Correlation of c-fos and p16 expression in oral squamous cell carcinoma with cell cycle and cell invasion

Jian Yang1, Tao Yuan1, Qing-Hui Meng2

1. Department of Stomatology, Zaozhuang Mining Group Central Hospital in Shandong Province, Zaozhuang, Shandong Province, 277000, China
2. Electrocardiography Room, Zaozhuang Mining Group Central Hospital in Shandong Province, Zaozhuang, Shandong Province, 277000, China

ARTICLE INFO

Article history:
Received 2 Jan 2018
Received in revised form 9 Jan 2018
Accepted 19 Jan 2018
Available online 28 Jan 2018

Keywords:
Oral squamous cell carcinoma
C-fos
p16
Cell cycle
Cell invasion

ABSTRACT

Objective: To study the correlation of c-fos and p16 expression in oral squamous cell carcinoma with cell cycle and cell invasion. Methods: Patients with oral squamous cell carcinoma who underwent surgical resection in Zaozhuang Mining Group Central Hospital between June 2014 and March 2017 were selected as OSCC group of the research, and patients who received impacted tooth extraction and provided normal gingival mucosa tissue in the Zaozhuang Mining Group Central Hospital during the same period were selected as the control group. The lesion tissue and normal tissue were obtained from OSCC group and control group respectively to determine the expression of c-fos, p16, cell cycle molecules and cell invasion molecules. Results: c-fos mRNA expression in lesion tissue of OSCC group was significantly higher than that of control group, p16 mRNA expression was significantly lower than that of control group, and the c-fos mRNA expression was negatively correlated with p16 mRNA expression; Chk1 and Chk2 mRNA expression in lesion tissue of OSCC group were significantly lower than those of control group whereas NEK2, CyclinD1, MALAT1, Periostin, β-catenin and Vimentin mRNA expression were significantly higher than those of control group; Chk1 and Chk2 mRNA expression in OSCC lesion tissue with high c-fos expression were significantly lower than those in OSCC lesion tissue with low c-fos expression whereas NEK2, CyclinD1, MALAT1, Periostin, β-catenin and Vimentin mRNA expression were significantly higher than those in OSCC lesion tissue with low c-fos expression. Conclusion: The high expression of c-fos and the low expression of p16 in oral squamous cell carcinoma can accelerate cell cycle and promote cell invasion.

1. Introduction

Oral squamous cell carcinoma (OSCC) is the most common malignant tumor in oral and maxillofacial region, its 5-year survival rate is less than 50%, and the local recurrence and distant metastasis are the important factors affecting prognosis. Cell cycle process acceleration and invasive ability enhancement are the important biological characteristics in oral squamous cell cancer lesion and also the important biological links causing the local recurrence and distant metastasis of tumors[1,2], but the specific regulatory mechanism of cell cycle and cell regulatory mechanism is still not clear. Proto-oncogene c-fos is a member of the immediate early gene family, its encoding product is the nucleoprotein with specific DNA sequences, and it can inhibit the expression of tumor suppressor gene p16 and participate in the regulation of cell cycle and cell invasion process. It has been reported that the expression of c-fos/ p16 pathway has changed in oral squamous cell carcinoma[3,4], but the effect of this pathway on cell cycle and cell invasion in the lesion is not clear. And the study specifically analyzed the correlation of c-fos and p16 expression in oral squamous cell carcinoma with cell cycle and cell invasion.
2. Materials and methods

2.1. General case information

Patients with oral squamous cell carcinoma who underwent surgical resection in Zaozhuang Mining Group Central Hospital between June 2014 and March 2017 were selected as the OSCC group of the research, and the lesion nature was proven to be oral squamous cell carcinoma by postoperative pathological examination; patients who received impacted tooth extraction and provided normal gingival mucosa tissue in the Zaozhuang Mining Group Central Hospital during the same period were selected as the control group, and the tissue nature was proven to be normal gingiva mucosa by pathological examination. There were 46 cases in OSCC group, including 29 males and 17 females who were 34-61 years old; there were 58 cases in the control group, including 32 males and 26 females who were 31-59 years old. There was no statistically significant difference in general information between the two groups (P > 0.05).

2.2. Research methods

2.2.1. Clinical sample collection

Oral squamous cell cancer lesion tissue was collected from OSCC group after surgical resection, normal gingiva mucosa tissue was collected from control group after impacted tooth extraction, the tissue was washed with saline, sealed in RNA later liquid seal and stored in -80°C refrigerator.

2.2.2. Gene expression detection

1-2 mm³ of tissue was cut off and added in Trizol lysis buffer, paramagnetic particle method was used to extract RNA, the cDNA first-strand synthesis kit was used to synthesize the RNA into cDNA by reverse transcription, then fluorescence quantitative PCR and pre-designed primers were used for PCR amplification, and the CT value of amplification curve was referred to calculate the c-fos, p16, Chk1, Chk2, NEK2, CyclinD1, MALAT1, Periostin, β-catenin and Vimentin mRNA expression.

2.3. Statistical methods

SPSS 17.0 software was used to input data, and the median of c-fos expression in OSCC tissues was calculated and used to divide the OSCC tissues into those with high and low c-fos expression respectively. The analysis of gene expression between two groups was by t test and P < 0.05 meant that the differences in test results were statistically significant.

3. Results

3.1. c-fos and p16 expression in lesion tissue and normal tissue

c-fos and p16 mRNA expression in lesion tissue of OSCC group were (3.15±0.51) and (0.31±0.07) respectively; c-fos and p16 mRNA expression in normal tissue of control group were (1.03±0.16) and (0.98±0.14) respectively. After t test, c-fos mRNA expression in lesion tissue of OSCC group was significantly higher than that of control group, p16 mRNA expression was significantly lower than that of control group, and the c-fos mRNA expression was negatively correlated with p16 mRNA expression.

3.2. Cell cycle molecule expression in lesion tissue and normal tissue

Analysis of cell cycle molecules Chk1, Chk2, NEK2 and CyclinD1 expression in lesion tissue of OSCC group and normal tissue of control group was as follows (shown in Table 1): Chk1 and Chk2 mRNA expression in lesion tissue of OSCC group were significantly lower than those of control group whereas NEK2 and CyclinD1 mRNA expression were significantly higher than those of control group. Analysis of cell cycle molecules Chk1, Chk2, NEK2 and CyclinD1 expression in OSCC lesion tissues with different c-fos expression was as follows (shown in Table 2): Chk1 and Chk2 mRNA expression in OSCC lesion tissue with high c-fos expression were significantly lower than those in OSCC lesion tissue with low c-fos expression whereas NEK2 and CyclinD1 mRNA expression were significantly higher than those in OSCC lesion tissue with low c-fos expression.

Table 1

Comparison of cell cycle molecules in lesion tissue and normal tissue.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Chk1</th>
<th>Chk2</th>
<th>NEK2</th>
<th>CyclinD1</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSCC group</td>
<td>46</td>
<td>0.33±0.07</td>
<td>0.41±0.06</td>
<td>2.91±0.46</td>
<td>2.44±0.39</td>
</tr>
<tr>
<td>Control group</td>
<td>58</td>
<td>1.02±0.18</td>
<td>0.97±0.14</td>
<td>1.03±0.19</td>
<td>1.01±0.14</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>22.583</td>
<td>13.228</td>
<td>19.019</td>
<td>13.586</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 2

Comparison of cell cycle molecules in OSCC lesion tissues with different c-fos expression.

<table>
<thead>
<tr>
<th>c-fos</th>
<th>n</th>
<th>Chk1</th>
<th>Chk2</th>
<th>NEK2</th>
<th>CyclinD1</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>23</td>
<td>0.21±0.04</td>
<td>0.27±0.04</td>
<td>4.39±0.62</td>
<td>3.37±0.51</td>
</tr>
<tr>
<td>Low</td>
<td>23</td>
<td>0.45±0.09</td>
<td>0.56±0.09</td>
<td>1.56±0.26</td>
<td>1.48±0.22</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>12.384</td>
<td>10.928</td>
<td>17.537</td>
<td>12.586</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
3.3. Cell invasion molecule expression in lesion tissue and normal tissue

Analysis of cell invasion molecules MALAT1, Periostin, β-catenin and Vimentin expression in lesion tissue of OSCC group and normal tissue of control group was as follows (shown in Table 3): MALAT1, Periostin, β-catenin and Vimentin mRNA expression in lesion tissue of OSCC group were significantly higher than those of control group. Analysis of cell invasion molecules MALAT1, Periostin, β-catenin and Vimentin expression in OSCC lesion tissues with different c-fos expression was as follows (shown in Table 4): MALAT1, Periostin, β-catenin and Vimentin mRNA expression in OSCC lesion tissue with high c-fos expression were significantly higher than those in OSCC lesion tissue with low c-fos expression.

Table 3
Comparison of cell invasion molecules in lesion tissue and normal tissue.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>MALAT1</th>
<th>Periostin</th>
<th>β-catenin</th>
<th>Vimentin</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSCC group</td>
<td>46</td>
<td>2.66±0.41</td>
<td>2.91±0.46</td>
<td>2.33±0.35</td>
<td>3.12±0.46</td>
</tr>
<tr>
<td>Control group</td>
<td>58</td>
<td>1.03±0.17</td>
<td>0.99±0.14</td>
<td>1.02±0.15</td>
<td>0.97±0.16</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>16.549</td>
<td>22.315</td>
<td>12.576</td>
<td>21.219</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 4
Comparison of cell invasion molecules in OSCC lesion tissues with different c-fos expression.

<table>
<thead>
<tr>
<th>c-fos</th>
<th>n</th>
<th>MALAT1</th>
<th>Periostin</th>
<th>β-catenin</th>
<th>Vimentin</th>
</tr>
</thead>
<tbody>
<tr>
<td>High expression</td>
<td>23</td>
<td>3.72±0.51</td>
<td>4.03±0.62</td>
<td>3.14±0.49</td>
<td>4.59±0.62</td>
</tr>
<tr>
<td>Low expression</td>
<td>23</td>
<td>1.59±0.24</td>
<td>1.93±0.26</td>
<td>1.51±0.22</td>
<td>1.74±0.25</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>12.485</td>
<td>13.049</td>
<td>10.182</td>
<td>12.549</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

4. Discussion

The biological characteristics of the oral squamous cell carcinomas include the acceleration of cell cycle process and the enhancement of cell invasion capacity, but the specific regulatory mechanism is not yet clear. c-fos is a kind of proto-oncogene and belongs to the immediate early gene family, and its encoding product has specific DNA binding sequence and can regulate the expression of multiple genes downstream[5]. Tumor suppressor gene p16 is the target gene regulated by c-fos in cells, and its encoding products have negative regulatory effect on cell cycle and can restrained the activation of CDK4 and CDK6 and prevent the formation of cyclinD1 CDK4/CDK6 complexes in order to impede the process of cell cycle and inhibit the growth of cells[6,7]. The analysis of the changes of c-fos/p16 pathway in oral squamous cell cancer lesion tissue in the study showed that c-fos mRNA expression in lesion tissue of OSCC group was significantly higher than that of control group whereas p16 mRNA expression was significantly lower than that of control group. This indicates that the low expression of c-fos and the high expression of p16 are closely related to the occurrence of oral squamous cell carcinoma. Further analysis of the correlation between c-fos and p16 expression change showed that the c-fos mRNA expression in lesion tissue of OSCC group was negatively correlated with p16 mRNA expression. It means that the c-fos within the oral squamous cell cancer lesion participates in the regulation of p16 expression, and high expression of c-fos can inhibit the expression of p16 and antagonize the activity of p16 tumor suppressor gene so as to promote the process of the cell cycle and the invasion of the cells.

The acceleration of cell cycle process is an important biological link that causes the excessive cell proliferation in the oral squamous cell carcinoma, and various positive and negative cell cycle molecules are involved in the regulation of the link. Chk1 and Chk2 are important kinases for cell cycle checkpoints to play their role, and they can be activated by DNA damage and inhibit cell cycle operation to inhibit cell proliferation[8,9]; NEK2 is a member of the Nek family and has three types of Nek2A, Nek2B and Nek2C, which are involved in the regulation of chromosome aggregation and spindle formation in the cell cycle process[10]; cyclinD1 is the most critical regulatory molecule of the cell cycle, which can form complexes with CDK4 and CDK6 to promote the cells to cross the cell cycle checkpoints and accelerate cell cycle[11,12]. The analysis of the changes in above cell cycle molecule expression in oral squamous cell cancer lesion tissue in the study showed that Chk1 and Chk2 mRNA expression in lesion tissue of OSCC group were significantly lower than those of control group whereas NEK2 and CyclinD1 mRNA expression were significantly higher than those of control group. This indicates that the low expression of negative regulators of the cell cycle and the high expression of positive regulators are closely related to the occurrence of oral squamous cell carcinoma. Further analysis of c-fos effect on cell cycle molecule expression showed that Chk1 and Chk2 mRNA expression in OSCC lesion tissue with high c-fos expression were significantly lower than those in OSCC lesion tissue with low c-fos expression whereas NEK2 and CyclinD1 mRNA expression were significantly higher than those in OSCC lesion tissue with low c-fos expression. It means that the high expression of c-fos in oral squamous cell cancer lesion can inhibit the expression of a variety of negative regulators of cell cycle and increase the expression of a variety of positive regulators of cell cycle so as to accelerate the process of cell cycle and promote the proliferation of cells.

The cell invasion in oral squamous cell cancer lesion is closely related to the epithelial mesenchymal transition of cells, the epithelial phenotype of cells is lost, the mesenchymal phenotype is enhanced, and the intercellular polarity and adhesion reduce in the process, so the ability of the cells to migrate and invade to the nearby and distant tissues are enhanced[13,14]. MALAT1 and Periostin are the upstream regulatory molecules of epithelial mesenchymal.
transition of cells, MALAT1 is a kind of lncRNA, and it can increase the accumulation of β-catenin by downstream Wnt pathway and promote the β-catenin to transfer into the nucleus and start the expression of mesenchymal phenotype marker Vimentin[15,16]. Periostin is a kind of extracellular matrix protein that can increase the expression of mesenchymal phenotype marker Vimentin by the downstream integrin receptors vβ3, vβ5 and 6vβ5 [17,18].

Analysis of the changes in above cell invasion molecule expression in oral squamous cell cancer lesion tissue in the study showed that MALAT1, Periostin, β-catenin and Vimentin mRNA expression in lesion tissue of OSCC group were significantly higher than those of control group. It indicates that the high expression of cell invasion molecules is closely related to the occurrence of oral squamous cell carcinoma. The effects of c-fos on the expression of cell invasion molecules were further analyzed, and the results showed that MALAT1, Periostin, β-catenin and Vimentin mRNA expression in OSCC lesion tissue with high c-fos expression were significantly higher than those in OSCC lesion tissue with low c-fos expression. This indicates that the high expression of c-fos in the oral squamous cell cancer lesion can increase the expression of multiple cell invasion molecules to promote the epithelial mesenchymal transition and invasion of the cells.

c-fos expression is high and p16 expression is low in oral squamous cell cancer, and the highly expressed c-fos and the lowly expressed p16 could regulate the expression of cell cycle molecules and cell invasion molecules so as to accelerate cell cycle and promote cell invasion.

References