreatic cancer tissue with low TWEAK expression. This means that the highly expressed apoptosis regulation gene TWEAK in pancreatic cancer tissue can increase the expression of a variety of anti-apoptotic genes, thereby enhancing the anti-apoptotic effect of corresponding proteins, and increase the stability of various anti-apoptotic proteins, and thus enhance the anti-apoptotic effect of corresponding proteins.

3.3 Invasion—related gene expression and the correlation with Del-1

Analysis of invasion-related genes CD44v9, GPSM2, MMP9, Snail and Vimentin expression in pancreatic cancer tissue and normal pancreatic tissue was as follows: CD44v9, GPSM2, MMP9, Snail and Vimentin mRNA expression in pancreatic cancer tissue were significantly higher than those in normal pancreatic tissue. Differences were statistically significant in CD44v9, GPSM2, MMP9, Snail and Vimentin mRNA expression in pancreatic cancer tissue and normal pancreatic tissue (P<0.05).

Analysis of invasion-related genes CD44v9, GPSM2, MMP9, Snail and Vimentin expression in pancreatic cancer tissue with different Del-1 expression was as follows: CD44v9, GPSM2, MMP9, Snail and Vimentin mRNA expression in pancreatic cancer tissue with high Del-1 expression were significantly higher than those in pancreatic cancer tissue with low Del-1 expression. Differences were statistically significant in CD44v9, GPSM2, MMP9, Snail and Vimentin mRNA expression in pancreatic cancer tissue with different Del-1 expression (P<0.05).

4. Discussion

Pancreatic cancer is with high malignant degree, low surgical resection rate and poor prognosis, cancer cell proliferation and invasion are the most prominent malignant biological behaviors of pancreatic cancer, but the specific molecular regulation mechanism has not been elucidated. TWEAK and Del-1 are newly discovered cell apoptosis and invasion regulators in recent years. TWEAK belongs to tumor necrosis factor superfamily, is a kind of type II transmembrane protein, and can be combined with the corresponding type I transmembrane protein Fn14 to activate NF-kB by adaptor molecule TRAF, which will regulate the expression of multiple genes, inhibit apoptosis and promote cell proliferation[5-6]. Del-1 is a type of extracellular matrix protein that causes tumor microenvironment changes by affecting the synthesis and degradation of extracellular matrix, which is beneficial to tumor cell invasion; the molecular structure of the protein contains RGD motifs, and the activation of the motif on integrin receptor αvβ3 can promote the cell adhesion[7]. In the study, analysis of the TWEAK and Del-1 expression in pancreatic cancer tissue and normal pancreatic tissue showed that TWEAK and Del-1 mRNA expression in pancreatic cancer tissue were significantly higher than those in normal pancreatic tissue. This means that the high expression of apoptosis regulation gene TWEAK and invasion regulation gene Del-1 are closely related to the occurrence of pancreatic cancer, and it can be speculated based on the analysis of biological function of genes that the highly expressed TWEAK and Del-1 in pancreatic cancer tissue can affect cancer cell proliferation and invasion to participate in the development and change of the disease.

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Table 3.

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>n</th>
<th>CD44v9</th>
<th>GPSM2</th>
<th>MMP9</th>
<th>Snail</th>
<th>Vimentin</th>
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<tbody>
<tr>
<td>Pancreatic cancer</td>
<td>54</td>
<td>2.09±0.35</td>
<td>2.65±0.42</td>
<td>2.88±0.41</td>
<td>3.51±0.52</td>
<td>3.11±0.46</td>
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<tr>
<td>Normal pancreas</td>
<td>32</td>
<td>1.04±0.15</td>
<td>0.96±0.12</td>
<td>1.07±0.17</td>
<td>0.98±0.11</td>
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<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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Table 4.

<table>
<thead>
<tr>
<th>Del-1</th>
<th>n</th>
<th>CD44v9</th>
<th>GPSM2</th>
<th>MMP9</th>
<th>Snail</th>
<th>Vimentin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low expression</td>
<td>27</td>
<td>1.40±0.26</td>
<td>1.82±0.25</td>
<td>1.76±0.20</td>
<td>2.36±0.42</td>
<td>2.03±0.36</td>
</tr>
<tr>
<td>High expression</td>
<td>27</td>
<td>2.73±0.42</td>
<td>3.41±0.57</td>
<td>3.91±0.62</td>
<td>4.79±0.62</td>
<td>4.21±0.55</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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antiapoptotic genes to hinder the pancreatic cancer cell apoptosis process and promote pancreatic cancer cell proliferation.

The main biological function of Del-1 is to influence the synthesis and degradation of extracellular matrix, and thereby promote cell migration and invasion. CD44v9, GPSM2, MMP9, Snail and Vimentin are currently known to be associated with pancreatic cancer cell invasion and migration. CD44v9 is the allosteric body of CD44v, is the adhesion molecule on the cell surface and can mediate the adhesion between cells and extracellular matrix[13]; GPSM2 is a kind of G protein signal regulatory protein that can regulate the activity of G-protein-coupled receptors to influence β-catenin expression, and then promote tumor cells to break from the primary lesion and invade towards the adjacent tissue through the β-catenin function changes[14]; MMP9 is a MMPs family member with specific degradation on type IV collagen, and after the cell and extracellular matrix adhesion, it can hydrolyze the collagen in extracellular matrix to cause the cells to further invade adjacent tissues[15]; Snail is a transcription factor that regulates the epithelial mesenchymal transition, it is combined with the promoter region of epithelial marker gene E-cadherin and block gene expression so as to further weaken the epithelial phenotype of cells, enhance the mesenchymal phenotype and increase mesenchymal gene Vimentin protein, and it is conducive to enhancing the movement performance of cells[16,17]. In the study, analysis of the invasion-related gene expression in pancreatic cancer tissue and normal pancreatic tissue showed that CD44v9, GPSM2, MMP9, Snail and Vimentin mRNA expression in pancreatic cancer tissue were significantly higher than those in normal pancreatic tissue. It suggests that the increased expression of invasion-related genes is closely related to the occurrence of pancreatic cancer. Further analysis of the correlation between Del-1 and invasion-related genes in pancreatic cancer tissue showed that CD44v9, GPSM2, MMP9, Snail and Vimentin mRNA expression in pancreatic cancer tissue with high Del-1 expression were significantly higher than those in pancreatic cancer tissue with low Del-1 expression. This suggests that the highly expressed apoptosis regulation gene Del-1 in pancreatic cancer tissue can increase the expression of multiple invasion-related genes to promote the invasion of pancreatic cancer cells.

The TWEAK and Del-1 expression significantly increase in pancreatic cancer tissue; highly expressed TWEAK can increase anti-apoptosis gene expression, inhibit pancreatic cancer cell apoptosis and promote pancreatic cancer cell proliferation, and the highly expressed Del 1 can increase the expression of invasion-related genes and promote pancreatic cancer cell invasion.

References