Relationship between miR-100 expression in cervical cancer tissue and cisplatin resistance

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ABSTRACT

Objective: To study the relationship between miR-100 expression in cervical cancer tissue and cisplatin resistance. Methods: A total of 107 cases of cervical cancer tissues from those who received cisplatin chemotherapy in Huanggan Central Hospital between May 2013 and October 2016 were collected as the clinical samples and divided into chemotherapy-responsive cisplatin-sensitive cervical cancer tissue and chemotherapy-irresponsive cisplatin-sensitive cervical cancer tissue according to the curative effect of chemotherapy. The miR-100 expression as well as the expression of drug resistance-related genes, cell cycle-related genes and cell invasion-related genes was determined. Results: The miR-100 expression in cisplatin-resistant cervical cancer tissue was significantly lower than that in cisplatin-sensitive cervical cancer tissue while Nek2, P-gp, GST-π, Topo-II, SP2, CyclinD1, CyclinG1, CDK4, CDK5, MMP1, PAR1, RbAp48, Vimentin and N-cadherin expression were significantly higher than those in cisplatin-sensitive cervical cancer tissue; the miR-100 expression was negatively correlated with Nek2, P-gp, GST-π, Topo-II, SP2, CyclinD1, CyclinG1, CDK4, CDK5, MMP1, PAR1, RbAp48, Vimentin and N-cadherin expression. Conclusion: The lower expression of miR-100 in cervical cancer tissue is closely associated with cisplatin resistance.

1. Introduction

Cervical cancer is one of the malignant tumors of female reproductive system with the highest incidence, and the middle-advanced cervical cancer is mainly treated with radiotherapy and chemotherapy. Cisplatin is a common chemotherapy drug for clinical treatment of cervical cancer, and the cisplatin-based chemotherapy regimen is the first-line solution for advanced cervical cancer chemotherapy. In clinical practice, cervical cancer sensitivity to cisplatin chemotherapy varies, and cisplatin resistance may occur in some patients and influence chemotherapy effect[1]. Drug resistance gene expression changes are the important factors influencing the cervical cancer sensitivity to cisplatin[2], but the mechanism adjusting the drug resistance gene expression is not yet clear. microRNA (miRNA) is the non-coding RNA that has received more and more attention in recent years, it participates in the posttranscriptional regulation of multiple genes in the body, and it has inhibitory effect on the translation of mRNA from target gene transcription. MiR-100 is a class of miRNA closely related to malignant tumors, and raising the miR-100 can inhibit cervical cancer cell proliferation and invasion and improve the sensitivity of cisplatin[3]. In the following study, the relationship between the miR-100 expression in cervical cancer tissue and cisplatin resistance was analyzed, and the miR-100 expression changes in cisplatin-resistant and cisplatin-sensitive cervical cancer tissue as well as their correlation with drug resistance genes, cell cycle genes and invasion genes were specifically explored.

2. Clinical sample information and experimental methods

2.1 Clinical sample information

A total of 107 cases of cervical cancer tissues from those who received cisplatin chemotherapy in Huanggan Central Hospital...
between May 2013 and October 2016 were collected as the clinical samples, all cervical cancer patients never received radiotherapy and chemotherapy or targeted drug treatment before inclusion, and they were diagnosed with cervical cancer after pathological biopsy. Pathology biopsy cervical cancer tissues were collected before chemotherapy, and according to the curative effect of chemotherapy, they were divided into the chemotherapy-responsive cisplatin-sensitive cervical cancer tissue and the chemotherapy-irresponsive cisplatin-sensitive cervical cancer tissue. Chemotherapy effect evaluation standard was as follows: the shrinking of maximum tumor diameter before and after chemotherapy more than 30% or the shrinking of maximum diameter and vertical diameter product more than 50% was judged as valid, and the rest was invalid.

2.2 Experimental methods

2.2.1 miR–100 expression detection methods

Cervical cancer tissue was collected, the miRNA extraction kits were used to separate the miRNA in tissue, the miRNA-dedicated cDNA synthesis kits were used for reverse transcription from miRNA into cDNA, the miR-100 and U6 primers were designed, fluorescence quantitative PCR was performed and the miR-100 expression was calculated.

2.2.2 Gene protein expression detection methods

Cervical cancer tissue was taken, added in RIPA lysis buffer and homogenized to extract total protein, enzyme-linked immunosorbent assay kits were used to determine the serine/threonine-protein kinase (Nek2), P-glycoprotein (P-gp), glutathione S transferase -π (GST-π), DNA topoisomerase II (Topo-II), SP2, CyclinD1, CyclinG1 of protein, cyclin-dependent kinase 4 (CDK4), CDK5, matrix metalloproteinase 1 (MMP1), protease-activated receptor 1 (PAR1), Rb-associated protein 48 (RbAp48), Vimentin and N-cadherin content.

2.3 Statistical methods

SPSS 18.0 software was used for data analysis, data between two groups was analyzed by t test and correlation was analyzed by Pearson test.

3. Results

3.1 miR–100 expression in cervical cancer tissue

miR-100 expression in cisplatin-resistant and cisplatin-sensitive cervical cancer tissue were (1.02±0.19) and (2.37±0.52) respectively. After t test, miR-100 expression in cisplatin-resistant cervical cancer tissue was significantly lower than that in cisplatin-sensitive cervical cancer tissue. Differences were statistically significant in miR-100 expression in cisplatin-resistant and cisplatin-sensitive cervical cancer tissue (P<0.05).

3.2 Drug resistance gene expression in cervical cancer tissue

Analysis of drug resistance genes Nek2 (μg/L), P-gp (ng/L), GST-π (ng/L) and Topo-II (ng/L) expression in cisplatin-resistant and cisplatin-sensitive cervical cancer tissue was as follows: Nek2, P-gp, GST-π and Topo-II expression in cisplatin-resistant cervical cancer tissue were significantly higher than those in cisplatin-sensitive cervical cancer tissue. Differences were statistically significant in Nek2, P-gp, GST-π and Topo-II expression in cisplatin-resistant and cisplatin-sensitive cervical cancer tissue (P<0.05). Pearson test showed that the miR-100 expression was negatively correlated with Nek2, P-gp, GST-π and Topo-II expression, and the correlation coefficient was -0.663, -0.596, -0.708 and -0.681 respectively.

3.3 Cell cycle–related gene expression in cervical cancer tissue

Analysis of cell cycle-related genes SP2 (μg/L), CyclinD1 (μg/L), CyclinG1 (μg/L), CDK4 (ng/L) and CDK5 (ng/L) expression in cisplatin-resistant and cisplatin-sensitive cervical cancer tissue was as follows: SP2, CyclinD1, CyclinG1, CDK4 and CDK5 expression in cisplatin-resistant cervical cancer tissue were significantly higher than those in cisplatin-sensitive cervical cancer tissue. Differences were statistically significant in SP2, CyclinD1, CyclinG1, CDK4 and CDK5 expression in cisplatin-resistant cervical cancer tissue (P<0.05). Pearson test showed that the miR-100 expression was negatively correlated with SP2, CyclinD1, CyclinG1, CDK4 and CDK5 expression, and the correlation coefficient was -0.629, -0.527, -0.611, -0.657 and -0.724 respectively.

Table 1.

<table>
<thead>
<tr>
<th>Cisplatin sensitivity</th>
<th>n</th>
<th>Nek2</th>
<th>P-gp</th>
<th>GST-π</th>
<th>Topo-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin-sensitive</td>
<td>69</td>
<td>1.42±0.18</td>
<td>103.52±15.38</td>
<td>88.97±12.41</td>
<td>146.39±22.14</td>
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<tr>
<td>Cisplatin-resistant</td>
<td>38</td>
<td>3.54±0.55</td>
<td>247.69±32.58</td>
<td>167.73±22.15</td>
<td>357.54±41.20</td>
</tr>
<tr>
<td>T</td>
<td>14.592</td>
<td>15.609</td>
<td>9.583</td>
<td>16.801</td>
<td></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 2.

<table>
<thead>
<tr>
<th>Cisplatin sensitivity</th>
<th>n</th>
<th>SP2</th>
<th>CyclinD1</th>
<th>CyclinG1</th>
<th>CDK4</th>
<th>CDK5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin-sensitive</td>
<td>69</td>
<td>0.78±0.09</td>
<td>2.14±0.32</td>
<td>1.76±0.31</td>
<td>145.25±22.12</td>
<td>121.29±16.72</td>
</tr>
<tr>
<td>Cisplatin-resistant</td>
<td>38</td>
<td>1.89±0.22</td>
<td>5.58±0.77</td>
<td>4.57±0.62</td>
<td>357.86±52.59</td>
<td>231.85±33.58</td>
</tr>
<tr>
<td>T</td>
<td>13.482</td>
<td>15.028</td>
<td>15.782</td>
<td>16.108</td>
<td></td>
<td></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>
<td></td>
</tr>
</tbody>
</table>
sensitivity to cisplatin, and the lowly expressed miR-100 in local changes are the important factors influencing the cervical lesion sensitive cervical cancer tissue. This means that miR-100 expression showed that miR-100 expression in cisplatin-resistant cervical cancer tissue in the study, and the analysis of the miR-100 into cisplatin-sensitive cervical cancer tissue and cisplatin-resistant the cervical cancer tissue after cisplatin chemotherapy was divided miR-100 expression and cisplatin chemotherapy for cervical cancer, sensitivity to cisplatin. In order to define the relationship between miR-100 might have regulating effect on the cervical cancer lesion sensitive cervical cancer tissue. The results indicate that the abnormal expression of drug resistance genes in cervical cancer cells will not only affect the sensitivity of chemotherapy drugs, but can also affect the cell proliferation and invasion; at the same time, a variety of cell cycle-related genes mediating cell proliferation are also under the targeted regulation of miR-100. SP2 is and important transcription factor regulating cell proliferation and cell cycle, and it is combined with the GC/TC-rich sequence to increase the downstream CyclinD1 and CyclinG1 expression; CyclinD1 and CyclinG1 can form complexes with CDK4 and CDK5 so as to accelerate the cell cycle over G1 phase and promote cell proliferation[11,12]. In the study, analysis of the cell cycle-related gene expression showed that SP2, CyclinD1, CyclinG1, CDK4 and CDK5 expression in cisplatin-resistant cervical cancer tissue were significantly higher than those in cisplatin-sensitive cervical cancer tissue. This means that high expression of Nek2, P-gp, GST-π and Topo-II is closely associated with cervical cancer resistance to cisplatin. Further analysis of the correlation between miR-100 and drug resistance gene expression indicated that lower expression of Nek2, P-gp, GST-π and Topo-II expression in cisplatin-resistant cervical cancer tissue were significantly higher than those in cisplatin-sensitive cervical cancer tissue. This means that high expression of Nek2, P-gp, GST-π and Topo-II is closely associated with cervical cancer resistance to cisplatin. Further analysis of the correlation between miR-100 and drug resistance gene expression indicated that miR-100 expression was negatively correlated with MMP1, PAR1, RbAp48, Vimentin and N-cadherin expression, and the correlation coefficient was -0.633, -0.578, -0.548, -0.615 and -0.648 respectively.

4. Discussion

MiRNAs is the small non-coding RNA that has received more and more attention in recent years, there are the binding sites of different miRNAs in 3’ non-coding region of mRNA obtained from the transcription of a variety of genes, and miRNA combination with 3’ non-coding region can cause mRNA degradation, inhibit mRNA translation, and then generate the corresponding biological effects through the change of the target gene expression. MiR-100 is a kind of miRNAs closely related to the occurrence of gastric cancer, liver cancer, ovarian cancer and other malignant tumors[4-6]. The study of domestic scholar LIU Yun-yun[3] about miR-100 in cervical cancer Hela and Siha cell lines confirmed that raising miR-100 in cervical cancer cells can inhibit cancer cell invasion and improve the sensitivity of cancer cells to cisplatin. The results indicate that miR-100 might have regulating effect on the cervical cancer lesion sensitivity to cisplatin. In order to define the relationship between miR-100 expression and cisplatin chemotherapy for cervical cancer, the cervical cancer tissue after cisplatin chemotherapy was divided into cisplatin-sensitive cervical cancer tissue and cisplatin-resistant cervical cancer tissue in the study, and the analysis of the miR-100 expression showed that miR-100 expression in cisplatin-resistant cervical cancer tissue was significantly lower than that in cisplatin-sensitive cervical cancer tissue. This means that miR-100 expression changes are the important factors influencing the cervical lesion sensitivity to cisplatin, and the lowly expressed miR-100 in local lesions can cause the occurrence of cisplatin resistance.

The biological effect of miRNAs is mainly achieved by regulating the expression of target genes, and cervical cancer cell resistance to chemotherapy drugs is closely related to the abnormal expression of Nek2, P-gp, GST-π, Topo-II and other drug resistance genes. Nek2 is a kind of serine/threonine protein kinase that causes P-gp, GST-π and Topo-II activation through the phosphorylation activation of downstream signaling molecules[7]. P-gp participates in the active transport of chemotherapy drugs from inside of the cells to outside of the cells, and can reduce the concentration of chemotherapeutic drugs in the cells and cause the development of drug resistance[8]; GST-can promote glutathione combination with the chemotherapy drug inside the cells, and thus inhibit the toxic effects of chemotherapy drugs on cells[9]. Topo-II is a cell cycle-dependent catalytic enzyme that can promote DNA replication and repair, and thus mitigate the effects of chemotherapy drugs on DNA replication[10]. In the study, analysis of the drug resistance gene expression indicated that Nek2, P-gp, GST-π and Topo-II expression in cisplatin-resistant cervical cancer tissue were significantly higher than those in cisplatin-sensitive cervical cancer tissue. This means that high expression of Nek2, P-gp, GST-π and Topo-II is closely associated with cervical cancer resistance to cisplatin.

### Table 3.

<table>
<thead>
<tr>
<th>Cisplatin sensitivity</th>
<th>n</th>
<th>MMP1</th>
<th>PAR1</th>
<th>RbAp48</th>
<th>Vimentin</th>
<th>N-cadherin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin-sensitive</td>
<td>69</td>
<td>4.29±0.72</td>
<td>2.42±0.41</td>
<td>325.63±47.59</td>
<td>10.28±1.65</td>
<td>6.38±0.94</td>
</tr>
<tr>
<td>Cisplatin-resistant</td>
<td>38</td>
<td>10.22±1.52</td>
<td>6.57±0.94</td>
<td>885.62±102.52</td>
<td>26.79±4.21</td>
<td>14.58±1.78</td>
</tr>
</tbody>
</table>

### 3.4 Cell invasion-related gene expression in cisplatin-resistant and cisplatin-sensitive cervical cancer tissue

Analysis of cell invasion-related genes MMP1, PAR1, RbAp48, Vimentin and N-cadherin expression in cisplatin-resistant and cisplatin-sensitive cervical cancer tissue was as follows: MMP1, PAR1, RbAp48, Vimentin and N-cadherin expression in cisplatin-resistant cervical cancer tissue were significantly higher than those in cisplatin-sensitive cervical cancer tissue. Differences were statistically significant in MMP1, PAR1, RbAp48, Vimentin and N-cadherin expression in cisplatin-resistant and cisplatin-sensitive cervical cancer tissue (\(P<0.05\)). Pearson test showed that the miR-100 expression was negatively correlated with MMP1, PAR1, RbAp48, Vimentin and N-cadherin expression, and the correlation coefficient was -0.633, -0.578, -0.548, -0.615 and -0.648 respectively.
Further analysis of the correlation between miR-100 and cell cycle-related gene expression indicated that miR-100 expression was negatively correlated with SP2, CyclinD1, CyclinG1, CDK4 and CDK5 expression. It means that lower expression of miR-100 is closely related to the high expression of Cyclins/CDKs mediated by SP2, and the lowly expressed miR-100 in cervical cancer lesions can increase the expression of SP2 and increase the Cyclins/CDKs expression so as to cause the cancer cell resistance to cisplatin.

Cervical cancer cell resistance to chemotherapy drugs can cause the invasive growth of cancer cells, and eventually result in distant metastasis or abdominal spreading and implantation. Cancer cell invasion is an important biological behavior causing invasive cervical cancer growth, and the extracellular matrix degradation mediated by MMP1 and the cellular epithelial-mesenchymal transition mediated by RhAp48 can significantly promote cell invasion. MMP1 combination with membrane receptor PAR1 can not only cause the degradation of a variety of ingredients in the extracellular matrix, but can also promote cancer cell adhesion and infiltration to the surrounding tissue[13]; RhAp48 can promote the transition from epithelial phenotype of cells to mesenchymal phenotype of cells[14], increase the expression of Vimentin, N-cadherin and other mesenchymal cell marker molecule and decrease intercellular adhesion performance, which is advantageous to the distant metastasis of the cancer cells[15-17]. In the study, analysis of the expression of above cell invasion-related genes showed that MMP1, PAR1, RhAp48, Vimentin and N-cadherin expression in cisplatin-resistant cervical cancer tissue were significantly higher than those in cisplatin-sensitive cervical cancer tissue. This means that the high expression of a variety of invasion genes is closely associated with cervical cancer resistance to cisplatin. Further analysis of the correlation between miR-100 and the cell invasion-related gene expression indicated that the miR-100 expression was negatively correlated with MMP1, PAR1, RhAp48, Vimentin and N-cadherin expression. It means that lower expression of miR-100 can promote the extracellular matrix degradation mediated by RhAp48 and the cellular epithelial-mesenchymal transition mediated by RhAp48 so as to cause cancer cell resistance to cisplatin.

Above all, it is believed that miR-100 shows a trend of lower expression in cisplatin-resistant cervical cancer tissue; the lowly expressed miR-100 can increase the expression of a variety of drug resistance genes, the expression of Cyclins/CDKs mediated by SP2, the extracellular matrix degradation mediated by MMP1 and the cellular epithelial-mesenchymal transition mediated by RhAp48.

References


