Relationship of PDCD5 expression with apoptosis, inflammatory factors and MMPs/TIMPs expression in degenerated intervertebral disc tissue

Xiao-Hua Gu, Di-Ping Cao, Xiao-Chun Chen, Jian-Hua Xu, Hai-Tao Jiang, Wei-Wei Ma, Hai-Jian Lu, Chao Hong, Si-bo Li

Department of Orthopedics and Rehabilitation, the Seventh People’s Hospital of Shanghai University of Traditional Chinese Medicine, Shanghai, 200137

ARTICLE INFO

Article history:
Received 2 Nov 2017
Received in revised form 9 Nov 2017
Accepted 12 Nov 2017
Available online 28 Nov 2017

Keywords:
Lumbar disc herniation
Programmed cell death 5
Apoptosis
Inflammatory response
Matrix metalloproteinase

ABSTRACT

Objective: To study the relationship of PDCD5 expression with apoptosis, inflammatory factors and MMPs/TIMPs expression in degenerated intervertebral disc tissue. Methods: Patients with lumbar disc herniation who were treated in the Seventh People’s Hospital of Shanghai between March 2015 and February 2017 were selected as the LDH group and patients with violent thoracolumbar vertebral fracture were selected as the control group. The intervertebral disc tissue was collected to determine the mRNA expression of PDCD5 as well as the protein levels of apoptosis molecules, inflammatory factors and MMPs/TIMPs molecules. Results: PDCD5 mRNA expression in intervertebral disc tissue of LDH group was significantly higher than that of control group; Caspase-3, Caspase-8, Fas, Caspase-9, Bax, SDF-1, CXCR-4, TNF-α, PGE2, MMP1, MMP2, MMP8 and MMP9 protein levels in intervertebral disc tissue of LDH group were significantly higher than those of control group and positively correlated with PDCD5 mRNA expression while TIMP1 and TIMP2 protein levels were significantly lower than those of control group and negatively correlated with PDCD5 mRNA expression. Conclusion: The high expression of PDCD5 in degenerated intervertebral disc tissue can activate apoptosis and inflammatory response and cause MMPs/TIMPs imbalance.

1. Introduction

Lumbar disc herniation (LDH) is the most common clinical degenerative lumbar disease that can cause obvious back and leg pain and affect daily life, and limb dysfunction can appear in severe cases and will cause deformity and greatly increase the burden on the family and society. The aging of cells and the loss of collagen in the nucleus pulposus are the important pathologic features of lumbar degenerative disease, the excessive apoptosis, excessive activation of inflammatory response and excessive degradation of extracellular matrix are believed to be closely related to the aging of the nucleus pulposus cells and the loss of collagen components[1,2], but the specific regulatory mechanism remains unclear. Programmed cell death 5 (PDCD5) is an important regulatory molecule in the process of apoptosis, existing clinical studies have confirmed that PDCD5 is highly expressed in intervertebral disc tissue of patients with lumbar disc herniation[3], but there is no report about the biological effects of PDCD5 expression change. In the following studies, we specifically analyzed the relationship of PDCD5 expression with apoptosis, inflammatory factors and MMPs/TIMPs expression in degenerated intervertebral disc tissue.

2. Case information and research methods

2.1 Clinical case information

Patients with lumbar disc herniation who were treated in the Seventh People’s Hospital of Shanghai between March 2015 and
February 2017 were selected as the LDH group, and all patients were in accordance with the diagnosis for lumbar disc herniation. Patients with violent thoracolumbar vertebral fracture who received surgical treatment in the Seventh People's Hospital of Shanghai during the same period were selected as the control group, all patients had clear history of trauma proven to be thoracolumbar vertebral fracture by imaging examination, and those who were with the history of lumbar disc herniation were excluded. There were a total of 58 cases in LDH group, including 38 male cases and 20 female cases that were 42-65 years old; there were 36 cases in control group, including 24 male cases and 12 female cases that were 39-61 years old. There was no statistically significant difference in general information between the two groups ( \( P > 0.05 \)).

2.2 Research methods

2.2.1 Intervertebral disc tissue collection

After the completion of operation, intervertebral disc tissue was collected from the diseased segment of LDH group, intervertebral disc tissue was collected from the fracture segment of control group, and the tissue was washed with saline to remove the residual blood, quickly frozen in liquid nitrogen for 10 min, and then stored in a -70°C cryogenic refrigerator.

2.2.2 mRNA expression detection

Appropriate amount of intervertebral disc tissue was collected from LDH group and control group, RNA extraction kit and cDNA synthesis kit were used to separate RNA and synthesize it into cDNA; the PDCD5 primers were designed, cDNA samples were given fluorescence quantitative PCR amplification, and the mRNA expression of PDCD5 was calculated according to the amplification curve.

2.2.3 Protein level detection

Appropriate amount of intervertebral disc tissue was collected from LDH group and control group, RIPA lysate was used to extract protein, and then enzyme-linked immunosorbent assay kit was used to detect Caspase-3, Caspase-8, Fas, Caspase-9, Bax, SDF-1, CXCR-4, TNF-α, PGE2, MMP1, MMP2, MMP8, MMP9, TIMP1 and TIMP2 protein levels.

2.3 Statistical methods

SPSS 20.0 software was used to input and analyze data, measurement data analysis between two groups was by \( t \) test, correlation analysis was by Pearson test and \( P < 0.05 \) meant statistical significance in differences.

3. Results

3.1 PDCD5 mRNA expression in intervertebral disc

PDCD5 mRNA expression in intervertebral disc tissue of LDH group was (3.58±0.58); PDCD5 mRNA expression in intervertebral disc tissue of control group was (1.04±0.18). \( t \) test analysis of the differences in PDCD5 mRNA expression in intervertebral disc tissue between LDH group and control group showed that PDCD5 mRNA expression in intervertebral disc tissue of LDH group was significantly higher than that of control group.

3.2 Apoptosis molecule protein levels in intervertebral disc

Analysis of apoptosis molecules Caspase-3 (ng/mL), Caspase-8 (ng/mL), Fas (pg/mL), Caspase-9 (ng/mL) and Bax (pg/mL) protein levels in intervertebral disc tissue between LDH group and control group was as follows: Caspase-3, Caspase-8, Fas, Caspase-9 and Bax protein levels in intervertebral disc tissue of LDH group were significantly higher than those of control group. Pearson test showed that Caspase-3, Caspase-8, Fas, Caspase-9 and Bax protein levels in intervertebral disc tissue of LDH group were positively correlated with PDCD5 mRNA expression.

3.3 Inflammatory factor protein levels in intervertebral disc

Analysis of inflammatory factors SDF-1 (ng/mL), CXCR-4 (ng/mL), TNF-α (pg/mL) and PGE2 (pg/mL) protein levels in intervertebral disc tissue between LDH group and control group was as follows: SDF-1, CXCR-4, TNF-α and PGE2 protein levels in intervertebral disc tissue of LDH group were significantly higher than those of control group. Pearson test showed that SDF-1, CXCR-4, TNF-α and PGE2 protein levels in intervertebral disc tissue of LDH group were positively correlated with PDCD5 mRNA expression.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Caspase-3 (ng/mL)</th>
<th>Caspase-8 (ng/mL)</th>
<th>Fas (pg/mL)</th>
<th>Caspase-9 (ng/mL)</th>
<th>Bax (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH group</td>
<td>58</td>
<td>2.02±0.35</td>
<td>1.74±0.25</td>
<td>95.58±11.27</td>
<td>0.93±0.15</td>
<td>164.48±20.82</td>
</tr>
<tr>
<td>Control group</td>
<td>36</td>
<td>0.92±0.14</td>
<td>0.83±0.10</td>
<td>39.52±5.59</td>
<td>0.39±0.07</td>
<td>80.30±9.39</td>
</tr>
<tr>
<td>( t )</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>
<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>

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>SDF-1 (ng/mL)</th>
<th>CXCR-4 (ng/mL)</th>
<th>TNF-α (pg/mL)</th>
<th>PGE2 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH group</td>
<td>58</td>
<td>4.85±0.76</td>
<td>3.74±0.59</td>
<td>265.49±30.39</td>
<td>213.49±29.58</td>
</tr>
<tr>
<td>Control group</td>
<td>36</td>
<td>2.25±0.35</td>
<td>1.57±0.20</td>
<td>147.61±19.39</td>
<td>86.95±9.95</td>
</tr>
<tr>
<td>( t )</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</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>
</tr>
</tbody>
</table>
The apoptosis process in nucleus pulposus involves both membrane receptor apoptosis pathway and mitochondrial apoptosis pathway, and the excessively high expression of PDCD5 in intervertebral disc tissue was significantly higher than that of control group. Pearson test showed that MMP1, MMP2, MMP8 and MMP9 protein levels in intervertebral disc tissue of LDH group were positively correlated with PDCD5 expression whereas TIMP1 and TIMP2 protein levels were negatively correlated with PDCD5 expression.

3.4 MMPs/TIMPs molecule protein levels in intervertebral disc

Analysis of MMPs/TIMPs molecules MMP1 (ng/mL), MMP2 (ng/mL), MMP8 (pg/mL), MMP9 (ng/mL), TIMP1 (pg/mL) and TIMP2 (pg/mL) protein levels in intervertebral disc tissue between LDH group and control group was as follows: MMP1, MMP2, MMP8 and MMP9 protein levels in intervertebral disc tissue of LDH group were significantly higher than those of control group whereas TIMP1 and TIMP2 protein levels were significantly lower than those of control group. Pearson test showed that MMP1, MMP2, MMP8 and MMP9 protein levels in intervertebral disc tissue of LDH group were positively correlated with PDCD5 expression whereas TIMP1 and TIMP2 protein levels were negatively correlated with PDCD5 expression.

4. Discussion

Lumbar disc herniation lesions mainly involve the central nucleus pulposus and surrounding fiber ring, and the degenerated nucleus pulposus and fiber ring cannot take buffer spinal normal physiological strain, which will result in nucleus pulposus herniation and nerve root compression. During the degeneration of nucleus pulposus and fibrous ring, the cells in the nucleus pulposus are aging, which is accompanied by a significant loss of collagens in the nucleus pulposus and fiber ring[4,5]. The aging of cells and the loss of collagen are related to the pathophysiological links of apoptosis, inflammatory response and intercellular matrix hydrolysis, but the specific regulation mechanism is not clear. PDCD5 is an important regulatory element in cell apoptosis. During the activation of apoptosis, it can be transferred from the plasma to the nucleus and affect the function of multiple apoptosis pathways. It has been reported that PDCD5 is highly expressed in degenerated intervertebral disc tissue. In the study, analysis of PDCD5 expression in intervertebral disc tissue of patients with lumbar disc herniation also confirmed that PDCD5 mRNA expression in intervertebral disc tissue of LDH group was significantly higher than that of control group. It means that the high expression of PDCD5 in intervertebral disc is associated with the occurrence of lumbar disc herniation, and the downstream biological links influenced by PDCD5 may include the apoptosis, inflammatory reaction activation and extracellular matrix degradation.

The apoptosis process in nucleus pulposus involves both membrane receptor pathway and mitochondrial pathway, and the excessively apoptotic nucleus pulposus cells will directly affect the stability of intervertebral disc and cause the occurrence of intervertebral disc herniation. Caspase-3 is a common downstream molecule of cell membrane receptor apoptosis pathway and mitochondrial apoptosis pathway, and activated Caspase-3 can induce apoptotic body formation and induce apoptosis[6]. Caspase-3 activation in the cells depends on the cascade amplification activation reaction of various upstream Caspase molecules, Caspase-9 cascade amplification reactions are caused by the mitochondrial pathway of apoptosis mediated by the mitochondrial membrane proteins Bax, and Caspase-8 cascade amplification reactions are caused by the membrane receptor apoptosis pathway mediated by Fas the membrane receptor[7–9]. In the study, analysis of apoptosis pathway molecule levels in intervertebral disc tissue of patients with lumbar disc herniation showed that Caspase-3, Caspase-8, Caspase-9 and Bax protein levels in intervertebral disc tissue of LDH group were significantly higher than those of control group. This shows that the activation of membrane receptor apoptosis pathway and mitochondrial apoptosis pathway are both related to the occurrence of lumbar disc herniation. Further analysis of the correlation between PDCD5 and above apoptosis molecules showed that Caspase-3, Caspase-8, Caspase-9 and Bax protein levels in intervertebral disc tissue of LDH group were positively correlated with PDCD5 expression. This indicates that PDCD5 can promote the membrane receptor apoptosis pathway and mitochondrial apoptosis pathway in the degenerated intervertebral disc.

The nucleus pulposus cells only account for 1% of the volume of intervertebral disc nucleus pulposus, and the remaining components are extracellular matrix. During the degeneration of the intervertebral disc, the extracellular matrix of the nucleus pulposus cells is obviously lost, and the activation of inflammatory response is closely related to the loss of extracellular matrix. Activation of inflammatory response can on the one hand, lead to the nucleus pulposus cell damage and apoptosis and influence the secretion of extracellular matrix, and on the other hand, can promote the hydrolysis of extracellular matrix. SDF-1/CXCR-4 is the signal axis regulating the inflammation in intervertebral disc tissue, and the combination between SDF-1 and CXCR-4 can not only induce inflammatory cell accumulation in local area[10], but can also promote the NF-κB activation and enter the nucleus, which further increases the secretion of TNF-α, PGE2 and other inflammatory mediators[11,12]. In the research, analysis of inflammatory factor levels in intervertebral disc tissue of patients with lumbar disc herniation showed that SDF-1, CXCR-4, TNF-α and PGE2 protein levels in intervertebral disc tissue of LDH group were significantly higher than those of control group. This indicates that the inflammatory response activation
mediated by SDF-1/CXCR-4 in the intervertebral disc is associated with the occurrence of lumbar disk herniation. Further analysis of the correlation between PDCD5 and the above inflammatory molecules showed that SDF-1, CXCR-4, TNF-α and PGE2 protein levels in intervertebral disc tissue of LDH group were positively correlated with PDCD5 expression. This indicates that PDCD5 has a promoting effect on the inflammatory response mediated by SDF-1/CXCR-4 in degenerated intervertebral disc.

The important pathway for inflammatory response to cause extracellular matrix is to activate protease and induce the hydrolysis of protein components in extracellular matrix. The main component of extracellular matrix in nucleus pulposus tissue are collagen, including type I, II, III, IV, V and others[13]; MMP1 and MMP8 in MMPs family can hydrolyze type I-III collagen, and the MMP2, MMP9 can hydrolyze type IV and V collagen. The activation of inflammatory response and the infiltration of inflammatory cells in the nucleus pulposus will secrete a large amount of MMPs and cause the hydrolysis of collagen in the extracellular matrix, resulting in degeneration of the nucleus pulposus[14,15]. TIMP1 and TIMP2 are MMPs family-specific suppressors that can form covalent binding with multiple MMP molecules and inhibit their hydrolytic activity[16]. In the study, analysis of MMPs/TIMPs molecule levels in intervertebral disc tissue of patients with lumbar disk herniation showed that MMP1, MMP2, MMP8 and MMP9 protein levels in intervertebral disc tissue of LDH group were significantly higher than those of control group whereas TIMP1 and TIMP2 protein levels were significantly lower than those of control group. This indicates that the MMPs/TIMPs imbalance in the intervertebral disc is associated with the occurrence of lumbar disk herniation. Further analysis of the correlation between PDCD5 and above MMPs/TIMPs molecules showed that MMP1, MMP2, MMP8 and MMP9 protein levels in intervertebral disc tissue of LDH group were positively correlated with PDCD5 expression whereas TIMP1 and TIMP2 protein levels were negatively correlated with PDCD5 expression. This indicates that PDCD5 has promoting effect on the imbalance of MMPs/TIMPs and the hydrolysis of extracellular matrix in degenerated intervertebral disc.

To sum up, it can be concluded that the PDCD5 is highly expressed in degenerated disc tissue; highly expressed PDCD5 can activate the apoptosis and inflammatory response in intervertebral disc, and can also cause MMPs/TIMPs imbalance and lead to excessive loss of extracellular matrix.

References


