LMP2A, AnnexinA2, Rad52 and Gli1 expression in nasopharyngeal carcinoma and their correlation with tumor malignancy

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ABSTRACT

Objective: To study the LMP2A, AnnexinA2, Rad52 and Gli1 expression in nasopharyngeal carcinoma and their correlation with tumor malignancy. Methods: Nasopharyngeal cancer tissue confirmed by fibreoptic nasopharyngoscopic biopsy and mild chronic rhinitis mucosa inflammation tissue in Renmin Hospital of Wuhan University between May 2014 and February 2017 were selected to extract the RNA, and then fluorescence quantitative PCR kits were used to determine the expression of LMP2A, AnnexinA2, Rad52, Gli1, epithelial-mesenchymal transition genes and cell cycle genes. Results: LMP2A, AnnexinA2, Rad52 and Gli1 mRNA expression in nasopharyngeal cancer lesions were significantly higher than those in rhinitis mucosa inflammation lesions, and the higher the clinical stage, the higher the LMP2A, AnnexinA2, Rad52 and Gli1 mRNA expression in nasopharyngeal cancer lesions; E-cadherin mRNA expression in nasopharyngeal cancer lesions was significantly lower than that in rhinitis mucosa inflammation lesions and negatively correlated with LMP2A and Gli1 while N-cadherin, Vimentin and ZEB2 mRNA expression were significantly higher than those in rhinitis mucosa inflammation lesions and positively correlated with LMP2A and Gli1; CyclinD1, CyclinE and PCNA mRNA expression in nasopharyngeal cancer lesions were significantly higher than those in rhinitis mucosa inflammation lesions and positively correlated with with AnnexinA2 and Rad52. Conclusion: The high expression of LMP2A, AnnexinA2, Rad52 and Gli1 in nasopharyngeal carcinoma can promote epithelial-mesenchymal transition and cell cycle process in cancer cells.

1. Introduction

Nasopharyngeal carcinoma is one of the most common cervical malignancies in China, and southern area is its high-prevalence area. At present, radiotherapy is the preferred therapy for nasopharyngeal carcinoma, and radiation can effectively kill the cancer cells and make the 5-year local disease control rate reach 80-90%. Nevertheless, the prognosis is still poor in some patients with nasopharyngeal carcinoma, and local recurrence and infiltration as well as distant metastasis are important factors affecting the prognosis[1,2]. In the process of tumor recurrence, infiltration and metastasis, the proliferation and invasion of cancer cells are the closely related biological behaviors, but the regulatory mechanism of nasopharyngeal carcinoma cell proliferation and invasion is not yet clear. Latent membrane protein 2A (LMP2A) and glioma-associated oncogene homologue 1 (Gli1) are the important proteins involved in the regulation of epithelial-mesenchymal transition (EMT), and can promote cell invasion by inducing EMT process[3,4]; AnnexinA2 and Rad52 are the important proteins involved in the regulation of cell cycle processes, which can promote cell proliferation by accelerating cell cycle[5,6]. The LMP2A, AnnexinA2, Rad52 and Gli1 expression in nasopharyngeal carcinoma and their correlation with tumor malignancy were specifically analyzed in the following study.
2. Tissue sample origin and research methods

2.1 Tissue sample origin and general information

Patients who were diagnosed with nasopharyngeal carcinoma and patients who were diagnosed with mild chronic rhinitis mucosa inflammation by fibreoptic nasopharyngoscopic biopsy in Renmin Hospital of Wuhan University between May 2014 and February 2017 were selected, and the corresponding tissue samples were collected. Patients with nasopharyngeal carcinoma \((n=78)\) included 53 male cases and 25 female cases that were 41-64 years old; patients with mild chronic rhinitis mucosa inflammation \((n=45)\) included 28 male cases and 17 female cases that were 38-62 years old. There was no significant difference in the general data of nasopharyngeal cancer and rhinitis mucosa inflammation lesion tissue samples \((P>0.05)\).

2.2 Tissue sample collection

In fibreoptic nasopharyngoscopic biopsy, moderate amount of nasopharyngeal carcinoma lesion and rhinitis mucosa inflammation lesion tissue were collected and washed with saline for several times, filter paper was used to absorb the moisture, and then the tissue was saved in the frozen pipe, shortly frozen in liquid nitrogen for 20 min, then taken out and saved in the -70 \(^\circ\)C refrigerator.

2.3 Gene expression detection

Proper amount of tissue samples from different sources were taken and added in appropriate amount of RNAiso lysate from Takara to extract the RNA from the tissue, and then the reverse transcription kit from Promega was used to synthesize RNA by reverse transcription. The primers for LMP2A, AnnexinA2, Rad52, Gli1, E-cadherin, N-cadherin, Vimentin, ZEB2, CyclinD1, CyclinE and PCNA were designed, fluorescence quantitative PCR amplification of cDNA samples were conducted, and the mRNA expression of corresponding genes were calculated according to the amplification curve.

2.4 Statistical processing methods

SPSS 22.0 software was used to input and analyze data, data between two groups were by t test, data among four groups were by variance analysis, and test results \(P<0.05\) indicated statistical significance in differences.

3. Results

3.1 LMP2A, AnnexinA2, Rad52 and Gli1 expression in nasopharyngeal cancer lesions and rhinitis mucosa inflammation lesions

Analysis of LMP2A, AnnexinA2, Rad52 and Gli1 expression in nasopharyngeal cancer lesions and rhinitis mucosa inflammation lesions was as follows: LMP2A, AnnexinA2, Rad52 and Gli1 mRNA expression in nasopharyngeal cancer lesions were significantly higher than those in rhinitis mucosa inflammation lesions. Differences were statistically significant in LMP2A, AnnexinA2, Rad52 and Gli1 expression in nasopharyngeal cancer lesions and rhinitis mucosa inflammation lesions \((P<0.05)\).

3.2 LMP2A, AnnexinA2, Rad52 and Gli1 expression in nasopharyngeal cancer lesions with different clinical tumor staging

Analysis of LMP2A, AnnexinA2, Rad52 and Gli1 expression in I stage, II stage, III stage and IV stage nasopharyngeal cancer lesions was as follows: LMP2A, AnnexinA2, Rad52 and Gli1 mRNA expression in II stage, III stage and IV stage nasopharyngeal cancer lesions were significantly higher than those in I stage nasopharyngeal cancer lesions, LMP2A, AnnexinA2, Rad52 and Gli1 mRNA expression in III stage and IV stage nasopharyngeal cancer lesions were significantly higher than those in II stage nasopharyngeal cancer lesions, and LMP2A, AnnexinA2, Rad52 and Gli1 mRNA expression in IV stage nasopharyngeal cancer lesions were significantly higher than those in III stage nasopharyngeal cancer lesions. Differences were statistically significant in pair-wise comparison of LMP2A, AnnexinA2, Rad52 and Gli1 expression in nasopharyngeal cancer lesions with different clinical tumor staging \((P<0.05)\).

Table 1.

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>n</th>
<th>LMP2A</th>
<th>AnnexinA2</th>
<th>Rad52</th>
<th>Gli1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasopharyngeal cancer</td>
<td>78</td>
<td>2.19±0.33</td>
<td>2.45±0.31</td>
<td>1.94±0.22</td>
<td>2.86±0.39</td>
</tr>
<tr>
<td>Rhinitis mucosa</td>
<td>45</td>
<td>1.06±0.13</td>
<td>1.01±0.12</td>
<td>0.95±0.14</td>
<td>1.03±0.16</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>10.948</td>
<td>13.285</td>
<td>10.221</td>
<td>15.585</td>
</tr>
<tr>
<td></td>
<td>(P)</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 EMT gene expression in nasopharyngeal cancer lesions and rhinitis mucosa inflammation lesions as well as their correlation with LMP2A and Gli1

Analysis of EMT genes E-cadherin, N-cadherin, Vimentin and ZEB2 expression in nasopharyngeal cancer lesions and rhinitis...
mucosa inflammation lesions was as follows: E-cadherin mRNA expression in nasopharyngeal cancer lesions was significantly lower than that in rhinitis mucosa inflammation lesions while N-cadherin, Vimentin and ZEB2 mRNA expression were significantly higher than those in rhinitis mucosa inflammation lesions. Differences were statistically significant in E-cadherin, N-cadherin, Vimentin and ZEB2 expression in nasopharyngeal cancer lesions and rhinitis mucosa inflammation lesions \( P<0.05 \). Pearson test showed that LMP2A and Gli1 mRNA expression were negatively correlated with E-cadherin, and positively correlated with N-cadherin, Vimentin and ZEB2.

### 3.4 Cell cycle gene expression in nasopharyngeal cancer lesions and rhinitis mucosa inflammation lesions as well as their correlation with AnnexinA2 and Rad52

Analysis of cell cycle genes CyclinD1, CyclinE and PCNA expression in nasopharyngeal cancer lesions and rhinitis mucosa inflammation lesions was as follows: CyclinD1, CyclinE and PCNA mRNA expression in nasopharyngeal cancer lesions were significantly higher than those in rhinitis mucosa inflammation lesions. Differences were statistically significant in CyclinD1, CyclinE and PCNA expression in nasopharyngeal cancer lesions and rhinitis mucosa inflammation lesions \( P<0.05 \). Pearson test showed that AnnexinA2 and Rad52 mRNA expression were positively correlated with E-cadherin, and negatively correlated with N-cadherin, Vimentin and ZEB2.

### Table 2.

<table>
<thead>
<tr>
<th>Clinical stage</th>
<th>( n )</th>
<th>LMP2A ( \text{mean} \pm \text{SD} )</th>
<th>Annexin2 ( \text{mean} \pm \text{SD} )</th>
<th>Rad52 ( \text{mean} \pm \text{SD} )</th>
<th>Gli1 ( \text{mean} \pm \text{SD} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>I stage</td>
<td>13</td>
<td>1.42±0.18</td>
<td>1.63±0.20</td>
<td>1.39±0.17</td>
<td>1.72±0.18</td>
</tr>
<tr>
<td>II stage</td>
<td>26</td>
<td>1.89±0.22 (^{1} )</td>
<td>2.03±0.27 (^{1} )</td>
<td>1.73±0.22 (^{1} )</td>
<td>2.41±0.31 (^{1} )</td>
</tr>
<tr>
<td>III stage</td>
<td>21</td>
<td>2.31±0.35 (^{1} )</td>
<td>2.76±0.33 (^{1} )</td>
<td>2.31±0.34 (^{1} )</td>
<td>3.15±0.41 (^{1} )</td>
</tr>
<tr>
<td>IV stage</td>
<td>18</td>
<td>2.87±0.42 (^{1} )</td>
<td>3.51±0.41 (^{1} )</td>
<td>2.80±0.45 (^{1} )</td>
<td>3.88±0.52 (^{1} )</td>
</tr>
</tbody>
</table>

\( ^{1} \) : compared with I stage nasopharyngeal cancer lesions, \( P<0.05 \); \( ^{2} \) : compared with II stage nasopharyngeal cancer lesions, \( P<0.05 \); \( ^{3} \) : compared with III stage nasopharyngeal cancer lesions, \( P<0.05 \).

### 4. Discussion

Invasive growth is an important pathologic factor causing the development and poor prognosis of nasopharyngeal carcinoma. LMP2A is the product of EB virus latent gene expression, its chemical nature is a kind of phosphorylation membrane protein, and the 74- and 85-bit tyrosine residues on intracellular fragments can form the ITAM motif and can identify a variety of tyrosine kinase of containing SH2 domain structure so as to regulate downstream gene expression and influence cell invasion and migration process[7]. Gli1 is a nuclear transcription factor that adjusts the Hedgehog signaling pathway, and Hedgehog can be combined with membrane receptors Smo and Pch to promote Gli1 activation and combination with the corresponding target genes so as to regulate gene expression and affect cell invasion and migration process[8]. In recent years, studies on LMP2A and Gli1 function have shown that the cellular epithelial mesenchymal transition (EMT) process is regulated by LMP2A and Gli1. In the study, analysis of the expression of these two genes closely related to the EMT in nasopharyngeal carcinoma lesions showed that LMP2A and Gli1 mRNA expression in nasopharyngeal cancer lesions were significantly higher than those in rhinitis mucosa inflammation lesions, and the higher the clinical stage, the higher the LMP2A and Gli1 mRNA expression in nasopharyngeal cancer lesions. This indicates that the high expression of LMP2A and Gli1 is closely related to the occurrence of nasopharyngeal carcinoma and the increase of its malignancy, and regulating EMT is a possible pathway for LMP2A and Gli1 to participate in the pathogenesis of nasopharyngeal carcinoma.

EMT is the process that epithelial phenotype in tissue is gradually replaced by the mesenchymal phenotype, and this process can enhance cell migration and movement ability, promote cells to escape from the primary site, infiltrate towards the surrounding and transfer to the distance[9]. E-cadherin is a marker of epithelial phenotype, which can maintain the adhesiveness and polarity of epithelial cells, and make the cells anchor in the primary site[10]; N-cadherin and Vimentin are the markers of the mesenchymal phenotype, which can reduce the intercellular polarity and promote cell migration and movement[11]; ZEB2 is a transcription factor with zinc finger structure, which inhibits the expression of E-cadherin, and thereby reduces the epithelial phenotype[12]. In the study, analysis of EMT gene expression in nasopharyngeal carcinoma lesions showed that E-cadherin mRNA expression in nasopharyngeal cancer lesions was significantly lower than that in rhinitis mucosa inflammation lesions while N-cadherin, Vimentin and ZEB2 mRNA expression were significantly higher than those in rhinitis mucosa inflammation lesions. This indicates that mesenchymal cell phenotype enhancement and epithelial cell phenotype attenuation are closely related to the occurrence of nasopharyngeal carcinoma. Further analysis of the correlation of LMP2A and Gli1 with EMT

### Table 3.

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>( n )</th>
<th>E-cadherin ( \text{mean} \pm \text{SD} )</th>
<th>N-cadherin ( \text{mean} \pm \text{SD} )</th>
<th>Vimentin ( \text{mean} \pm \text{SD} )</th>
<th>ZEB2 ( \text{mean} \pm \text{SD} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasopharyngeal cancer</td>
<td>78</td>
<td>0.41±0.06</td>
<td>2.65±0.35</td>
<td>3.15±0.51</td>
<td>2.04±0.31</td>
</tr>
<tr>
<td>Rhinitis mucosa inflammation</td>
<td>45</td>
<td>1.05±0.13</td>
<td>1.02±0.14</td>
<td>0.97±0.12</td>
<td>1.08±0.11</td>
</tr>
<tr>
<td>( T )</td>
<td>13.272</td>
<td>16.029</td>
<td>14.587</td>
<td>9.287</td>
<td>&lt;0.05</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>

### Table 4.

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>( n )</th>
<th>CyclinD1 ( \text{mean} \pm \text{SD} )</th>
<th>CyclinE ( \text{mean} \pm \text{SD} )</th>
<th>PCNA ( \text{mean} \pm \text{SD} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasopharyngeal cancer</td>
<td>78</td>
<td>1.98±0.24</td>
<td>2.96±0.42</td>
<td>2.21±0.32</td>
</tr>
<tr>
<td>Rhinitis mucosa inflammation</td>
<td>45</td>
<td>1.03±0.16</td>
<td>1.01±0.15</td>
<td>0.98±0.14</td>
</tr>
<tr>
<td>( T )</td>
<td>9.875</td>
<td>18.572</td>
<td>13.215</td>
<td>&lt;0.05</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>
</tr>
</tbody>
</table>
processes showed that LMP2A and Gli1 mRNA expression were negatively correlated with E-cadherin, and positively correlated with N-cadherin, Vimentin and ZEB2. The results show that the highly expressed LMP2A and Gli1 in nasopharyngeal carcinoma lesions could affect the EMT process, promote the transition from epithelial phenotype to mesenchymal phenotype, and enhance the invasion ability of the cells.

The pathogenesis of nasopharyngeal carcinoma is not only related to the migration and invasion of cancer cells, but also related to the enhancement of cell proliferation activity. AnnexinA2 is a calcium-dependent phospholipid binding protein, which mainly locates in cytoplasm and cell membrane, and can influence the expression of a variety of cyclin through Wnt/β-catenin pathway, promote cyclin to form complexes with CDK, accelerate the process of cell cycle and promote cell proliferation[13]. Rad52 is a molecule involved in DNA damage repair, which is closely related to the repair after DNA double-strand break; DNA double-strand break is the most serious form of DNA damage, which can polymerize with Rad52 after cut into ssDNA by the Rad50-Mre11-NBS1 complex, and then conduct DNA replication and promote the cell cycle process under the action of Rad52[14]. In the study, analysis of the expression of these two genes closely related to cell cycle in nasopharyngeal carcinoma lesions showed that AnnexinA2 and Rad52 mRNA expression in nasopharyngeal cancer lesions were significantly higher than those in rhinitis mucosa inflammation lesions, and the higher the clinical stage, the higher the AnnexinA2 and Rad52 mRNA expression in nasopharyngeal cancer lesions. This indicates that the high expression of AnnexinA2 and Rad52 is closely related to the occurrence of nasopharyngeal carcinoma and the increase of the malignant degree, and regulating cell cycle process is a possible pathway for AnnexinA2 and Rad52 to participate in the pathogenesis of the nasopharyngeal carcinoma.

The regulation of cell cycle depends on the interaction between Cyclin, CDK and corresponding suppressor CDKI. CyclinD1 and CyclinE are the important molecules involved in cell cycle regulation in nasopharyngeal carcinoma. CyclinD1 can form complexes with CDK4 and CDK6 to promote the cell cycle from G0 to G1, and CyclinE can form complexes with CDK2 to promote cell cycle from G1 to S phase; at the same time, the formation of Cyclin/CDKs complexes can also cause Rb phosphorylation, increase E2F release and regulate the expression of multiple target genes, thereby promoting cell proliferation[15]. PCNA mainly participates in the regulation of DNA replication process, and can affect the process of cell cycle[16]. In the study, analysis of cell cycle gene expression in nasopharyngeal carcinoma lesion showed that CyclinD1, CyclinE and PCNA mRNA expression in nasopharyngeal cancer lesions were significantly higher than those in rhinitis mucosa inflammation lesions. This indicates that the accelerated cell cycle is closely related to the occurrence of nasopharyngeal carcinoma. Further analysis of the correlation of AnnexinA2 and Rad52 with cell cycle process showed that AnnexinA2 and Rad52 mRNA expression were positively correlated with CyclinD1, CyclinE and PCNA. Therefore, the highly expressed AnnexinA2 and Rad52 in nasopharyngeal carcinoma can accelerate cell cycle process and promote cell proliferation.

The expression of LMP2A, AnnexinA2, Rad52, and Gli1 increase significantly in nasopharyngeal cancer; highly expressed LMP2A, AnnexinA2, Rad52 and Gli1 can promote the epithelial-mesenchymal transition of cancer cells as well as the cell cycle process.

References