The correlation of miR-106b in nasopharyngeal carcinoma with cell cycle progression and cell invasion

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ABSTRACT

Objective: To study the correlation of miR-106b in nasopharyngeal carcinoma with cell cycle progression and cell invasion. Methods: Patients who were diagnosed with nasopharyngeal carcinoma by pathological biopsy in Shenmu Hospital between March 2013 and February 2018 were included in the study, and the nasopharyngeal carcinoma tissue and tissue adjacent to nasopharyngeal carcinoma obtained from biopsy were collected, miRNA was extracted to determine miR-106b expression, and RNA was extracted to determine the mRNA expression of cell cycle-related genes and cell invasion-related genes. Results: miR-106b expression as well as FOXC1, CyclinD1, CyclinE, TRIM27, uPAR, PARP1 and MTA1 mRNA expression in nasopharyngeal carcinoma tissues were higher than those in adjacent tissues, CYB5R2, p16, IRF5 and E-cadherin mRNA expression were significantly lower than those in adjacent tissues whereas; CYB5R2, p16, IRF5 and E-cadherin mRNA expression in nasopharyngeal carcinoma tissues with high miR-106b expression were lower than those in nasopharyngeal carcinoma tissues with low miR-106b expression whereas FOXC1, CyclinD1, CyclinE, TRIM27, uPAR, PARP1 and MTA1 mRNA expression were higher than those in nasopharyngeal carcinoma tissues with low miR-106b expression. Conclusion: The lowly expressed miR-106b in nasopharyngeal carcinoma can promote the cell cycle progression and cell invasion in the lesion.

1. Introduction

Nasopharyngeal carcinoma is a common malignant tumor in the head and neck, and the incidence is higher in South Asia and southern China. At present, the main methods for clinical treatment of nasopharyngeal carcinoma are radiotherapy and chemotherapy, their killing effect on cancer cells is accurate, but the overall five-year survival rate is not ideal[1,2]. Nasopharyngeal carcinoma pathogenesis has not been clarified so far, the accelerated cell cycle and active cell invasion in the lesion are the pathological links closely related to the occurrence of disease, and multiple genes are involved in the regulation of nasopharyngeal carcinoma cell cycle and cell invasion[3]. MiRNA is the non-coding small RNA that has received more and more attention in recent years, which has regulating effect on the expression of multiple genes in cells, and influences the expression of corresponding genes to produce different biological effects. MiR-106b is a type of miRNA with proto-oncogene activity, and can positively regulate the expression of a variety of genes that promote growth. Related cytological experiments have confirmed that miR-106b has promoting effect on the cell cycle and invasion of nasopharyngeal carcinoma cells cultured in vitro[4], but it is not yet clear about the change of miR-106b expression in nasopharyngeal carcinoma lesions and its relationship with the cell cycle and cell invasion. In the following studies, we took nasopharyngeal carcinoma tissues and adjacent tissues as the research objects and analyzed the correlation of micro-106b in nasopharyngeal carcinoma with cell cycle progression and cell invasion in the lesion.

2. Data and methods

2.1 Clinical data

Patients who were diagnosed with nasopharyngeal carcinoma by pathological biopsy in Shenmu Hospital between March 2013 and February 2018 were included in the study, all patients were...
diagnosed with nasopharyngeal carcinoma for the first time, the patients who received chemoradiotherapy before inclusion were ruled out, and a total of 40 patients were enrolled, including 18 males and 22 females who were 42-65 years old. After obtaining informed consent, the nasopharyngeal carcinoma tissues and the adjacent tissues from biopsy were obtained for test.

2.2 miR-106b expression detection

The right amount of nasopharyngeal carcinoma tissue and adjacent tissue were collected, the tissue miRNA extraction kit was used for experiment, the miRNA was separated from the tissue and then synthesized into cDNA with the specific reverse transcription kit, then fluorescence quantitative PCR kit manual was used to configure the reaction system, it included cDNA 2 μL, reaction mixture 10 μL from PCR kit, primers 0.8 μL and deionized water 7.2 μL, the reaction was conducted on the PCR apparatus and then U6 was used as reference to calculate the miR-106b expression.

2.3 Cell cycle and invasion-related gene expression detection

The right amount of nasopharyngeal carcinoma tissue and adjacent tissue were collected, the tissue RNA extraction kit was used for experiment, the RNA was separated from the tissue and synthesized into cDNA by reverse transcription, then fluorescence quantitative PCR kit manual was followed to configure the reaction system, it included cDNA 2 μL, reaction mixture 10 μL from PCR kit, primers 0.8 μL and deionized water 7.2 μL, the reaction was conducted in PCR apparatus, and then β -actin was used as reference to calculate CYB5R2, p16, FOXC1, CyclinD1, CyclinE, TRIM27, uPAR, IRF5, PARP1, MTA1 and E-cadherin mRNA expression.

2.4 Statistical methods

Software SPSS 22.0 was used to input data, the differences in the measurement data between groups were analyzed by t test and P<0.05 meant statistical significance in the differences.

3. Results

3.1 miR–106b expression in nasopharyngeal carcinoma lesion

miR-106b expression in nasopharyngeal carcinoma tissue was (2.12±0.37) and miR-106b expression in adjacent tissue was (1.02±0.16). The t test analysis of the differences in miR-106b expression in nasopharyngeal carcinoma tissue and adjacent tissue showed that miR-106b expression in nasopharyngeal carcinoma tissue was significantly higher than that in adjacent tissue.

3.2 Cell cycle–related gene expression in nasopharyngeal carcinoma lesions and their correlation with miR–106b

Analysis of cell cycle-related genes CYB5R2, p16, FOXC1, CyclinD1 and CyclinE expression in nasopharyngeal carcinoma tissues and adjacent tissues was as follows: CYB5R2 and p16 mRNA expression in nasopharyngeal carcinoma tissues were lower than those in adjacent tissues whereas FOXC1, CyclinD1 and CyclinE mRNA expression were higher than those in adjacent tissues (P<0.05).

Analysis of cell cycle-related genes CYB5R2, p16, FOXC1, CyclinD1 and CyclinE expression in nasopharyngeal carcinoma tissues with different miR-106b expression was as follows: CYB5R2 and p16 mRNA expression in nasopharyngeal carcinoma tissues with high miR-106b expression were lower than those in nasopharyngeal carcinoma tissues with low miR-106b expression whereas FOXC1, CyclinD1 and CyclinE mRNA expression were higher than those in nasopharyngeal carcinoma tissues with low miR-106b expression (P<0.05).

3.3 Cell invasion–related gene expression in nasopharyngeal carcinoma lesions and their correlation with miR–106b

Analysis of cell invasion-related genes TRIM27, uPAR, IRF5, PARP1, MTA1 and E-cadherin mRNA expression in nasopharyngeal carcinoma tissues and adjacent tissues was as follows: TRIM27, uPAR, PARP1 and MTA1 mRNA expression in nasopharyngeal carcinoma tissues and adjacent tissues was as follows: TRIM27, uPAR, PARP1 and MTA1 mRNA expression in nasopharyngeal carcinoma tissues and adjacent tissues was as follows: TRIM27, uPAR, PARP1 and MTA1 mRNA expression in nasopharyngeal carcinoma tissues and adjacent tissues was as follows: TRIM27, uPAR, PARP1 and MTA1 mRNA expression in nasopharyngeal carcinoma tissues and adjacent tissues was as follows: TRIM27, uPAR, PARP1 and MTA1 mRNA expression in nasopharyngeal carcinoma tissues and adjacent tissues was as follows: TRIM27, uPAR, PARP1 and MTA1 mRNA expression in nasopharyngeal carcinoma tissues and adjacent tissues was as follows: TRIM27, uPAR, PARP1 and MTA1 mRNA expression in nasopharyngeal carcinoma tissues and adjacent tissues was as follows: TRIM27, uPAR, PARP1 and MTA1 mRNA expression in nasopharyngeal carcinoma tissues and adjacent tissues was as follows: TRIM27, uPAR, PARP1 and MTA1 mRNA expression in nasopharyngeal carcinoma tissues and adjacent tissues was as follows: TRIM27, uPAR, PARP1 and MTA1 mRNA expression in nasopharyngeal carcinoma tissues and adjacent tissues was as follows: TRIM27, uPAR, PARP1 and MTA1 mRNA expression in nasopharyngeal carcinoma tissues and adjacent tissues was as follows: TRIM27, uPAR, PARP1 and MTA1 mRNA expression in nasopharyngeal carcinoma tissues and adjacent tissues was as follows: TRIM27, uPAR, PARP1 and MTA1 mRNA expression in nasopharyngeal carcinoma tissues and adjac

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>n</th>
<th>CYB5R2</th>
<th>p16</th>
<th>FOXC1</th>
<th>CyclinD1</th>
<th>CyclinE</th>
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<tr>
<td>Nasopharyngeal carcinoma</td>
<td>40</td>
<td>0.45±0.07</td>
<td>0.39±0.06</td>
<td>1.88±0.25</td>
<td>2.06±0.32</td>
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<td>Adjacent tissue</td>
<td>40</td>
<td>1.03±0.15</td>
<td>1.01±0.16</td>
<td>0.98±0.13</td>
<td>0.96±0.17</td>
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<td>15.839</td>
<td>18.964</td>
<td>17.022</td>
<td>13.485</td>
<td>16.387</td>
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<table>
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<tr>
<th>MiR-106b</th>
<th>n</th>
<th>CYB5R2</th>
<th>p16</th>
<th>FOXC1</th>
<th>CyclinD1</th>
<th>CyclinE</th>
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<tr>
<td>High expression</td>
<td>20</td>
<td>0.28±0.05</td>
<td>0.22±0.04</td>
<td>2.32±0.34</td>
<td>2.78±0.39</td>
<td>3.04±0.46</td>
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<td>Low expression</td>
<td>20</td>
<td>0.63±0.09</td>
<td>0.58±0.08</td>
<td>1.41±0.20</td>
<td>1.36±0.23</td>
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<td>23.922</td>
<td>17.855</td>
<td>10.938</td>
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<td>15.586</td>
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<tr>
<td>P</td>
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<td>&lt;0.05</td>
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</table>
Comparison of cell invasion-related genes in nasopharyngeal carcinoma tissues with different miR-106b expression.

Table 3

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>n</th>
<th>TRIM27</th>
<th>uPAR</th>
<th>IRF5</th>
<th>PARP1</th>
<th>MTA1</th>
<th>E-cadherin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasopharyngeal carcinoma</td>
<td>40</td>
<td>1.73±0.26</td>
<td>2.05±0.36</td>
<td>0.39±0.06</td>
<td>2.31±0.36</td>
<td>1.88±0.24</td>
<td>0.49±0.07</td>
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<tr>
<td>Adjacent tissue</td>
<td>40</td>
<td>1.02±0.14</td>
<td>0.97±0.15</td>
<td>1.04±0.16</td>
<td>0.99±0.11</td>
<td>1.06±0.15</td>
<td>0.95±0.13</td>
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</tbody>
</table>

*p < 0.05

IRF5 and E-cadherin mRNA expression were lower than those in adjacent tissues whereas FOXC1, CyclinD1 and CyclinE mRNA expression in nasopharyngeal carcinoma tissues with low miR-106b expression were significantly lower whereas CYB5R2, CyclinD1 and CyclinE mRNA expression were significantly higher. This indicates that the low expression of negative regulatory genes of cell cycle and the high expression of positive regulatory genes are closely related to the occurrence of nasopharyngeal carcinoma. Further analysis of the correlation between highly expressed miR-106b in nasopharyngeal carcinoma tissues and cell cycle-related genes showed that CYB5R2 and p16 mRNA expression in nasopharyngeal carcinoma tissues were significantly lower whereas FOXC1, CyclinD1 and CyclinE mRNA expression were significantly higher. This indicates that the low expression of negative regulatory genes of cell cycle and the high expression of positive regulatory genes are closely related to the occurrence of nasopharyngeal carcinoma.

Comparison of cell invasion-related genes in nasopharyngeal carcinoma tissues with different miR-106b expression.

Table 4

<table>
<thead>
<tr>
<th>MiR-106b</th>
<th>n</th>
<th>TRIM27</th>
<th>uPAR</th>
<th>IRF5</th>
<th>PARP1</th>
<th>MTA1</th>
<th>E-cadherin</th>
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<tbody>
<tr>
<td>High expression</td>
<td>20</td>
<td>2.08±0.33</td>
<td>2.68±0.42</td>
<td>0.25±0.04</td>
<td>3.09±0.49</td>
<td>2.21±0.30</td>
<td>0.29±0.04</td>
</tr>
<tr>
<td>Low expression</td>
<td>20</td>
<td>1.39±0.21</td>
<td>1.47±0.22</td>
<td>0.55±0.08</td>
<td>1.61±0.22</td>
<td>1.56±0.18</td>
<td>0.70±0.09</td>
</tr>
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</table>

*p < 0.05

4. Discussion

The accelerated cell cycle and active cell invasion in nasopharyngeal carcinoma lesions are the important pathologic links causing disease progression, but the specific regulatory mechanism of cell cycle and invasion is still not clarified. It has been discovered in recent years that miRNA is the non-coding small RNA that plays an important role in cell proliferation, differentiation, invasion and many other biological links, and its biological activity is dependent on the negative regulation of miRNA on target gene expression. mir-106b is a newly discovered miRNA with proto-oncogene activity, and mir-106b shows the trend of high expression in lung cancer, breast cancer, kidney cancer and various other malignant tumor lesions[5-7]. In the cellular study related to nasopharyngeal carcinoma, up-regulation of mir-106b can promote the proliferation and invasion of nasopharyngeal carcinoma cells, and inhibiting mir-106b can hinder the proliferation and invasion of nasopharyngeal carcinoma cells[4]. The above results indicate that mir-106b has a significant promoting effect on the proliferation and invasion of nasopharyngeal carcinoma cells, but it is still unclear about the changes of mir-106b expression and the specific biological effects in the nasopharyngeal carcinoma lesions. In the above study, we analyzed the difference in miR-106b expression in nasopharyngeal carcinoma tissues and adjacent tissues at first, and the results showed that miR-106b expression in nasopharyngeal carcinoma tissue was significantly higher than that in adjacent tissue. This means that the high expression of miR-106b in the local lesion is closely related to the occurrence of nasopharyngeal carcinoma, and the analysis based on the biological effects of miR-106b in nasopharyngeal carcinoma cells in vitro shows that the highly expressed miR-106b in nasopharyngeal carcinoma tissue may be involved in the positive regulation of cancer cell proliferation and invasion.

During the proliferation of cancer cells in nasopharyngeal carcinoma, the acceleration of cell cycle is a biological link closely related to cell proliferation. CYB5R2 is a negative regulatory molecule of cell cycle that can increase the expression of p16 gene and antagonize the activation of a variety of cyclins through the biological activities of p16 so as to cause cell cycle arrest and inhibit cell proliferation[8,9]. FOXC1 is a fork head transcription factor, and it can increase the expression of a variety of cyclins such as CyclinD1 and CyclinE after it enters the nucleus, which will form complexes with corresponding kinases and accelerate the transformation rate of cell cycle[10,11]. Analysis of the changes of above cell cycle-related gene expression in nasopharyngeal carcinoma tissue showed that CYB5R2 and p16 mRNA expression in nasopharyngeal carcinoma tissues were significantly lower whereas FOXC1, CyclinD1 and CyclinE mRNA expression were significantly higher. This indicates that the low expression of negative regulatory genes of cell cycle and the high expression of positive regulatory genes are closely related to the occurrence of nasopharyngeal carcinoma. Further analysis of the correlation between highly expressed miR-106b in nasopharyngeal carcinoma tissues and cell cycle-related genes showed that CYB5R2 and p16 mRNA expression in nasopharyngeal carcinoma tissues with high miR-106b expression were significantly lower whereas FOXC1, CyclinD1 and CyclinE mRNA expression were significantly higher. This means that the highly expressed miR-106b in nasopharyngeal carcinoma tissues can inhibit the expression of negative regulatory genes of cell cycle, and promote the expression of positive regulatory genes of cell cycle to accelerate the cell cycle and promote cell proliferation. On the basis of proliferation, the tumor cells in nasopharyngeal carcinoma lesion will continuously invade the surrounding tissue, which leads to infiltration and diffusion of tumor lesions. TRIM27 is a type of trp3 motif highly conserved in evolution, which can enhance the invasion ability of cells through the activation of downstream Ras/MAPK pathway[12]; uPAR can activate fibrinolytic enzyme in extracellular matrix and degrade the matrix and basement membrane components surrounding tumor cells and facilitate cell invasion.
invasion[13]; IFR5 is a molecule with immunomodulatory activity, which inhibits the self-repair and invasion process of cells through the negative regulation of PARP1 in the nucleus[14,15]; MTA1 is a kind of tumor progression-related gene, and directly participates in the regulation of tumor cell migration, movement and invasion[16,17]; E-cadherin is the marker gene of epithelial cells and has important value for maintaining the intercellular polarity and adhesion, and its expression trend will weaken the cell polarity and enhance its invasion[18]. Analysis of the changes in above cell invasion-related gene expression in nasopharyngealcarcinoma tissue showed that TRIM27, uPAR, PARP1 and MTA1 mRNA expression in nasopharyngealcarcinoma tissues were significantly higher whereas IFR5 and E-cadherin mRNA expression were significantly lower. This indicates that the high expression of the positive regulatory genes of cell invasion and the low expression of negative regulatory genes are closely related to the occurrence of nasopharyngealcarcinoma. Further analysis of the correlation between highly expressed miR-106b in nasopharyngealcarcinoma tissues and cell invasion-related genes showed that TRIM27, uPAR, PARP1 and MTA1 mRNA expression in nasopharyngealcarcinoma tissues with high miR-106b expression were significantly higher whereas IFR5 and E-cadherin mRNA expression were significantly lower. This indicates that the highly expressed miR-106b in nasopharyngealcarcinoma can increase the expression of positive regulatory genes of cell invasion and inhibit the expression of negative regulatory genes of cell invasion to promote the invasion of cells.

Based on the analysis and discussion about the expression of miR-106b as well as cell cycle-related genes and cell invasion-related genes, it can be preliminarily concluded that miR-106b shows the trend of high expression in nasopharyngeal carcinoma tissues; the highly expressed miR-106b can regulate the expression of cell cycle-related genes and cell invasion-related genes to accelerate cell cycle progression and promote cell invasion.

References