Correlation of Runt-related transcription factor gene 3 expression in osteosarcoma tissue with cell proliferation and angiogenesis

Bin Xie

Orthopedics Department No. 3 Ward, Yan’an People’s Hospital, Yan’an City 716000, China

1. Introduction

Osteosarcoma is the malignant bone tumor with a variety of gene mutations involved in its occurrence and development, which commonly occurs in adolescents who are under the age of 20. For recent years, many scholars have believed that Runt-related transcription factor 3 (RUNX3) expression change may be directly involved in the malignant progress of osteosarcoma[1,2]. RUNX3 belongs to Runt-related transcription factor family. Cell research has demonstrated that RUNX3 gene knockout can lead to the occurrence of a variety of tumors[3,4], but there is not much research about the relationship between RUNX3 and osteosarcoma at present. In order to define the role of RUNX3 in the osteosarcoma progression, the expression of RUNX3 gene in osteosarcoma tissue and adjacent tissue were compared in the study, and the effect of differential expression of RUNX3 gene in osteosarcoma tissue on tumor proliferation, angiogenesis and other malignant behaviors was further explored, hereby reported as follows.

2. Materials and methods

2.1. General information

A total of 80 cases of patients with osteosarcoma who were treated in our hospital between February 2014 and February 2017 were enrolled as research subjects, and the families of the patients have signed informed consent. The enrolled patients included 43 male cases and 37 female cases that were 11-25 years old, and the study was approved by the hospital ethics committee. The inclusion criteria were as follows: (1) diagnosed with osteosarcoma by histopathology; (2) receiving examination and treatment for the first time, and without surgery and drug treatment history; (3) cooperating with related clinical examination and with complete data. The exclusion criteria were as follows: (1) with metastatic osteosarcoma; (2) combined with malignant tumors of other tissue organs.
2.2. RUNX3 expression

Osteosarcoma tissue and adjacent tissue were taken, added in Trizol reagent (produced by Shanghai Xinfan Biotechnology Co., Ltd., the article number 15596-026) to split cells and then centrifuged at high speed to get upper clear water, the same volume of isopropanol (produced by Nanjing, Mr Ng Biotechnology Co., Ltd., the article number 0918) was used to precipitate the total RNA, and then it was cleaned and air-dried at room temperature. Sample cDNA was synthesized according to the operation instructions of reverse transcription kit (produced by Biomiga company, article number RT021301). 50 μg of cDNA specimen was taken, and the fluorescence quantitative PCR kit (Shenzhen Ziker Biological Technology Co., Ltd., the article number zk7340) instructions were followed for the RUNX3 mRNA amplification.

2.3. Proliferation gene and angiogenesis gene expression

Osteosarcoma tissue was collected to amplify the mRNA of proliferation genes: KISS-1, VCP, RanBP9, Six1 and S100A6 as well as angiogenesis genes: HIF-1α, MMP-14, bFGF and Ang-2, and the specific steps were the same as in 2.2.

2.4. Statistical processing

Statistical software was SPSS 20.0. RUNX3 expression, tumor cell proliferation gene expression and angiogenesis gene expression belonged to measurement data and were in terms of mean ± standard deviation, and $P<0.05$ was set as the standard of statistical significance in differences.

3. Results

3.1. RUNX3 expression

RUNX3 mRNA expression in tissue adjacent to osteosarcoma was (93.26±11.53) and RUNX3 mRNA expression in osteosarcoma tissue was (27.51±4.09). RUNX3 mRNA expression in osteosarcoma tissue was significantly lower than that in adjacent tissue. Differences were statistically significant in RUNX3 mRNA expression in osteosarcoma tissue and adjacent tissue ($P<0.05$).

3.2. RUNX3 expression and tumor cell proliferation

Analysis of proliferation genes KISS-1, VCP, RanBP9, Six1 and S100A6 mRNA expression in osteosarcoma tissue and adjacent tissue was as follows: KISS-1 and RanBP9 mRNA expression in osteosarcoma tissue were significantly lower than those in adjacent tissue while VCP, Six1 and S100A6 mRNA expression were significantly higher than those in adjacent tissue, and differences were statistically significant in KISS-1, VCP, RanBP9, Six1 and S100A6 mRNA expression in osteosarcoma tissue and adjacent tissue ($P<0.05$), shown in Table 1.

Analysis of proliferation genes KISS-1, VCP, RanBP9, Six1 and S100A6 mRNA expression in osteosarcoma tissue with different RUNX3 expression was as follows: KISS-1 and RanBP9 mRNA expression in osteosarcoma tissue of high RUNX3 expression group were significantly higher than those of low RUNX3 expression group while VCP, Six1 and S100A6 mRNA expression were significantly lower than those of low RUNX3 expression group. Differences were statistically significant in proliferation genes KISS-1, VCP, RanBP9, Six1 and S100A6 mRNA expression in high RUNX3 expression group and low RUNX3 expression group ($P<0.05$), shown in Table 2.

3.3. RUNX3 expression and angiogenesis

Analysis of angiogenesis genes HIF-1α, MMP-14, bFGF and Ang-2 mRNA expression in osteosarcoma tissue and adjacent tissue was as follows: HIF-1α, MMP-14, bFGF and Ang-2 mRNA expression in osteosarcoma tissue were significantly higher than those in adjacent tissue, and differences were statistically significant in HIF-1α, MMP-14, bFGF and Ang-2 mRNA expression in osteosarcoma tissue and adjacent tissue ($P<0.05$), shown in Table 3.

<p>| Table 1 | Comparison of proliferation gene expression in osteosarcoma tissue and adjacent tissue. |</p>
<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>n</th>
<th>KISS-1</th>
<th>VCP</th>
<th>RanBP9</th>
<th>Six1</th>
<th>S100A6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteosarcoma</td>
<td>80</td>
<td>45.37±5.21</td>
<td>199.84±22.16</td>
<td>61.75±8.28</td>
<td>228.53±30.47</td>
<td>203.21±25.88</td>
</tr>
<tr>
<td>Adjacent tissue</td>
<td>80</td>
<td>104.29±13.73</td>
<td>101.73±8.59</td>
<td>102.36±12.51</td>
<td>101.48±12.53</td>
<td>102.64±11.52</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>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

<p>| Table 2 | Comparison of proliferation gene expression in osteosarcoma tissue with different RUNX3 expression. |</p>
<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>KISS-1</th>
<th>VCP</th>
<th>RanBP9</th>
<th>Six1</th>
<th>S100A6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low RUNX3 expression group</td>
<td>40</td>
<td>20.12±3.48</td>
<td>262.35±31.58</td>
<td>42.31±5.68</td>
<td>302.37±48.84</td>
<td>203.21±25.88</td>
</tr>
<tr>
<td>High RUNX3 expression group</td>
<td>40</td>
<td>79.41±9.35</td>
<td>137.54±15.69</td>
<td>77.65±9.42</td>
<td>142.58±19.39</td>
<td>141.25±15.58</td>
</tr>
<tr>
<td>$t$</td>
<td></td>
<td>27.948</td>
<td>9.182</td>
<td>7.884</td>
<td>12.485</td>
<td>8.685</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>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
Ang-2 mRNA expression in osteosarcoma tissue with different RUNX3 expression was as follows: IF-1, MMP-14, bFGF and Ang-2 mRNA expression in osteosarcoma tissue of high RUNX3 expression group were significantly lower than those of low RUNX3 expression group. Differences were statistically significant in angiogenesis genes IF-1, MMP-14, bFGF and Ang-2 mRNA expression in high RUNX3 expression group and low RUNX3 expression group (P<0.05), shown in Table 4.

### 4. Discussion

Osteosarcoma is a tumor with high clinical malignant degree, whose early metastasis is one of the important causes to death for patients. Therefore, looking for the genes closely related to the malignant degree of tumor is the key to the future target therapy practice. RUNX3 gene plays an important role in the process of embryonic development, its expression reduction or even deletion has now been confirmed in cervical cancer, gastric cancer and other tumor tissues, and therefore, some scholars think that the RUNX3 is a new tumor suppressor gene[5-7]. At present, there is not much research about the RUNX3 expression change in osteosarcoma tissue and the influence on the malignant biological behavior of tumor cells, it is essential to conduct the study focusing on it. The relationship of RUNX3 expression change with the occurrence and development of osteosarcoma will be discussed in the following paragraphs in order to provide practice basis for the mechanism of long-term osteosarcoma and gene therapy. In the study, the differences in RUNX3 gene expression in osteosarcoma tissue and adjacent tissue were compared at first, and it was found that RUNX3 mRNA expression in osteosarcoma tissue was lower than that in adjacent tissue, which is the same as the change trend of RUNX3 gene expression in cervical cancer. Therefore, it is confirmed that the reduced expression of RUNX3 gene is involved in the occurrence of osteosarcoma, but the effect of RUNX3 gene expression changes on malignant behavior of tumor tissue remains to be confirmed below.

Tumor cell pro-proliferation/anti-proliferation gene expression imbalance is the primary cause of their infinite proliferation, and detection of proliferation-related genes expression in osteosarcoma tissue can quantifiably reflect tumor proliferation activity[8]. The expression of KISS-1 has a certain inhibitory effect on tumor cell proliferation, which activates Rho and its related kinase and induces tumor necrosis factor expression[9]. VCP is involved in the regulation of various cell proliferation and apoptosis activities, and the studies have shown that the proliferation activity of osteosarcoma cells decreases after silencing VCP expression[10,11]. RanBP9, closely related to cell adhesion migration and widely expressed in different human tissues, is found to be lowly expressed in osteosarcoma cells, and it can act as a cancer-suppressor gene to be involved in osteosarcoma[12]. Six1 gene mainly involved in the development of eyes, ears, muscles and other tissues has been confirmed in the latest study that Six1 gene is highly expressed in many kinds of malignant tumors and inhibiting its proliferation can reduce the proliferation and metastasis capacity of the tumor cells[13]. The S100A6 can stimulate proliferation and invasion of osteosarcoma cells by activating PI3K/Akt signaling pathway[14]. In the study, analysis of the proliferation gene expression in osteosarcoma tissue showed that KISS-1 and RanBP9 mRNA expression in osteosarcoma tissue were significantly significantly lower than those in adjacent tissue while VCP, Six1 and S100A6 mRNA expression were significantly higher than those in adjacent tissue, and further analysis of the proliferation gene expression in osteosarcoma tissues with different RUNX3 expression showed that KISS-1 and RanBP9 mRNA expression in osteosarcoma tissue were significantly lower than those in adjacent tissue while VCP, Six1 and S100A6 mRNA expression were significantly lower than those of low RUNX3 expression group. The combination with the physiological function of these genes can confirms that the lowly expressed RUNX3 can promote the proliferation activity of osteosarcoma cells.

Vigorous angiogenesis provides oxygen and nutrients to the
proliferation and invasion of tumor cells. Therefore, the expression of pro-angiogenesis indexes in tumor tissues can indirectly reflect the malignancy of tumor. The tumor tissue is in a hypoxia state, which can induce HIF-1α overexpression and induce VEGF gene expression to accelerate the angiogenesis within tumor[15]. MMP-14 is a kind of transmembrane proteolytic enzyme, which degrades the extracellular matrix and also promotes tumor angiogenesis. bFGF is one of the most important angiogenesis factors, it is closely related to tumor angiogenesis, and bFGF expression increases with the increase of tumor malignancy[16]. Ang-2 is a specific pro-angiogenesis factor for vascular endothelial cells, which can promote the vascular endothelial cell survival and induce vascular maturation[17]. In the study, analysis of the angiogenesis gene expression in osteosarcoma tissue showed that IF-1α, MMP-14, bFGF and Ang-2 mRNA expression in osteosarcoma tissue were significantly higher than those in adjacent tissue. Further analysis of the angiogenesis gene expression in osteosarcoma tissue with different RUNX3 expression showed that angiogenesis gene IF-1α, MMP-14, bFGF and Ang-2 mRNA expression in osteosarcoma tissue of high RUNX3 expression group were significantly lower than those of low RUNX3 expression group. This confirms that lowly expressed RUNX3 can promote angiogenesis in osteosarcoma.

To sum up, it can be concluded as follows: RUNX3 gene expression decreases in osteosarcoma tissue, and the RUNX3 gene expression is negatively correlated with tumor cell proliferation activity and angiogenesis activity.

References


