ZEB2, AnnexinA2 and LMP2A expression in nasopharyngeal carcinoma and their correlation with prognosis and malignant molecules

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ABSTRACT

Objective: To study the ZEB2, AnnexinA2 and latent membrane protein 2A (LMP2A) expression in nasopharyngeal carcinoma and their correlation with prognosis and malignant molecules.  
Methods: Patients who underwent local tissue biopsy under nasopharyngeal fiberscope in Xuhui Dahua Hospital between March 2015 and February 2018 were selected as the research subjects, and the pathological results of biopsy were referred to enroll the patients with nasopharyngeal carcinoma into nasopharyngeal carcinoma group and enroll those with nasal mucosal inflammation into control group of the research. The biopsy tissue was collected to detect the mRNA expression of ZEB2, AnnexinA2, LMP2A and malignant molecules related to proliferation and invasion.  
Results: ZEB2, AnnexinA2, LMP2A, β-catenin, CyclinD1, iASPP, N-cadherin, PARP-1 and MMP9 mRNA expression in tumor tissue of nasopharyngeal carcinoma group were significantly higher than those in rhinitis mucosa tissue of control group whereas RASSF1A, p21WAF1, E-cadherin, IFR5 and TIMP1 mRNA expression were significantly lower than those in rhinitis mucosa tissue of control group; ZEB2, AnnexinA2 and LMP2A mRNA expression in tumor tissue of nasopharyngeal carcinoma group were positively correlated with β-catenin, CyclinD1, iASPP, N-cadherin, PARP-1 and MMP9, and negatively correlated with RASSF1A, p21WAF1, E-cadherin, IFR5 and TIMP1.  
Conclusion: The high expression of ZEB2, AnnexinA2 and LMP2A in nasopharyngeal carcinoma tissue is closely related to the changes of tumor staging and malignant molecule expression.

1. Introduction

Nasopharyngeal carcinoma is a common malignant tumor in E.N.T. Department with rising incidence year by year, its occurrence is closely related to EB infection, genetic factors, environmental factors and so on[1], but the exact pathogenesis has not been elucidated so far. Cancer cell proliferation and invasion are the important malignant biological behaviors in the course of nasopharyngeal carcinoma, they are closely related to the growth and infiltration of tumor lesion, and the expression of a variety of malignant molecules is related to proliferation and invasion significant changes during the process, which can cause tumor stage progression and poor prognosis[2,3]. ZEB2 protein (ZEB2), AnnexinA2 and latent membrane protein 2A (LMP2A) have been the newly discovered malignant tumor-related molecules in recent years, and they participate in the regulation of cancer cell proliferation and invasion in the progression of a variety of malignant tumors. Relevant clinical studies have confirmed that ZEB2, AnnexinA2 and LMP2A are highly expressed in the nasopharyngeal carcinoma lesions, but the specific biological effect and clinical value are still unclear. In the following studies, we specifically analyzed the expression of ZEB2, AnnexinA2 and LMP2A in nasopharyngeal carcinoma tissues and their correlation with prognosis and malignant molecules.

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2. General information and research methods

2.1 General case information

A total of 144 patients who underwent local tissue biopsy under nasopharyngeal fiberscope in Xuhui Dahua Hospital between March 2015 and February 2018 were chosen as the research subjects. The pathological results after biopsy were referred to group these patients, those conforming to the diagnosis of nasopharyngeal carcinoma were enrolled in nasopharyngeal carcinoma group and included a total of 52 cases, and those conforming to the diagnosis of nasal mucosal inflammation were enrolled in control group and included a total of 92 cases. There were 22 males and 30 females in the nasopharyngeal carcinoma group, and they were 39-62 years old; there were 43 males and 49 females in the control group, and they were 41-61 years old. There was no significant difference in the general data between the two groups ($P>0.05$).

2.2 Laboratory detection methods

2.2.1 Tissue preservation

Proper amount of tumor tissue was collected from nasopharyngeal carcinoma group and proper amount of rhinitis mucosa tissue was collected from control group during biopsy, and the tissues were washed with saline, then joined by Trizol lysate and preserved at -80℃.

2.2.2 Gene expression detection

The tissues preserved at -80℃ were taken, unfrozen and then fully split, and then RNA extraction kit was used to separate RNA from the tissue; the RNA was taken, the reverse transcription kit and fluorescence quantitative PCR kit manuals were referred to configure the reaction system, the reverse transcription reaction was completed and followed by the PCR reaction, and PCR reaction curve was referred to calculate the mRNA expression of ZEB2, AnnexinA2, LMP2A, β-catenin, CyclinD1, iASPP, RASSF1A, p21WAF1, N-cadherin, E-cadherin, IFR5, PARP-1, MMP9 and TIMP1.

2.3 Statistical methods

Software SPSS 21.0 was used to input data, the comparison between two groups was by t test, the comparison among four groups was by variance analysis, correlation analysis was by Pearson test and $P<0.05$ meant statistical significance in differences.

3. Results

3.1 Changes of ZEB2, AnnexinA2 and LMP2A expression in nasopharyngeal carcinoma tissues and their correlation with AJCC staging

Analysis of ZEB2, AnnexinA2 and LMP2A expression in tissues between the two groups of patients was as follows: ZEB2, AnnexinA2 and LMP2A mRNA expression in tumor tissue of nasopharyngeal carcinoma group were significantly higher than those in rhinitis mucosa tissue of control group ($P<0.05$). Analysis of ZEB2, AnnexinA2 and LMP2A expression in nasopharyngeal carcinoma group of tumor tissues with different AJCC stages was as follows: the higher the AJCC staging of nasopharyngeal carcinoma, the higher the ZEB2, AnnexinA2 and LMP2A mRNA expression in tumor tissue ($P<0.05$).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>ZEB2</th>
<th>AnnexinA2</th>
<th>LMP2A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasopharyngeal carcinoma group</td>
<td>52</td>
<td>2.42±0.36</td>
<td>1.91±0.27</td>
<td>2.03±0.31</td>
</tr>
<tr>
<td>Control group</td>
<td>92</td>
<td>1.05±0.16</td>
<td>0.98±0.11</td>
<td>0.99±0.13</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>27.892</td>
<td>17.689</td>
<td>20.338</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 1

Changes of ZEB2, AnnexinA2 and LMP2A in nasopharyngeal carcinoma group of tumor tissues with different AJCC stages.

<table>
<thead>
<tr>
<th>AJCC staging</th>
<th>n</th>
<th>ZEB2</th>
<th>AnnexinA2</th>
<th>LMP2A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>10</td>
<td>1.42±0.17</td>
<td>1.38±0.15</td>
<td>1.46±0.20</td>
</tr>
<tr>
<td>Stage II</td>
<td>18</td>
<td>1.89±0.24</td>
<td>1.76±0.22</td>
<td>1.93±0.25</td>
</tr>
<tr>
<td>Stage III</td>
<td>13</td>
<td>2.55±0.35</td>
<td>2.03±0.28</td>
<td>2.39±0.32</td>
</tr>
<tr>
<td>Stage IV</td>
<td>11</td>
<td>3.26±0.47</td>
<td>2.89±0.41</td>
<td>2.98±0.33</td>
</tr>
</tbody>
</table>

$^a$: compared with nasopharyngeal carcinoma tissues at AJCC I stage, $P<0.05$;

$^b$: compared with nasopharyngeal carcinoma tissues at AJCC II stage, $P<0.05$;

$^c$: compared with nasopharyngeal carcinoma tissues at AJCC III stage, $P<0.05$.

3.2 Proliferation-related malignant molecule expression in nasopharyngeal carcinoma tissues and their correlation with ZEB2, AnnexinA2 and LMP2A

Analysis of proliferation-related malignant molecules β-catenin, CyclinD1, iASPP, RASSF1A and p21WAF1 expression in tissues between the two groups of patients was as follows: β-catenin, CyclinD1 and iASPP mRNA expression in tumor tissue of nasopharyngeal carcinoma group were significantly higher than those in rhinitis mucosa tissue of control group whereas RASSF1A and p21WAF1 mRNA expression were significantly lower than those in rhinitis mucosa tissue of control group ($P<0.05$). Pearson test
showed that the ZEB2, AnnexinA2 and LMP2A mRNA expression in tumor tissues of nasopharyngeal carcinoma group were positively correlated with β-catenin, CyclinD1 and iASPP, and negatively correlated with RASSF1A and p21W AF1.

3.3 Invasion-related malignant molecule expression in nasopharyngeal carcinoma tissues and their correlation with ZEB2, AnnexinA2 and LMP2A

Analysis of invasion-related malignant molecules N-cadherin, E-cadherin, IFR5, PARP-1, MMP9 and TIMP1 expression in tissues between the two groups of patients was as follows: N-cadherin, PARP-1 and MMP9 mRNA expression in tumor tissue of nasopharyngeal carcinoma group were significantly higher than those in rhinitis mucosa tissue of control group whereas E-cadherin, IFR5 and TIMP1 mRNA expression were significantly lower than those in rhinitis mucosa tissue of control group (P<0.05). Pearson test showed that the ZEB2, AnnexinA2 and LMP2A mRNA expression in tumor tissues of nasopharyngeal carcinoma group were positively correlated with N-cadherin, PARP-1 and MMP9, and negatively correlated with E-cadherin, IFR5 and TIMP1.

4. Discussion

Nasopharyngeal carcinoma is the most common malignant tumor in the E.N.T. Department. It is prone to recurrence and metastasis in the course of disease, but the specific mechanism remains unclear. ZEB2, AnnexinA2 and LMP2A have been the newly discovered molecules related to malignant tumor in recent years, and participate in the regulation of malignant biological behaviors such as cancer cell proliferation and invasion. ZEB2 is the TCF/Zfh1 protein family member that is able to activate the Wnt/β-catenin pathway in cells and the epithelial mesenchymal transition (EMT) of cells, promote cell proliferation through the biological effect of β-catenin, and facilitate cell invasion through the biological effect of EMT pathway[11]; AnnexinA2 is a type of annexin of bioactivity-dependent calcium ions located in the cell matrix, and it can activate the Wnt/β-catenin pathway and promote cell proliferation[5,6]; LMP2A is a gene closely related to the latent infection of EB virus, and the infection of EB virus in the course of nasopharyngeal carcinoma can induce the expression of LMP2A to enhance the EMT process of cells and promote cell invasion[7,8]. Analysis of the changes of above malignant tumor-related molecule expression in nasopharyngeal carcinoma tissues showed that ZEB2, AnnexinA2 and LMP2A mRNA expression in tumor tissue of nasopharyngeal carcinoma group were significantly higher than those of control group. It indicates that the high expression of ZEB2, AnnexinA2 and LMP2A is related to the occurrence of nasopharyngeal carcinoma. Further analysis of the relationship between the malignant tumor-related molecule expression and AJCC staging showed that the higher the AJCC staging of nasopharyngeal carcinoma, the higher the ZEB2, AnnexinA2 and LMP2A mRNA expression in tumor tissue. It indicates that the high expression of ZEB2, AnnexinA2 and LMP2A is not only involved in the occurrence of nasopharyngeal carcinoma, but also related to the progression of nasopharyngeal carcinoma stage, and it can affect the prognosis of the disease.

In the progression of nasopharyngeal carcinoma stage, tumor lesions are continuously growing and expanding, and the completion of this process is closely related to the abnormal expression of various proliferation-associated malignant molecules. β -catenin is the effector molecule of Wnt pathway, and it is combined with GSK-3 β and constantly degraded in the resting state[9,10], but after the Wnt is activated, β -catenin is dissociated with GSK-3 β and constantly moves to the nuclei, and then it regulates the expression of CyclinD1 to accelerate the process of cell cycle and achieve the positive regulation on cell proliferation[11,12]; iASPP is a negative regulator of p53 tumor suppressor pathway, which achieves the positive regulation on cell proliferation by weakening the anticancer activity mediated by p53 gene. RASSF1A and p21W AF1 are negative regulatory molecules of cell cycle, which can interact with various cyclins and kinases in cell cycle progression to inhibit cell cycle development and cell proliferation[13]. In the study, analysis of the changes in above proliferation-related malignant molecule expression in nasopharyngeal carcinoma tissue showed that β-catenin, CyclinD1 and iASPP mRNA expression in tumor tissue of nasopharyngeal carcinoma group were significantly higher than those in rhinitis mucosa tissue of control group whereas RASSF1A and p21W AF1 mRNA expression were significantly lower than those in rhinitis mucosa tissue of control group. This indicates that the up-regulated expression of positive regulatory molecules of proliferation and the down-regulated expression of negative regulatory molecules
of proliferation are related to the occurrence of nasopharyngeal carcinoma. Further analysis of the correlation of ZEB2, AnnexinA2 and LMP2A with malignant molecules related to proliferation showed that ZEB2, AnnexinA2 and LMP2A mRNA expression in tumor tissues of nasopharyngeal carcinoma group were positively correlated with β-catenin, CyclinD1 and iASPP, and negatively correlated with RASSF1A and p21WAF1. This means that the high expression of ZEB2, AnnexinA2 and LMP2A in nasopharyngeal carcinoma can regulate the expression of positive and negative regulatory molecules of proliferation to promote the proliferation of tumor cells.

The nasopharyngeal carcinoma lesions are characterized by infiltrative growth during the disease progression, and cell invasion is the biological behavior most closely related to the infiltrative growth of tumor. The process of cell invasion involves the cell movement and migration ability obtained from EMT process and the hydrolysis of various protein components in the extracellular matrix. N-cadherin and E-cadherin are the markers of cell EMT process, the former is mesenchymal cell marker and can promote cell migration and movement, and the latter is the epithelial cell marker and can enhance the intercellular polarity and prevent cell migration and movement[14,15]. IFR5 is the upstream regulatory molecule of extracellular matrix degradation process. It not only induce the secretion of IFN-α, IFN-β and other immunoactive substances, but also block the MMP9 expression mediated by downstream PARP-1 to curb cell invasion[16]; MMP9 is powerful proteolytic enzyme that can hydrolyze various elements in extracellular matrix and promote cell invasion, and TIMP1 is the specific inhibitor of MMP9 and can hinder its hydrolytic activity directly. Analysis of the changes in above invasion-related malignant molecule expression in nasopharyngeal carcinoma tissue showed that N-cadherin, PARP-1 and MMP9 mRNA expression in tumor tissue of nasopharyngeal carcinoma group were significantly higher than those in rhinitis mucosa tissue of control group whereas E-cadherin, IFR5 and TIMP1 mRNA expression were significantly lower than those in rhinitis mucosa tissue of control group. It means that the up-regulated expression of positive regulatory molecules of invasion and the down-regulated expression of negative regulatory molecules of invasion are associated with the incidence of nasopharyngeal carcinoma. Further analysis of the correlation of ZEB2, AnnexinA2 and LMP2A with malignant molecules related to invasion showed that ZEB2, AnnexinA2 and LMP2A mRNA expression in tumor tissues of nasopharyngeal carcinoma group were positively correlated with N-cadherin, PARP-1 and MMP9, and negatively correlated with E-cadherin, IFR5 and TIMP1. It indicates that the high expression of ZEB2, AnnexinA2 and LMP2A in nasopharyngeal carcinoma can modulate the expression of positive and negative regulatory molecules of invasion to promote the invasion of tumor cells.

Based on the analysis of above gene and molecule expressions, it can be concluded that the ZEB2, AnnexinA2 and LMP2A expression are increasing in nasopharyngeal carcinoma tissues; the highly expressed ZEB2, AnnexinA2 and LMP2A are closely related to the tumor stage progression as well as the change in the expression of malignant molecules related to proliferation and invasion.

References