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## Role of IL-17 in nucleus pulposus cell proliferation and metabolism cultured *in vitro*

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## ABSTRACT

**Objective:** To explore the role of cytokine, interleukin-17 (IL-17) in human degenerative disc disease. **Methods:** Through magnetic resonance imaging, human degenerative disc tissues were confirmed from the isolated nucleus pulposus cells, which were then cultured *in vitro*. The cells were cultured with and without different concentrations of IL-17. 2 ng/mL, 5 ng/mL, 10 ng/mL, 15 ng/mL and 20 ng/mL IL-17 concentrations were used for stimulation. After 72 hours, the inhibition rate of proliferation was measured by MTS method. For 48 and 96 hours, the nucleus pulposus cells were cultured with and without the appropriate IL-17 concentrations. The mRNA and protein expression levels of the matrix macromolecules and degrading tissue genes were measured by Real-time PCR and Western blot analysis. **Results:** It was noted that nucleus pulposus cell proliferation was inhibited after culturing *in vitro* with IL-17 stimulation, and it was further observed that the inhibition effect was significantly stronger with 15 ng/mL IL-17 concentration. With the dosage of 15 ng/mL, IL-17 stimulation induced multiple cellular responses, such as the significant increase in mRNA expression for both aggrecan (*ACAN*) and type I collagen (*COL1A1*) genes ( $P < 0.05$ ), and the significant decrease in mRNA expression of both degrading tissue genes, MMP3 and TIMP3 ( $P < 0.05$ ). Western blot results also showed that the protein level of COL1A1 was significantly decreased ( $t = 3.199$ ,  $P = 0.006$ ), while the protein level of one peptidases (ADAMTS5) significantly increased ( $t = 2.667$ ,  $P = 0.021$ ). **Conclusions:** These findings suggest that IL-17 can inhibit proliferation and affect the metabolism of the cultured nucleus pulposus cells *in vitro*, and these findings could possibly contribute to the degenerative changes that occur in DDD through extracellular matrix synthesis inhibition, promoting nucleus pulposus extracellular matrix degradation and disrupting the metabolic balance.

### 1. Introduction

Degenerative Disc Disease (DDD) is a common health problem in invertebrates, including humans[1]. It is a chronic disease that involves multiple-factors that progressively destroys the composition, structure and function of the disc[2,3]. The main pathological features that characterize DDD include collagen type conversion, progressive loss

of extracellular matrix proteoglycans, disc dehydration, increased expression and activity of matrix-degrading enzymes and inflammatory factors accumulation[1,4,5]. Immune cell accumulation and increased inflammatory cytokine expressions have been identified in degenerative disc tissues[6–10], indicating that inflammatory cytokines may play an important role in the disc degeneration process[11,12].

Interleukin-17 (IL-17), a powerful inflammatory cytokine, plays critical roles in the pathogenesis of various inflammatory disorders[13]. Studies on arthritis found that IL-17 can, by itself or combined with other inflammatory cytokines, induce inflammatory cytokine

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aggregation, inhibit aggrecan synthesis, and up-regulate the aggrecan enzyme and degrading collagen molecules; thus, contributing to joint degeneration<sup>[14,15]</sup>. Chondrocyte-like cells, similar to articular chondrocytes, is the main cell type found in the nucleus pulposus (NP) of the human disc, suggesting that IL-17 may be involved in disc degeneration similar to joint degeneration. Scuderi *et al* revealed that the epidural space lavage in patients with lumbar intervertebral disk herniation and radiculopathy contained an increased expression of IL-17<sup>[16]</sup>, suggesting that IL-17 may be involved in intervertebral disc tissue degeneration which may also be associated with the development of pain. With further analysis, Shamji *et al*<sup>[17]</sup> identified that nucleus pulposus cells cultured *in vitro* not only responded with a catabolic phenotype to IL-17 stimulation, but also expressed surface ligands with a consequential potential to recruit additional lymphocytes and immune cells into the DDD microenvironment. Perhaps IL-17 may be an important inflammation regulator in DDD pathologies. So far, studies have shown that IL-17 may be involved in the process of disc degeneration; however, the contribution of inflammatory IL-17 cytokines in disc degeneration through inhibiting cell proliferation, the extracellular matrix synthesis of intervertebral disc cells, as well as promoting the degradation of aggrecan enzyme expressions and collagen molecules, still needs to be investigated.

Based on this background, we found the importance of examining the response of isolated nucleus pulposus cells from IL-17 stimulated degenerative disc tissues; and hypothesized that IL-17 can inhibit the proliferation and destruction of extracellular matrix tissue homeostasis in nucleus pulposus cells cultured *in vitro*.

## 2. Materials and methods

### 2.1. Clinical cases

Out of the 36 patients that visited our hospital for lumbar disc herniation surgery, between December 2013 and March 2014, 20 patients (12 males, 8 females) were enrolled as subjects for this study, after obtaining informed consents. The patients had average age of (68.4±7.7) years old. The extent of disc degeneration of each subject was from levels III to IV, which was graded using Thompson's classification by magnetic resonance imaging<sup>[18]</sup>. The subjects had no prior history of surgery, serious infection, cancer, hypotension, and diabetes, which was confirmed by questionnaire and pathological examination after operation.

### 2.2. Cell separation and culturing

During surgery, NP tissues was dissected, stripped from the annulus and cartilage tissues, and immersed in saline for subsequent analysis, within 30 minutes. Using scissors, the collected human NP tissues were cut into small pieces. The NP cells were enzymatically released from the NP tissues by digestion, using a combined solution of 0.25% trypsin and 0.025% collagenase for half an hour at 37 °C. After 10 minutes of centrifugation at 1 000 rpm, the supernatant was removed. Subsequently, cell clumps were resuspended with Ham's F12 media (Gibco), combined with 10% fetal bovine serum, 100 μg/mL of penicillin and 100 μg/mL of streptomycin (culture medium), for cell counting; and it was inoculated on a gelatin-coated tissue culture plastic with 5% CO<sub>2</sub> at 37 °C. All cells were seeded at an approximate density of 25 000 cells per cm<sup>2</sup>, the medium was changed every other day. Cell morphology and density were observed under an inverted microscope DIAVERT. Cell passage was established when approximately 80% cell fusion was achieved<sup>[19]</sup>. Cells after three passages were used in all experiments.

### 2.3. IL-17 stimulation

For cell proliferation analysis: NP cells were plated at a density of 50 000 cells per well in 48-well plates (*n*=6 replicates) with an overlaid culture medium of 150 μL. In order to determine the optimal IL-17 dosage for all experiments, the inhibition effects of IL-17 on NP cells were first evaluated over a broad range of doses (0–20 ng/mL). After 72 hours, the cultured cells were collected and the proliferation rate were measured by MTS analysis.

For cell metabolism analysis: NP cells were plated at a density of 50 000 cells per well in 48-well plates (*n*=6 replicates), overlaid with 300 μL of the following culture mediums: fresh culture medium without IL-17 (unstimulated group), fresh culture medium with concentrations identified during the IL-17 cell proliferation analysis (stimulated group). After culturing for 48 and 96 hours, the mRNA expression and protein levels were measured by real-time PCR and Western blot analysis.

### 2.4. Cell proliferation and metabolism measurement

#### 2.4.1. Cell proliferation measurement

Cell proliferation was determined by MTS assay; the CellTiter 96® Aqueous One Solution Cell Proliferation Assay kit was used, based on manufacturer's instructions. Briefly, 3 hours before each of the desired time points, 20 μL of

MTS reagent was added into each well, and the cells were incubated at 37 °C for 3 hours. Then, a 550 BioRad plate–reader (Bio–Rad, Hertfordshire, UK) was used, and an optical density (OD) value of OD<sub>490</sub> was detected. All experiments were repeated three times<sup>[20]</sup>. Cell suppression ratio was calculated, according to the following formula: (stimulated group OD – unstimulated group OD)/(unstimulated group OD – background OD).

#### 2.4.2. Reagents and instruments

DMEM/F12 Medium (Gibco, USA); fetal bovine serum (Gibco, USA); 0.25% trypsin (Invitrogen, USA); Collagenase II (Invitrogen, USA); DMSO (Invitrogen, USA); Penicillin and Streptomycin (Invitrogen, USA); SuperScript® III First Strand kits (Takara, China); CellTiter 96® AQueous One Solution Cell Proliferation Assay kit (Promega, USA).

Cell incubator (Heraeus, Germany); Inverted microscope (Olympus, Japan); Clean bench (Shengggong Tecnology Company, Shanghai); Digital thermostat water bath (Shengggong Tecnology Company, Shanghai); Spectrophotometer.

#### 2.4.3. Total RNA extraction and RT–PCR analysis

Total RNA was isolated from the two groups of cells using TRIzol Reagent (Gibco) and the cells were immediately reverse–transcribed using oligo (dT); then, cDNA was generated from 5 μg of total RNA in 20 μL reaction mixes using SuperScript® III First Strand kits (Takara), and stored at –20 °C for later use. All procedures were conducted

according to manufacturer’s instructions. Primers for the following genes were designed by using Oligo 6.0 (National Biosciences Inc., Plymouth, MN) and synthesized by Sangon Biotech (Shanghai, China): glyceraldehyde–3–phosphate dehydrogenase (*GAPDH*), aggrecan (*ACAN*), collagen, type I, alpha 1 (*COL1A1*), collagen, type II, alpha 1 (*COL2A1*), matrix metalloproteinase–3 (*MMP–3*), stromolysin, matrix metalloproteinase–1 (*MMP–1*), collagenase, a disintegrin and metalloproteinase with thrombospondin motifs 4 (*ADAMTS4*), a disintegrin and metalloproteinase with thrombospondin motifs 5 (*ADAMTS5*), tissue inhibitor of matrix metalloproteinase–1 (*TIMP–1*), tissue inhibitor of matrix metalloproteinase–13 (*TIMP–13*). The detailed sequences of the primer pairs are shown in Table 1.

The mRNA expression levels were quantified by real–time PCR using the Mas tercyler®ep Rea lplex Thermal Cycler (Eppendorf, Hamburg, Germany) and Thunderbird SYBR® qPCR Mix (Toyobo, Osaka, Japan) to detect PCR products. The 25 μL PCR reaction mixture contained 5 μL of cDNA, 12.5 μL of Thunderbird SYBR® qPCR Mix, 0.2 mM of dNTP, and 0.5 μM of each primer (Toyobo). The reaction mixture was denatured at 95 °C for 2 minutes, followed by 40 cycles of PCR reactions in the following settings: 95 °C, 15 seconds; 60 °C, 15 seconds; and 72 °C, 45 seconds. The expression level of each target gene was normalized to *GAPDH* expression. Normalized mRNA expression (in ΔC<sub>t</sub> units) was calculated by subtracting the cycle threshold (C<sub>t</sub>) of the target gene from the C<sub>t</sub> value of the house–keeping genes: ΔC<sub>t</sub> (target gene)=C<sub>t</sub> (target gene) – C<sub>t</sub> (*GAPDH*).

**Table 1**

Gene sequences and primer information.

Gene	GenBank Accession NO.	Primer pair (5′–3′)	Length of product (bp)
<i>ACAN</i>	NM_001135	For: CAGAATCAACTGCTGCAGACCA Rev: TTCGATGGTCCCTGTCGTTCCAG	227
<i>COL1A1</i>	NM_000088	For: AGTGGTTTGGATGGTGCCAA Rev: GCACCATCATTTCACAGGAGC	170
<i>COL2A1</i>	NM_001844	For: GGGACTGtTCCTCTGCGACGACA Rev: CTTTGGTCCCTGGTTGCCCACT	172
<i>MMP3</i>	NM002422	For: TGAGGACACCAGCATGAACC Rev: ACTTCGGGATGCCAGGAAAG	248
<i>MMP13</i>	NM002427	For: CATGAGTTCGGCCACTCCTT Rev: CCTCGGAGACTGGTAATGGC	230
<i>ADAMTS4</i>	NM005099	For: CACATTCTTGTCGGCAGCA Rev: CCCCTCCCCACTGAGTCTTA	489
<i>ADAMTS5</i>	NM007038	For: ACTTTCCTTTGGCACACCCA Rev: ACGTCACTGACCACTGTTGG	230
<i>TIMP1</i>	NM003254	For: GCGGATACTTCCACAGGTCC Rev: CTGGTCCGTCCACAAGCAAT	242
<i>TIMP3</i>	NM000362	For: CCCTCCCACAAGTGGACATC Rev: ACTCCGCAGGGAAACTTCAG	139
<i>GAPDH</i>	NM002046	For: TCAAGATCATCAGCAATGCC Rev: TGTGGTCATGAGTCCTTCCA	227

Note: For, forward; Rev, reverse.

#### 2.4.4. Western blot analysis

After the IL-17 stimulation of NP cells cultured *in vitro*, a Western blot analysis was conducted to measure the protein levels of *ACAN*, *COL1A1*, *COL2A1*, *ADAMTS4*, and *ADAMTS5* genes with polyclonal goat anti-*ACAN* antibody, polyclonal goat anti-*COL1A1* antibody, polyclonal goat anti-*COL2A1* antibody, polyclonal goat anti-*ADAMTS4* antibody, and polyclonal goat anti-*ADAMTS5* antibody (sc-16492, sc-8784, sc-7764, sc-16534, Santa Cruz Biotechnology Company; LS-C124924-100, LifeSpanBioSciences Inc.).

Cells were harvested and washed twice with ice-cold PBS. Lysates were obtained with a TEN-T buffer (150 mM NaCl, 10 mM Tris-HCl, pH 7.4, 5 mM EDTA, pH 8.0, 1% Triton X-100, 1 mM PMSF, 2  $\mu$ g/mL aprotinin) and was subjected to 10 000 $\times$ g centrifugation at 4 °C for 20 minutes. The BCA Protein Assay Kit (Beyotime) was used to measure the protein concentration of the supernatants. Per lane, 50  $\mu$ g of protein was electrophoresed in 12% SDS polyacrylamide gels and transferred onto nitrocellulose membranes (Sigma). After blocking with TTBS (50 mM Tris-HCl, pH 7.4, 0.5 M NaCl, 0.05% Tween-20) containing 5% (w/v) skimmed milk, the membranes were incubated with first antibodies overnight at 4 °C, followed by horseradish peroxidase-conjugated second antibody incubation (Santa Cruz Biotechnology Company). Immunoreactive bands were detected by Super ECL Plus Detection Reagent (Amersham).

#### 2.5. Statistical analysis

All tests were repeated three times. Statistical analysis was carried out using Student's *t*-test with SPSS software. Results are presented as mean $\pm$ SD.  $P < 0.05$  was considered statistically significant.

### 3. Results

#### 3.1. IL-17 stimulation effects on cell proliferation

In 72 hours, IL-17 stimulation induced a dose-dependent

decrease in proliferation rate. With the IL-17 dosage range (0–20 ng/mL), the suppression ratios were 3.89% $\pm$ 0.65%, 6.87% $\pm$ 1.05%, 8.35% $\pm$ 0.77%, 12.89% $\pm$ 0.64%, and 12.80% $\pm$ 1.56%, respectively. The 15 ng/mL concentration showed significantly stronger inhibition effect on nucleus pulposus cell proliferation than 2 ng/mL ( $t=17.089$ ,  $P=0.000$ ), 5 ng/mL ( $t=8.479$ ,  $P=0.001$ ), and 10 ng/mL ( $t=7.854$ ,  $P=0.001$ ); however, the difference was not significant compared with 20 ng/mL ( $t=0.015$ ,  $P=0.99$ ). These results suggest that IL-17 stimulation induced the proliferation rate to decrease in cultured NP cells, and the inhibition effect on 15 ng/mL of IL-17 was significantly stronger. A 15 ng/mL concentration of IL-17 was used to stimulate nucleus pulposus cells cultured *in vitro* for metabolic analysis.

#### 3.2. IL-17 stimulation effects on cell metabolism

##### 3.2.1. IL-17 stimulation effects on gene mRNA expression

After 48 and 96 hours of treatment with 15 ng/mL IL-17, a significant decrease on *ACAN* expression levels were observed; *MMP3* expression levels were significantly elevated; a significant decrease of *COL1A1* mRNA was noted after 48 hours ( $P < 0.05$ ), and the significance disappeared after 96 hours; a significant increase of *TIMP3* mRNA was noted after 48 hours ( $P < 0.05$ ), and the significance also disappeared after 96 hours. The expression levels of *COL2A1*, *MMP13*, *ADAMTS4*, *ADAMTS5* and *TIMP1* also responded to IL-17 stimulation, but no significant changes were noted (Table 2).

##### 3.2.2. IL-17 stimulation effects on protein expression

The effects of IL-17 (15 ng/mL) on the protein levels of *ACAN*, *COL1A1*, *COL2A1*, *ADAMTS4* and *ADAMTS5* were measured by Western blot analysis (Figure 3). NP cells demonstrated a greater decrease in *COL1A1* protein expression after 48 hours ( $t=3.199$ ,  $P=0.006$ ) and a significant increase in *ADAMTS5* protein expression ( $t=2.667$ ,  $P=0.021$ ), compared with the unstimulated group. However, both

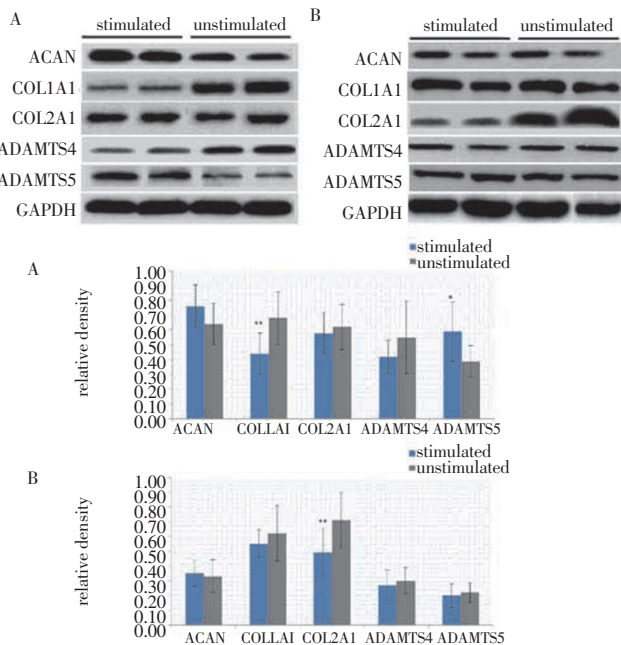
**Table 2**

Normalized mRNA expressions ( $\Delta C_t$ , unit) in annulus fibrosus cells exposed to interleukin-17 for (A) 48 hrs and (B) 96 hrs compared with unstimulated cells.

Time	Gene	<i>ACAN</i>	<i>COL1A1</i>	<i>COL2A1</i>	<i>MMP3</i>	<i>MMP13</i>	<i>ADAMTS4</i>	<i>ADAMTS5</i>	<i>TIMP1</i>	<i>TIMP3</i>
48 h	S	7.22 $\pm$ 2.08	11.90 $\pm$ 3.85	8.82 $\pm$ 3.02	5.44 $\pm$ 2.27	7.43 $\pm$ 3.16	11.19 $\pm$ 4.11	13.44 $\pm$ 3.67	10.33 $\pm$ 2.33	8.22 $\pm$ 3.29
	US	4.55 $\pm$ 2.66	8.20 $\pm$ 1.43	7.44 $\pm$ 2.69	8.89 $\pm$ 3.88	8.22 $\pm$ 2.77	13.22 $\pm$ 3.88	11.55 $\pm$ 4.22	11.47 $\pm$ 3.05	12.02 $\pm$ 4.00
	<i>t</i>	2.372	2.703	1.024	2.302	0.564	1.077	1.014	0.891	2.201
	<i>P</i>	0.031*	0.022*	0.321	0.035*	0.581	0.297	0.326	0.386	0.043*
96 h	S	10.44 $\pm$ 3.34	8.22 $\pm$ 2.99	10.22 $\pm$ 3.55	7.03 $\pm$ 2.89	11.40 $\pm$ 4.25	10.22 $\pm$ 4.22	5.44 $\pm$ 2.34	8.39 $\pm$ 2.77	11.22 $\pm$ 4.00
	US	7.00 $\pm$ 2.27	9.33 $\pm$ 3.22	8.99 $\pm$ 3.96	10.55 $\pm$ 2.91	9.22 $\pm$ 3.55	12.25 $\pm$ 3.59	6.37 $\pm$ 3.12	6.88 $\pm$ 2.49	10.42 $\pm$ 2.98
	<i>t</i>	2.555	0.758	0.694	2.575	1.181	1.099	0.715	1.216	0.481
	<i>P</i>	0.021*	0.460	0.498	0.020*	0.255	0.288	0.485	0.242	0.637

Note: \*  $P < 0.05$ , compared with unstimulated cells. S: stimulated; US: unstimulated.

significant changes disappeared after 96 hours ( $t=0.359$ ,  $P=0.724$ ;  $t=0.538$ ,  $P=0.568$ ). At this time point, COL2A1 protein levels greatly increased, compared with the unstimulated group ( $t=4.411$ ,  $P=0.000$ ).



**Figure 3.** Protein expression in annulus fibrosus cells exposed to interleukin-17 for (A) 48 hrs and (B) 96 hrs compared with unstimulated cells.

\*  $P<0.05$ , compared with unstimulated cells; \*\*  $P<0.01$ , compared with unstimulated cells.

#### 4. Discussion

IL-17 is a cytokine factor that has recently shown to be elevated in degenerated human intervertebral disc tissues[6], suggesting that an in-depth investigation of its involvement in disc degeneration may help us better understand the DDD process. In this study, through establishing cultured nucleus pulposus cells *in vitro*, the inhibiting effects of different IL-17 concentrations on nucleus pulposus cell proliferation was analyzed. After IL-17 exposure, nucleus pulposus cell metabolic changes were explored in order to identify the possible role of IL-17 in the pathogenesis of disc degeneration from an extracellular matrix balance aspect.

Within 72 hours, IL-17 stimulation in cultured NP cells induced a dose-dependent proliferation inhibition rate. The suppression ratio increased as the concentrations were increased, and the suppression ratio of IL-17 at a concentration of 15 ng/mL had the highest results. Based on the analysis of Gabr *et al*, they found that NP cells increased its nitric oxide (NO) production over the 72 hour culture period in the presence of IL-17, with a maximal effect noted

for IL-17 doses higher than 10 ng/mL[17]. This result was basically consistent with the results of our experiments. The suppression ratio of IL-17 at 20 ng/mL concentration also showed no significant difference, perhaps due to the complete saturation of the IL-17 receptors in NP cells[21]. Until now, the mechanism of the proliferation inhibiting effects of IL-17 on NP cells is still unknown. However, it is known that NO, another inflammatory factor, is involved in DDD development, through promoting apoptosis[22], and IL-17 can significantly induce the expression of nitric oxide synthase, thereby, contributing to NO molecule generation[23]. IL-17 may also play a role in inhibiting cell proliferation by indirectly inducing apoptosis in the NP cells.

A IL-17 at 15 ng/mL concentration was used to stimulate the NP cells cultured *in vitro* for metabolic analysis. We found that *ACAN* and *COL1A1* mRNA levels were significantly down regulated by IL-17 after 48-h culture. After 96 hours, the *ACAN* mRNA levels in stimulated group were still significantly down regulated. Western blot results showed that *COL1A1* protein levels in stimulated group were significantly higher than the unstimulated group. Collagen type II mRNA expression was down regulated at two time points, but no significant differences were noted, compared with unstimulated group. Based on the experiments of Seguin *et al*, NP cells formed *in vitro* were treated with  $TNF-\alpha$ , a proinflammatory cytokine, over 48 hours[24], and the tissues were assessed for proteoglycan and collagen synthesis. They found that  $TNF-\alpha$  induced the decreased expressions of both aggrecan and type II collagen genes, suggesting that decreasing the synthesis of matrix macromolecules may be one of the main functions of inflammatory cytokines in disc degeneration. Besides the decrease in matrix macromolecules synthesis[25,26], a gradual transition from type I collagen to type II collagen was observed[27]. In our analysis, the down regulation of type I collagen was not observed after IL-17 stimulation, but the *COL2A1* gene protein level was significantly lower, compared with the unstimulated group (time point: 96 hours). One possible explanation is that within 48 hours, NP cells responded to IL-17 stimulation with a decreased type I collagen protein synthesis; and also, the collagen type transition enabled the observation of increased type I collagen protein level and decreased type II protein level. The results suggest that IL-17 may be involved in tissue matrix homeostasis destruction through inhibiting matrix macromolecules synthesis and collagen type transition.

Nucleus pulposus cells not only synthesize extracellular matrix macromolecules, but also synthesize a variety of enzymes for matrix degradation[28,29]. A variety of enzyme changes in IL-17 stimulated NP cells were noted in our

analysis. Compared with the unstimulated group, the mRNA levels of the *MMP3* gene significantly increased after 48 and 96 hours of stimulation; and the significant increase in *TIMP3* mRNA levels were noted at the 48-hour time point; an additional evidence that DDD develops, as specific gene/protein expression increases, such as the *MMP3* and *TIMP3* genes identified by Liang *et al.*<sup>[30,31]</sup>, which indicated that IL-17 may directly or indirectly contribute to disc degeneration through other mechanisms. *ADAMTS4*, *ADAMTS5*, and *TIMP1* mRNA levels also increased after IL-17 stimulation, but compared with the unstimulated group, no significant differences were noted. A possible reason, for the absence of significant response to IL-17 stimulation, is that the expression of these genes has already been up-regulated *in vivo* since the time the NP cells were isolated from degenerative human discs. After 48 hours of stimulation, *ADAM-TS5* gene protein levels were significantly up-regulated, probably due to the response of *ADAMTS5* mRNA translation from IL-17 stimulation. All these results have shown that IL-17 stimulation increased the synthesis and activity of degrading enzymes in NP cells, which therefore enhanced disc extracellular matrix degradation and further promoted the degradation process.

IL-17 stimulation effects on the proliferation and metabolism of nucleus pulposus cells cultured *in vitro* were analyzed. Our findings suggest that IL-17 can inhibit proliferation and possibly contribute to the degenerative changes that occur in disc disease, through inhibiting extracellular matrix synthesis, promoting nucleus pulposus extracellular matrix degradation and disrupting metabolic balance.

Despite the effectiveness of using a model where nucleus pulposus cells were cultured *in vitro* to study the process of disc degeneration, previous studies have also demonstrated gene expression changes in culturing intervertebral disc cells<sup>[32–35]</sup>. In order to minimize any potential phenotypic changes, we used third passage cells for all experiments. Clearly, the *in vitro* results presented in this study cannot be directly used to interpret the function of IL-17 *in vivo*; however, we hope that the identified responses of IL-17 stimulation would be further considered for DDD. Future studies are needed to identify the specific changes of normal and degenerative cells that respond to IL-17 only or in combining IL-17 with other inflammatory cytokines both *in vitro* and *in vivo*; and how these results may be further exploited to facilitate its beneficial effects on tissue matrix homeostasis.

### Conflict of interest statement

We declare that we have no conflict of interest.

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