CD80 and CD86 expression in nasopharyngeal carcinoma and their correlation with recurrence after endoscopic sinus surgery as well as tumor invasion and apoptosis

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Abstract

Objective: To study CD80 and CD86 expression in nasopharyngeal carcinoma and their correlation with recurrence after endoscopic sinus surgery as well as tumor invasion and apoptosis. Methods: Patients with nasopharyngeal carcinoma who received surgical resection in Nanchong Central Hospital between February 2013 and March 2014 were collected and divided into recurrence group and non-recurrence group according to the recurrence 3 years after surgery. Normal nasal mucosa tissue for biopsy during the same period was collected, and the expression levels of CD80, CD86 as well as apoptosis molecules and invasion molecules in lesions were detected. Results: CD80, ARD1, Survivin, Shh, Ptc1, S100A4, Ezrin and N-cadherin expression in lesions of recurrence group and non-recurrence group were significantly higher than those of control group, and CD80, ARD1, Survivin, Shh, Ptc1, S100A4, Ezrin and N-cadherin expression in lesions of recurrence group were significantly higher than those of non-recurrence group; CD86 expression in lesions were not significantly different among recurrence group, non-recurrence group and control group. ARD1, Survivin, Shh, Ptc1, S100A4, Ezrin and N-cadherin mRNA expression in nasopharyngeal carcinoma lesions with high CD80 expression were significantly higher than those in nasopharyngeal carcinoma lesions with low CD80 expression. Conclusion: The high expression of CD80 in nasopharyngeal carcinoma can participate in tumor recurrence through promoting the cell proliferation and invasion.

1. Introduction

Nasopharyngeal carcinoma is a common malignant tumor of the head and neck, and it has the characteristics of hidden onset, low early diagnosis rate and high recurrence rate after radiation therapy and surgical resection[1,2]. Immune escape is an important pathologic factor that causes the occurrence and development of malignant tumors and influences the prognosis. The T lymphocyte-mediated cellular immune response of is the main pathway for the body to exert antitumor immune response[3,4]. CD80 and CD86 are the costimulatory factors that play an important role in the T lymphocyte differentiation and maturation process, CD80 can be combined with ligand cytotoxic T lymphocyte-associated antigen to restrain the differentiation and maturation of Th1 cells, and CD86 can be combined with ligand cytotoxic T lymphocyte-associated antigen to promote the differentiation and maturation of Th2 cells. The abnormal expression of CD80 and CD86 as well as the change of the immune response mediated by them are closely related to the occurrence of multiple malignant tumors. In order to define the relationship of CD80 and CD86 expression with the occurrence, development and postoperative recurrence of nasopharyngeal carcinoma, the correlation of CD80 and CD86 expression in nasopharyngeal carcinoma tissue with recurrence after endoscopic sinus surgery as well as tumor invasion and apoptosis was analyzed in the study.

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2. Clinical sample information and research methods

2.1 Clinical sample information

Patients with nasopharyngeal carcinoma who received surgical resection in Nanchong Central Hospital between February 2013 and March 2014 were selected, postoperative pathology confirmed the diagnosis of nasopharyngeal carcinoma, the recurrence 3 years after operation was followed up, and then the nasopharyngeal carcinoma lesions were divided into recurrence group and non-recurrence group. The nasal mucosa biopsy tissue from patients with abnormal nasopharyngeal performance and high risk factors for nasopharyngeal carcinoma during the same period were selected, they were confirmed as normal mucosa after pathology biopsy, and they were as control group. Recurrence group (n=38) included 28 male cases and 10 female cases; non-recurrence group (n=44) included 31 male cases and 13 female cases; control group (n=28) included 19 male cases and 9 female cases. The difference was not statistically significant in the information of patients corresponding to the three groups of clinical samples (P>0.05).

2.2 Research methods

2.2.1 Protein expression detection methods

Nasopharyngeal carcinoma lesions and normal nasal mucosa tissues were taken and added in RIPA lysis buffer to extract the total protein in the tissue, and after the centrifuge and purification, the enzyme-linked immunosorbent assay kits were used to detect the content of CD80 and CD86.

2.2.2 mRNA expression detection methods

Nasopharyngeal carcinoma lesions and normal nasal mucosa tissues were taken and added in Trizol lysis buffer to extract the total RNA in the tissue and reversely transcribe it into cDNA, then fluorescence quantitative PCR kit was used to amplify ARD1, Survivin, Shh, Ptch1, S100A4, Ezrin, N-cadherin and β-actin, and the amplification curve was referred to calculate ARD1, Survivin, Shh, Ptch1 mRNA expression.

2.3 Statistical methods

SPSS 20.0 software was used to input and statistically process gene expression data, data comparison among three groups was by variance analysis, data comparison between two groups was by t test and P<0.05 indicated statistical significance in differences.

3. Results

3.1 CD80 and CD86 expression in lesions

CD80 protein expression of recurrence group, non-recurrence group and control group were (1.95±0.25) μg/L, (1.22±0.18) μg/L and (0.68±0.09) μg/L respectively, and CD86 protein expression of three groups were (0.94±0.11) μg/L, (1.02±0.15) μg/L and (0.98±0.14) μg/L respectively. Analysis of CD80 and CD86 expression in three groups of lesions was as follows: CD80 expression in lesions of recurrence group and non-recurrence group were significantly higher than that of control group, CD80 expression in lesions of recurrence group was significantly higher than that of non-recurrence group, and differences were statistically significant in pair-wise comparison of CD80 expression in three groups of lesions (P<0.05); CD86 expression in lesions were not significantly different among recurrence group, non-recurrence group and control group (P>0.05).

3.2 Cell apoptosis molecule expression in lesions

Analysis of apoptosis molecules ARD1, Survivin, Shh and Ptch1 mRNA expression in lesions of recurrence group, non-recurrence group and control group was as follows: ARD1, Survivin, Shh and Ptch1 mRNA expression in lesions of recurrence group and non-recurrence group were significantly higher than those of control group, ARD1, Survivin, Shh and Ptch1 mRNA expression in lesions of recurrence group were significantly higher than those of non-recurrence group, and differences were statistically significant in pair-wise comparison of ARD1, Survivin, Shh and Ptch1 mRNA expression in three groups of lesions (P<0.05).

Analysis of apoptosis molecules ARD1, Survivin, Shh and Ptch1 mRNA expression in nasopharyngeal carcinoma lesions with different CD80 expression was as follows: ARD1, Survivin, Shh and Ptch1 mRNA expression in nasopharyngeal carcinoma lesions with high CD80 expression were significantly higher than those in nasopharyngeal carcinoma lesions with low CD80 expression. Differences were statistically significant in ARD1, Survivin, Shh and Ptch1 mRNA expression in nasopharyngeal carcinoma lesions with high and low CD80 expression (P<0.05).

Table 1. Apoptosis molecule mRNA expression in three groups of lesions.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>ARD1</th>
<th>Survivin</th>
<th>Shh</th>
<th>Ptch1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrence group</td>
<td>38</td>
<td>2.27±0.33*</td>
<td>2.18±0.34*</td>
<td>2.42±0.51*</td>
<td>2.30±0.48*</td>
</tr>
<tr>
<td>Non-recurrence</td>
<td>44</td>
<td>1.45±0.18*</td>
<td>1.58±0.20*</td>
<td>1.73±0.22*</td>
<td>1.51±0.13*</td>
</tr>
<tr>
<td>Control group</td>
<td>28</td>
<td>1.03±0.16</td>
<td>1.07±0.18</td>
<td>1.02±0.15</td>
<td>0.98±0.11</td>
</tr>
</tbody>
</table>
* compared with control group, differences were statistically significant, P<0.05; * compared with non-recurrence group, differences were statistically significant, P<0.05.

Table 2. Apoptosis molecule mRNA expression in nasopharyngeal carcinoma lesions with different CD80 expression.

<table>
<thead>
<tr>
<th>CD80 expression</th>
<th>n</th>
<th>ARD1</th>
<th>Survivin</th>
<th>Shh</th>
<th>Ptch1</th>
</tr>
</thead>
<tbody>
<tr>
<td>High expression</td>
<td>41</td>
<td>2.09±0.32</td>
<td>2.21±0.31</td>
<td>2.58±0.55</td>
<td>2.37±0.51</td>
</tr>
<tr>
<td>Low expression</td>
<td>41</td>
<td>1.56±0.23</td>
<td>1.45±0.19</td>
<td>1.57±0.20</td>
<td>1.44±0.17</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>
3.3 Cell invasion molecule expression in lesions

Analysis of invasion molecules S100A4, Ezrin and N-cadherin mRNA expression in lesions of recurrence group, non-recurrence group and control group was as follows: S100A4, Ezrin and N-cadherin mRNA expression in lesions of recurrence group and non-recurrence group were significantly higher than those of control group, S100A4, Ezrin and N-cadherin mRNA expression in lesions of recurrence group were significantly higher than those of non-recurrence group, and differences were statistically significant in pair-wise comparison of S100A4, Ezrin and N-cadherin mRNA expression in three groups of lesions (P<0.05).

Analysis of invasion molecules S100A4, Ezrin and N-cadherin mRNA expression in nasopharyngeal carcinoma lesions with different CD80 expression was as follows: S100A4, Ezrin and N-cadherin mRNA expression in nasopharyngeal carcinoma lesions with high CD80 expression were significantly higher than those in nasopharyngeal carcinoma lesions with low CD80 expression. Differences were statistically significant in S100A4, Ezrin and N-cadherin mRNA expression in nasopharyngeal carcinoma lesions with high and low CD80 expression (P<0.05).

Table 3.
Invasion molecule mRNA expression in three groups of lesions.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>S100A4</th>
<th>Ezrin</th>
<th>N-cadherin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrence group</td>
<td>38</td>
<td>2.38±0.41*</td>
<td>2.16±0.32*</td>
<td>2.52±0.48*</td>
</tr>
<tr>
<td>Non-recurrence group</td>
<td>44</td>
<td>1.65±0.22</td>
<td>1.54±0.25</td>
<td>1.48±0.20</td>
</tr>
<tr>
<td>Control group</td>
<td>28</td>
<td>1.04±0.17</td>
<td>1.02±0.15</td>
<td>1.01±0.12</td>
</tr>
</tbody>
</table>

*: compared with control group, differences were statistically significant, P<0.05; &: compared with non-recurrence group, differences were statistically significant, P<0.05.

Table 4.
Invasion molecule mRNA expression in nasopharyngeal carcinoma lesions with different CD80 expression.

<table>
<thead>
<tr>
<th>CD80 expression</th>
<th>n</th>
<th>S100A4</th>
<th>Ezrin</th>
<th>N-cadherin</th>
</tr>
</thead>
<tbody>
<tr>
<td>High expression</td>
<td>41</td>
<td>2.44±0.45</td>
<td>2.24±0.36</td>
<td>2.41±0.42</td>
</tr>
<tr>
<td>Low expression</td>
<td>41</td>
<td>1.51±0.20</td>
<td>1.47±0.22</td>
<td>1.54±0.2</td>
</tr>
<tr>
<td>T</td>
<td>7.518</td>
<td>6.974</td>
<td>7.126</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05 &lt;0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Discussion

Immune escape is an important pathological link causing malignant tumor occurrence and development as well as recurrence and metastasis, and the abnormal expression of a variety of immune cell costimulatory molecules is closely related to the abnormal proliferation and invasion of cancer cells. CD80 and CD86 are the important costimulatory molecules in the differentiation and maturation process of T lymphocytes and participate in the differentiation of CD4+ T lymphocytes. Both CD80 and CD86 can be combined with ligand cytotoxic T lymphocyte-associated antigens, the former mediates the inhibiting effect on Th1 cell differentiation and maturation in CD4+T lymphocyte subsets, and the latter mediates the promoting effect on Th2 cell differentiation and maturation in CD4+T lymphocyte subsets[5]. Studies have shown that the abnormal expression of CD80 and CD86 is closely related to the occurrence of breast cancer, ovarian cancer and bladder cancer[6-8]. In order to define the relationship of T cell differentiation and maturation as well as antitumor immune response mediated by CD80 and CD86 with the occurrence and prognosis of nasopharyngeal carcinoma, CD80 and CD86 protein expression in nasopharyngeal carcinoma tissues and normal nasal mucosa tissues were analyzed in the study, and the results show that CD80 expression in lesions of recurrence group and non-recurrence group were significantly higher than that of control group while CD86 expression were not significantly different from that of control group. This shows that the abnormal high expression of the CD80 is closely related to the occurrence of nasopharyngeal carcinoma, and CD86 does not play a key role in the occurrence of nasopharyngeal carcinoma. Further analysis of the relationship between CD80 expression and postoperative nasopharyngeal carcinoma recurrence showed that CD80 expression in lesions of recurrence group was significantly higher than that of the non-recurrence group. This shows that the abnormal high expression of CD80 is not only related to the occurrence of nasopharyngeal carcinoma, but is also closely related to the postoperative recurrence of nasopharyngeal carcinoma. Abnormal cancer cell proliferation and apoptosis is a critical biological behavior that causes the occurrence and postoperative recurrence of nasopharyngeal carcinoma, and the unusual high expression of a variety of pro-proliferation and anti-apoptosis genes is related to nasopharyngeal carcinoma cell proliferation. ARD1 is a catalyzing enzyme with acetyl transferase activity, and it can catalyze the acetylation process of a variety of proteins and remove the blocking effects on cell cycle so as to accelerate the cell cycle progression and promote cell proliferation[9]. Survivin is an important member of anti-apoptosis protein family, it has antagonizing and inhibitory effect on several members in the caspase family, and it can block apoptosis signal cascade amplification mediated by caspase, thereby inhibit cell apoptosis and promote cell proliferation[10,11]. Shh is a member of the Hedgehog family, and it can form compound with the receptor Ptch1 to promote the cell proliferation process[12]. In the study, the analysis of above pro-proliferation and anti-apoptosis gene expression in nasopharyngeal carcinoma lesions showed that ARD1, Survivin, Shh and Ptch1 mRNA expression in lesions of recurrence group and non-recurrence group were significantly higher than those of control group, and ARD1, Survivin, Shh and Ptch1 mRNA expression in lesions of recurrence group were significantly higher than the non-recurrence group. This suggests that the high expression of pro-proliferation and anti-apoptosis genes is related to the occurrence and recurrence of nasopharyngeal carcinoma. Further analysis of the correlation of CD80 expression with above pro-proliferation and anti-apoptosis gene expression indicated that ARD1, Survivin, Shh...
and Pch1 mRNA expression in nasopharyngeal carcinoma lesions with high CD80 expression were significantly higher than those in nasopharyngeal carcinoma lesions with low CD80 expression. It means that high expression of CD80 is closely related to the high expression of pro-proliferation and anti-apoptosis genes, and highly expressed CD80 will inhibit the Th1 cell differentiation and maturation to cause immune escape, thus increase the expression of ARD1, Survivin, Shh and Pch1 and promote cell proliferation.

The occurrence and recurrence of nasopharyngeal carcinoma are not only related to the proliferation of cancer cells, but also associated with the aggressive growth of cancer cells. S100A4, Ezrin and N-cadherin are the important molecules that regulate the invasion of nasopharyngeal carcinoma cells. S100A4 is a kind of calcium-binding regulatory protein that has EF double helix structure domain, can form high-affinity binding with calcium ions, and plays a promoting role in cell migration, movement and invasion[13]. After Ezrin is activated, it can be combined with adhesion molecules to regulate the junction between the cell membrane and the cell skeleton and promote cell invasion[14]. N-cadherin is a marker of mesenchymal cell phenotype, it can weaken the adhesion between cells and make the cells obtain extremely high movement performance, and it is beneficial for cell invasion[15]. In the study, analysis of above invasion gene expression in nasopharyngeal carcinoma lesions showed that S100A4, Ezrin and N-cadherin mRNA expression in lesions of recurrence group and non-recurrence group were significantly higher than those of control group, and S100A4, Ezrin and N-cadherin mRNA expression in lesions of recurrence group were significantly higher than those of non-recurrence group. This suggests that the high expression of the invasion genes is related to the occurrence and recurrence of nasopharyngeal cancer. Further analysis of the correlation between CD80 expression and above pro-invasion gene expression indicated that S100A4, Ezrin and N-cadherin mRNA expression in nasopharyngeal carcinoma lesions with high CD80 expression were significantly higher than those in nasopharyngeal carcinoma lesions with low CD80 expression. It means that high expression of CD80 is closely related to the high expression of pro-invasion and genes, and highly expressed CD80 will inhibit Th1 cell differentiation and maturation to cause immune escape, thus increase the expression of S100A4, Ezrin and N-cadherin and promote cell invasion.

To sum up, it is believed that high expression of CD80 in nasopharyngeal carcinoma is closely related to the occurrence and recurrence of tumor; highly expressed CD80 can promote cancer cell proliferation, inhibit cancer cell apoptosis, and promote cancer cell invasion and movement.

References


