Tumor suppressor gene TAp73 and autophagy gene Beclin1 expression in osteosarcoma tissue and their correlation with tumor malignancy

Yun-Hua Li, Chun-Lei Liu, Jiang Zhu, Xiao-Bo Tan
Orthopedics Department Section 2, Qingyuan People’s Hospital in Guangdong Province, Qingyuan 511518, China

Objective: To study the correlation of tumor suppressor gene TAp73 and autophagy gene Beclin1 expression in osteosarcoma tissue with tumor malignancy. Methods: 56 cases of osteosarcoma tissues and peri-osteosarcoma tissues as well as 37 cases of giant cell tumor tissues surgically removed in our hospital between June 2013 and August 2016 were collected, immunohistochemical kits were used to detect the positive expression rate of TAp73 and Beclin1, and enzyme-linked immunosorbent assay kits were used to determine the protein expression TAp73, Beclin1 in osteosarcoma tissues were significantly lower than those in peri-osteosarcoma tissues and giant cell tumor tissues (P<0.05), and IRX2, JAK3, STAT5, CTGF, Wnt and β-catenin protein expression were significantly higher than those in peri-osteosarcoma tissues and giant cell tumor tissues (P<0.05); IRX2, JAK3, STAT5, CTGF, Wnt and β-catenin protein expression in osteosarcoma tissues with TAp73 and Beclin1 (+), (++) and (+++) were significantly lower than those in osteosarcoma tissues with TAp73 and Beclin1 (-), IRX2, JAK3, STAT5, CTGF, Wnt and β-catenin protein expression in osteosarcoma tissues with TAp73 and Beclin1 (+), (++) and (+++) were significantly lower than those in osteosarcoma tissues with TAp73 and Beclin1 (+), and IRX2, JAK3, STAT5, CTGF, Wnt and β-catenin protein expression in osteosarcoma tissues with TAp73 and Beclin1 (+++) were significantly lower than those in osteosarcoma tissues with TAp73 and Beclin1 (+++) (P<0.05). Conclusion: Lowly expressed tumor suppressor gene TAp73 and autophagy gene Beclin1 in osteosarcoma tissue can activate IRX2/JAK3/STAT5 and CTGF/ Wnt/β-catenin signaling pathways to promote cell proliferation and invasion.

1. Introduction

Osteosarcoma (OS) is the most common primary malignant tumor of bone tissue, it trends to occur in teenagers, the tumor cell proliferation and invasion are extremely strong, the recurrence rate and metastasis rate are high after surgery combined with radiotherapy and chemotherapy, and patients are with poor prognosis and high mortality[1,2]. At present, there are still no targeted drugs for clinical treatment of osteosarcoma, and the molecular mechanism of osteosarcoma cell proliferation and invasion has not been elucidated. Tumor suppressor gene TAp73 is a member of the p53 tumor suppressor gene family, and the protein encoded by it has negative regulating effect on cell cycle, cell proliferation and cell invasion[3]. The protein encoded by autophagy gene Beclin1 is an important protein that regulates the initial part of autophagosome formation, and it can induce autophagy to regulate cell proliferation, apoptosis and invasion[4]. In order to define the relationship of TAp73 and Beclin1 gene expression with the progression of osteosarcoma, the TAp73 and Beclin1 expression in osteosarcoma tissue and their correlation with tumor malignancy were analyzed in the following study.

2. Materials and methods

2.1. Clinical samples

56 cases of osteosarcoma tissues and peri-osteosarcoma tissues surgically removed in our hospital between June 2013 and August 2016 were collected, all patients with osteosarcoma didn’t receive radiotherapy and chemotherapy before surgery, and the
osteosarcoma tissue and peri-osteosarcoma tissue properties were confirmed by postoperative pathological examination; 37 cases of giant cell tumor tissues surgically removed in our hospital during the same period were collected, and the giant cell tumor tissue properties were confirmed by postoperative pathological examination. Patients with osteosarcoma included 36 male cases and 20 female cases that were 12–58 years old; patients with giant cell tumor included 22 male cases and 15 female cases that were 17–52 years old. Patients with osteosarcoma and patients with giant cell tumor were not significantly different in general data (P>0.05).

2.2. Experimental materials

SP immunohistochemical staining kits and 10% paraformaldehyde fixation were purchased from Fujian Maxin Biotechnology Company, polyclonal antibodies of TAp73 and Beclin1 were purchased from Abcam Company, protein lysis buffer and BCA total protein quantification were purchased from Shanghai Beyotime Company, and the TAp73 and Beclin1 enzyme-linked immunosorbent assay kits were purchased from Guangzhou Runkwon Biotech Co., LTD.

2.3. Experimental methods

2.3.1. Experimental methods of protein expression detection

Osteosarcoma tissues, peri-osteosarcoma tissues and giant cell tumor tissues were collected, shortly frozen in liquid nitrogen, then added in protein lysis buffer and split to extract protein, the extracted protein suspension was centrifuged in 4 ℃ centrifuge for 20 min at a speed of 12 000 r/min to separate the upper clear protein liquid, enzyme-linked immunosorbent assay kits were used to determine TAp73, Beclin1, IRX2, JAK3 and STAT5 levels, BCA kits were used to determine total protein content, and the TAp73, Beclin1, IRX2, JAK3 and STAT5 protein expression per unit mass total protein were calculated.

2.3.2. Experimental methods of positive protein expression rate detection

Osteosarcoma tissues, peri-osteosarcoma tissues and giant cell tumor tissues were collected, fixed with 10% paraformaldehyde, then embedded in paraffin and made into serial sections of 4 μm, then immunohistochemical kits were used to stain TAp73 and Beclin1, and the concentration of antibody incubation was 1:150; 10 high power fields in each section were randomly selected and observed, and the cells were scored according to the positive staining rate: 0 point for cells without positive staining, 1 point for cells with 1%–10% positive staining, 2 points for cells with 11%–50% positive staining, 3 points for cells with 51%–75% positive staining and 4 points for cells with >75% positive staining; cells were scored according to the staining intensity: 0 point for cells without staining, 1 point for cells with light yellow staining, 2 points for cells with brownish yellow staining and 3 points for cells with reddish brown staining. The protein staining extent was judged according to the product of positive staining rate score and staining intensity score: 0 point for negative (-), 1 point for weakly positive (+), 2 points for positive (++) and 3 points for strongly positive (+++).

2.4. Statistical analysis

SPSS17.0 software was used to input and process data, measurement data analysis among groups was by variance analysis, count data analysis was by chi-square test and P<0.05 indicated statistical significance in differences.

3. Results

3.1. TAp73 and Beclin1 expression in osteosarcoma tissues, peri-osteosarcoma tissues and giant cell tumor tissues

Analysis of the protein expression and positive expression rate of TAp73 and Beclin1 in osteosarcoma tissues, peri-osteosarcoma tissues and giant cell tumor tissues was as follows: protein expression and positive expression rate of TAp73 and Beclin1 in osteosarcoma tissues were significantly lower than those in peri-osteosarcoma tissues and giant cell tumor tissues, and protein expression and positive expression rate of TAp73 and Beclin1 in peri-osteosarcoma tissues were not significantly different from those in giant cell tumor tissues. Differences in protein expression and positive expression rate of TAp73 and Beclin1 were statistically significant in osteosarcoma tissues and peri-osteosarcoma tissues as well as in osteosarcoma tissues and giant cell tumor tissues (P<0.05) (Table 1).

3.2. Proliferation and invasion gene expression in osteosarcoma tissues, peri-osteosarcoma tissues and giant cell tumor tissues

Analysis of the proliferation genes IRX2, JAK3 and STAT5 as well as the invasion genes CTGF, Wnt and β-catenin expression in osteosarcoma tissues, peri-osteosarcoma tissues and giant cell tumor tissues was as follows: IRX2, JAK3, STAT5, CTGF, Wnt and β-catenin protein expression in osteosarcoma tissues were significantly higher than those in peri-osteosarcoma tissues and giant cell tumor tissues, and IRX2, JAK3, STAT5, CTGF, Wnt and β-catenin protein expression in peri-osteosarcoma tissues were not significantly different from those in giant cell tumor tissues. Differences in the IRX2, JAK3, STAT5, CTGF, Wnt and β-catenin protein expression were statistically significant in osteosarcoma tissues and peri-osteosarcoma tissues as well as in osteosarcoma tissues and giant cell tumor tissues (P<0.05) (Table 2).

3.3. Proliferation and invasion gene expression in osteosarcoma tissues with different TAp73 expression

Analysis of the proliferation genes IRX2, JAK3 and STAT5 as well as the invasion genes CTGF, Wnt and β-catenin expression in osteosarcoma tissues with different TAp73 expression was as

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>n</th>
<th>Protein expression</th>
<th>Positive protein expression rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TAp73</td>
<td>Beclin1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteosarcoma tissue</td>
<td>56</td>
<td>3.47±0.51*</td>
<td>1.89±0.22*</td>
</tr>
<tr>
<td>Peri-osteosarcoma tissue</td>
<td>56</td>
<td>8.51±0.93</td>
<td>5.24±0.67</td>
</tr>
<tr>
<td>Giant cell tumor tissue</td>
<td>37</td>
<td>8.84±1.02</td>
<td>5.41±0.69</td>
</tr>
</tbody>
</table>

*: comparison between osteosarcoma tissue and peri-osteosarcoma tissue, P<0.05; *: comparison between osteosarcoma tissue and giant cell tumor tissue, P<0.05.
follows: IRX2, JAK3, STAT5, CTGF, Wnt and β-catenin protein expression in osteosarcoma tissues with TAp73 (+), (+++) and (++++) were significantly lower than those in osteosarcoma tissues with TAp73 (-), IRX2, JAK3, STAT5, CTGF, Wnt and β-catenin protein expression in osteosarcoma tissues with TAp73 (+++) were significantly lower than those in osteosarcoma tissues with TAp73 (+), and IRX2, JAK3, STAT5, CTGF, Wnt and β-catenin protein expression in osteosarcoma tissues with TAp73 (+++) were significantly lower than those in osteosarcoma tissues with TAp73 (++). Differences in pair-wise comparison of IRX2, JAK3, STAT5, CTGF, Wnt and β-catenin protein expression were statistically significant in osteosarcoma tissues with different TAp73 expression (P<0.05) (Table 3).

3.4. Proliferation and invasion gene expression in osteosarcoma tissues with different Beclin1 expression

Analysis of the proliferation genes IRX2, JAK3 and STAT5 as well as the invasion genes CTGF, Wnt and β-catenin expression in osteosarcoma tissues with different Beclin1 expression was as follows: IRX2, JAK3, STAT5, CTGF, Wnt and β-catenin protein expression in osteosarcoma tissues with Beclin1 (+), (+++) and (++++) were significantly lower than those in osteosarcoma tissues with Beclin1 (+), IRX2, JAK3, STAT5, CTGF, Wnt and β-catenin protein expression in osteosarcoma tissues with Beclin1 (+) and (+++) were significantly lower than those in osteosarcoma tissues with Beclin1 (+), and IRX2, JAK3, STAT5, CTGF, Wnt and β-catenin protein expression in osteosarcoma tissues with Beclin1 (+++) were significantly lower than those in osteosarcoma tissues with Beclin1 (+++). Differences in pair-wise comparison of IRX2, JAK3, STAT5, CTGF, Wnt and β-catenin protein expression were statistically significant in osteosarcoma tissues with different Beclin1 expression (P<0.05) (Table 4).

4. Discussion

Osteosarcoma has the characteristics of high malignant degree as well as easy recurrence and metastasis after treatment, and neither overall prognosis nor survival is ideal[5]. Osteosarcoma cells proliferation and invasion is the biological behavior closely related to tumor recurrence and metastasis[6,7], but the key molecules that regulate cell proliferation and invasion in osteosarcoma tissue remain uncertain, and there are no targeted drugs for the clinical treatment of disease. TAp73 and Beclin1 are the new genes discovered in recent years that regulate cellular biological behaviors, and the proteins encoded by them can affect cell proliferation, apoptosis, invasion, migration and many other biological processes. TAp73 gene is a new member of p53 tumor suppressor gene family, and the protein encoded by it can antagonize the cyclin function and inhibit cell proliferation and invasion process[8-10]; the protein ended by Beclin1 gene encoding is mainly involved in regulating autophagy process, and can promote the autophagosome formation and induce the autophagy apoptosis of cells, thus inhibiting cell proliferation and invasion process[11-13]. In order to determine whether TAp73 and Beclin1 participated in the occurrence and development of osteosarcoma, TAp73 and Beclin1 expression levels in osteosarcoma tissue were analyzed in the study, and the results showed that the protein expression and positive expression rate of TAp73 and Beclin1 in osteosarcoma tissues were significantly lower than those in peri-osteosarcoma tissues and giant cell tumor tissues (P<0.05). This means that the TAp73 and Beclin1 expression decrease significantly in osteosarcoma tissues with different Beclin1 expression (P<0.05) (Table 4).
in osteosarcoma tissue, and lowly expressed TAp73 and Beclin1 may be associated with the abnormal cell proliferation and invasion in osteosarcoma tissue.

JAK3/STAT5 is an important signaling pathway that regulates cell proliferation and apoptosis in osteosarcoma tissues. IRX2 is the regulating and activating factor of JAK3/STAT5 signal pathway upstream[14]. IRX2 belongs to the IRX family, it can induce the phosphorylation of JAK3 in osteosarcoma cells and then promote STAT5 phosphorylation through JAK3, and the phosphorylated STAT5 can start the cyclin CyclinD1 expression, accelerate the cell cycle process and promote cell proliferation after transferring into the nucleus[15]. Existing cell experiment has proved that targeted knockdown of IRX2 expression can inhibit osteosarcoma cell proliferation process in vitro[16]. In the study, analysis of IRX2/JAK3/STAT5 signaling pathway expression in osteosarcoma tissues showed that IRX2, JAK3 and STAT5 protein expression in osteosarcoma tissues were significantly higher than those in peri-osteosarcoma tissues and giant cell tumor tissues (P<0.05). It means that the abnormal activation of IRX2/JAK3/STAT5 signaling pathway is associated with the pathogenesis of osteosarcoma. Further analysis of the relationship of TAp73 and Beclin1 expression in osteosarcoma tissues with IRX2/JAK3/STAT5 signaling pathway showed that IRX2, JAK3 and STAT5 protein expression in osteosarcoma tissues with positive TAp73 and Beclin1 expression were significantly lower than those in osteosarcoma tissues with negative TAp73 and Beclin1 expression (P<0.05); the lower the TAp73 and Beclin1 expression, the higher the IRX2, JAK3 and STAT5 protein expression in osteosarcoma tissues. This means that lowly expressed TAp73 and Beclin1 in osteosarcoma tissue are associated with the excessive activation of IRX2/JAK3/STAT5 signaling pathway, and can activate IRX2/JAK3/STAT5 signaling pathway to promote osteosarcoma cell proliferation.

Wnt/β-catenin is an important signaling pathway that adjusts the cell migration, invasion and movement in osteosarcoma tissue. CTGF is the cytokine of Wnt/β-catenin signaling pathway upstream that can activate the Wnt molecules to inhibit the β-catenin degradation in cells, and the β-catenin constantly accumulated in cytoplasm will transfer into the nucleus and then start the downstream MMPs gene expression, promote the degradation of a variety of protein components in extracellular matrix and then promote cell invasion and migration[17,18]. Existing cell experiment has confirmed that CTGF can promote the migration and invasion of osteosarcoma cells cultured in vitro[9]. In the study, analysis of CTGF/Wnt/β-catenin signaling pathway expression in osteosarcoma tissue showed that CTGF, Wnt and β-catenin protein expression in osteosarcoma tissues were significantly higher than those in peri-osteosarcoma tissues and giant cell tumor tissues (P<0.05). This means that the abnormal activation of CTGF/Wnt/β-catenin signaling pathway is associated with the pathogenesis of osteosarcoma. Further analysis of the relationship of TAp73 and Beclin1 expression in osteosarcoma tissues with CTGF/Wnt/β-catenin signaling pathway showed that CTGF, Wnt and β-catenin protein expression in osteosarcoma tissues with positive TAp73 and Beclin1 expression were significantly lower than those in osteosarcoma tissues with negative TAp73 and Beclin1 expression (P<0.05); the lower the TAp73 and Beclin1 expression, the higher the CTGF, Wnt and β-catenin protein expression in osteosarcoma tissue. This means that the lowly expressed TAp73 and Beclin1 in osteosarcoma tissue are associated with the excessive activation of CTGF/Wnt/β-catenin signaling pathway, and can activate the CTGF/Wnt/β-catenin signaling pathway to promote osteosarcoma cell invasion.

To sum up, it is believed that tumor suppressor gene TAp73 and autophagy gene Beclin1 expression significantly decrease in osteosarcoma tissue; lowly expressed TAp73 and Beclin1 are related to the excessive activation of IRX2/JAK3/STAT5 and CTGF/Wnt/β-catenin signaling pathways, and the activation of above signaling pathways can promote the osteosarcoma cell proliferation and invasion.

References