Cross-talk between synovial fibroblasts and chondrocytes in condylar hyperplasia: an in vitro pilot study

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Objective. Increasing evidence indicates an interaction between the synovium and the cartilage in the temporomandibular joint (TMJ) and other joints. We recently demonstrated that the expression of proangiogenic factors was enhanced and that of factors promoting matrix degradation was decreased in synovial fibroblasts in condylar hyperplasia (CH). The aim of this study was to explore whether CH chondrocytes can affect the expression of these factors of synovial fibroblasts in a co-culture system.

Study Design. The expressions of vascular endothelial growth factor (VEGF), cluster of differentiation 34 (CD34), fibroblast growth factor 2 (FGF-2), and tissue inhibitor of metalloproteinase 1 (TIMP1) from CH condylar tissues were observed by using immunohistochemical methods. Synovial fibroblasts of control tissues were co-cultured with the chondrocytes of CH, and protein expressions of VEGF, FGF-2, thrombospondin 1 (TSP1), matrix metalloproteinase 3 (MMP3), and TIMP1 were examined by using Western blotting.

Results. Positive staining for VEGF, CD34, FGF-2, and TIMP1 was found in the hypertrophic cartilage layer of CH condylar tissues. Protein expressions of VEGF, FGF-2, and TIMP1 were significantly increased in co-cultured synovial fibroblasts, but TSP1 and MMP3 expressions were decreased.

Conclusions. The angiogenic factors and matrix degradation–related factors in synovial fibroblasts co-cultured with CH chondrocytes showed the same trends as those in synovial fibroblasts from CH tissue, suggesting potential cross-talk between synovial fibroblasts and chondrocytes during CH progression. (Oral Surg Oral Med Oral Pathol Oral Radiol 2021;131:558–564)

Condylar hyperplasia (CH) of the temporomandibular joint (TMJ) is a rare but self-limiting disease, the main features of which are facial asymmetry and occlusal disturbance.[1,2] Most studies on CH have described cases, treatment proposals, and treatment outcomes; however, a systematic understanding of the pathogenesis of CH is still lacking. To date, the majority of past research has focused on the changes in the condylar cartilage and chondrocytes during disease progression. For instance, bone morphogenetic protein 2 (BMP-2) and transforming growth factor β1 (TGF-β1) have been confirmed to be highly expressed in the cartilage of CH.[3] In addition, insulin-like growth factor 1 (IGF-1) has been shown to promote CH development by facilitating chondrocyte proliferation.[4,5] Notably, our recent study revealed that angiogenesis-associated factors, such as vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF-2), cluster of differentiation 34 (CD34), and angiopoietin 1, were abundantly expressed in synovial fibroblasts in CH tissue. In contrast, the expressions of matrix degradation–related factors, such as matrix metalloproteinase (MMP)-1, MMP-3, and MMP-13, were downregulated, but those of tissue inhibitor of matrix metalloproteinase 1 (TIMP1) and TIMP3 were elevated in synovial fibroblasts in CH tissue.[6]

The cartilage and the synovium together form the internal environment of the TMJ, in which the mutual effect between the synovium and the cartilage occurs. Synovial fluid secreted by the synovium protects the surface of the articular cartilage.[7] However, in TMJ osteoarthritis (OA), the cartilage may be damaged by some of the growth factors and inflammatory cytokines in the synovial fluid.[8] Catabolic enzymes in the cartilage matrix, such as MMP and a disintegrin and metalloproteinase domain with thrombospondin motifs, can be detected in the synovial fluid of patients with symptomatic temporomandibular disorders.[9-11] With the advancement of specific co-culture devices, an increasing number of studies have been conducted on the cross-talk between synovial fibroblasts and chondrocytes during the progression or treatment of rheumatoid arthritis or OA in knee joints. For example, after co-culture of chondrocytes and synovial mesenchymal stem cells, chondrocytes showed enhanced ability for adherence,

Statement of Clinical Relevance

Proangiogenic factors and matrix degradation–related factors in synovial fibroblasts co-cultured with condylar hyperplasia (CH) chondrocytes showed the same trends as those in synovial fibroblasts from CH tissue, suggesting potential cross-talk between the cartilage and the synovium during CH progression.

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and the synovial mesenchymal stem cells displayed improved osteogenic performance.[12] In addition, chondrocytes co-cultured with synovial fibroblasts showed significantly lower amounts of native collagen type II in the extracellular matrix than did control chondrocytes.[13] When co-cultured with hypoxia-inducible factor 2 α-overexpressing chondrocytes, the expression of MMPs and inflammatory factors was enhanced in the synovial fibroblasts, which was similar to the effect induced by tumor necrosis factor -α treatment of the synovial fibroblasts.[14] However, to our knowledge, whether such cross-talk occurs between the cartilage and the synovium in TMJ CH is unknown.

The aim of the present study was to examine whether cross-talk occurs between chondrocytes and synovial fibroblasts in CH by employing an in vitro co-culture system. The expression of angiogenic factors and matrix degradation−related factors in the condylar tissue of CH and in synovial fibroblasts co-cultured with CH chondrocytes was investigated.

MATERIALS AND METHODS

Sample collection and clinical observations of patients with CH

Synovial and condylar samples of CH tissue were collected from 8 patients age 18−24 years (Table I) undergoing condylectomy and arthroplasty. Patients with CH typically displayed facial and craniofacial asymmetry from the frontal view (Figure 1A) and in 3-dimensional (3-D) reconstruction of the maxillofacial region from cone beam computed tomography (CBCT) images (Figure 1B). Single-photon emission computed tomography (SPECT) was used to confirm the growth activity of the affected TMJ before surgery. SPECT provided functional imaging and allowed for visualization of bone metabolism. In 1 representative patient with CH, SPECT bone scintigraphy showed that the right condyle was much deeper than the left (Figure 1C), revealing the enhanced metabolic activity of the affected condylar process. As controls, 4 patients with condylar fracture without accelerated growth activity were included (Table II). Informed consent was obtained from all patients, and the study was approved by the Human Research Ethics Committee, School & Hospital of Stomatology, Wuhan University (Wuhan, China).

Table I. CH samples used for analysis

Case	*Gender*	*Age (years)*	*Affected side*	*SPECT*
1	Female	18	Left	+
2	Female	24	Right	+
3	Male	19	Left	+
4	Female	24	Left	+
5	Female	23	Right	+
6	Male	19	Left	+
7	Male	21	Right	+
8	Male	21	Left	+

CH, condylar hyperplasia; *SPECT,* single-photon emission computed tomography.

Cell culture

The synovial specimens from patients with CH or condylar fracture were minced into small explants (1 mm^3) and cultured in Dulbecco's Modified Eagle's Medium (DMEM; HyClone, Logan, UT) supplemented with 15% fetal bovine serum (FBS; HyClone, Logan, UT), streptomycin (100 μg/mL; HyClone, Logan, UT) and penicillin (100 units/mL; HyClone, Logan, UT) as described previously.[15] The cartilage samples of CH were washed 3 times with phosphate buffered saline (PBS) and digested with 0.2% collagenase (Sigma, St. Louis, MO). The synovial explants and chondrocytes were maintained at 37°C in a 5% carbon dioxide incubator. When the cells became confluent, they were digested with 0.25% trypsin (HyClone, Logan, UT) and then plated in 10 cm^2 dishes containing DMEM supplemented with 10% FBS. Because multiple-passaged cells lose disease status and tend to resemble normal cells, cells between passages 1 and 3 were used for the experiment.

In the co-culture system, the chondrocytes of CH were cultured on the membranes of transwell cell culture inserts (pore size 0.4 μm; Corning, NY). Synovial fibroblasts of condylar fracture were plated on the bottom of wells of a 6-well transwell cell culture system. The cells were incubated for 2 days with complete medium and an appropriate culture environment. In addition, synovial fibroblasts of condylar fracture alone and CH alone were seeded as negative and positive controls, respectively.

Immunohistochemistry

Condylar tissues of CH were fixed, decalcified in 10% ethylenediaminetetraacetic acid, and embedded in paraffin by using standard procedures. After a series of routine treatments, 4-μm sections were cut and processed for immunohistochemical staining. Antigen retrieval was performed by using pepsin (DIG-3009; Maixin, Fuzhou, China) for 30 minutes at 37°C. Rabbit anti-VEGF polyclonal antibody (1:100, Catalog #BA0407; Boster Biological Technology, Wuhan, China); rabbit anti-TIMP1 monoclonal antibody (1:50, Catalog #BM4980; Boster Biological Technology, Wuhan, China); mouse anti-FGF-2 antibody (1:100, Catalog #BM0259; Boster Biological Technology, Wuhan, China); and rabbit anti-CD34 monoclonal antibody (1:100, Catalog #AB81289; Abcam, Cambridge, MA) were applied as primary antibodies, and the sections were incubated overnight at 4°C. Equal amounts of PBS without primary antibody were used as negative control. Then, the histologic sections were washed 3 times with

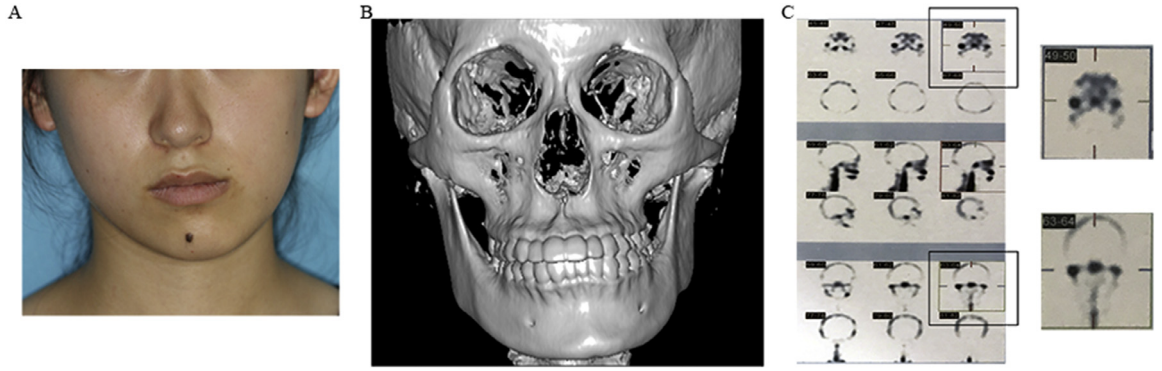

Fig. 1. The clinical features and radiologic observations of one representative patient with condylar hyperplasia (CH). **A**, A frontal view of the patient. **B**, A 3-dimensional reconstruction image of the maxillofacial region. **C**, A single-photon emission computed tomography (SPECT) scan showing that the bone activity in the affected condyle *(right side)* was strongly enhanced.

Table II. Control samples used for analysis

Case	*Gender*	*Age(years)*	*Affected side*
1	Male	28	Left
2	Male	30	Right
3	Female	15	Left
4	Male	18	Right

PBS and stained by using an antirabbit streptavidin-peroxidase kit (Kit-9706; Maixin, Fuzhou, China) or antimouse streptavidin-peroxidase kit (Kit-9701; Maixin, Fuzhou, China), according to the manufacturer's instructions. Finally, the sections were reacted with 3,3'-diaminobenzidine (DAB-0031, Maixin, Fuzhou, China). Counterstaining with hematoxylin was performed for light microscopy observation.

Western blotting

After the pretreatments described above, the cells from each group were rinsed 3 times in cold PBS and incubated with 30 μL lysis buffer. Lysis supernatant containing 20 μg protein was collected, as previously described.[6] The samples were then subjected to electrophoresis in 10% sodium dodecyl sulfate-polyacrylamide gels and then transferred to a 0.45-μm polyvinylidene difluoride membrane (Millipore, Bedford, MA) for 1 to 2 hours at 4°C. After being blocked with 5% skim milk for 1 hour at room temperature, the membrane was incubated overnight with the following primary antibodies at 4°C: rabbit anti-VEGF polyclonal antibody (dilution 1:1000, 19003-1-AP; Proteintech, Wuhan, China); rabbit anti-thrombospondin 1 (TSP1) polyclonal antibody (dilution 1:2000, 18304-1-AP; Proteintech, Wuhan, China); goat anti-FGF-2 polyclonal antibody (dilution 1:300, sc-1390; Santa Cruz Biotech, Santa Cruz, CA); rabbit anti-MMP3 monoclonal antibody (dilution 1:1000, 14351; Cell Signaling Technology [CST], Danvers, MA); rabbit anti-TIMP1 monoclonal antibody (dilution 1:1000, 8946; CST, Danvers, MA); and mouse anti-β-actin monoclonal antibody (dilution 1:10000, RM2001; Rayantibody, Beijing, China). Then, the membrane was treated with antirabbit or antimouse immunoglobulin G secondary antibody (1:10000; ThermoFisher Scientific, Rockford, IL) conjugated with horseradish peroxidase for 1 hour. The membrane was washed, and the bands were visualized on x-ray films by using Thermo Pierce ECL western blotting Substrate (ThermoFisher Scientific, Rockford, IL). β-actin was used as an internal control.

Statistical analysis

The results were analyzed by using the SPSS version 20 software program (SPSS Inc., Chicago, IL). The data were expressed as the mean ± standard deviation (SD) and tested for homogeneous variance and Gaussian distribution. The 2-sample Student *t* test was used to analyze the differences between group means. Differences were considered statistically significant at *P* value less than .05.

RESULTS

Expression of factors related to angiogenesis and matrix degradation in CH condylar tissues

With regard to angiogenic factors, positive staining for VEGF, CD34, and FGF-2 was observed mainly in the hypertrophic cartilage layer of CH tissue as a continuous layer. Some TIMP1-positive staining was also observed in the hypertrophic cartilage layer. Furthermore, some areas of positive staining for VEGF, CD34, and TIMP1 were found in the fibrous articular layer. These factors were not detected in the control condyle (Figure 2).

Morphologic observation of synovial fibroblasts and chondrocytes of CH tissues

As shown in Figure 3A, the CH synovial fibroblasts of primary culture migrated outward from the site of tissue inoculation. After passing through the culture, the

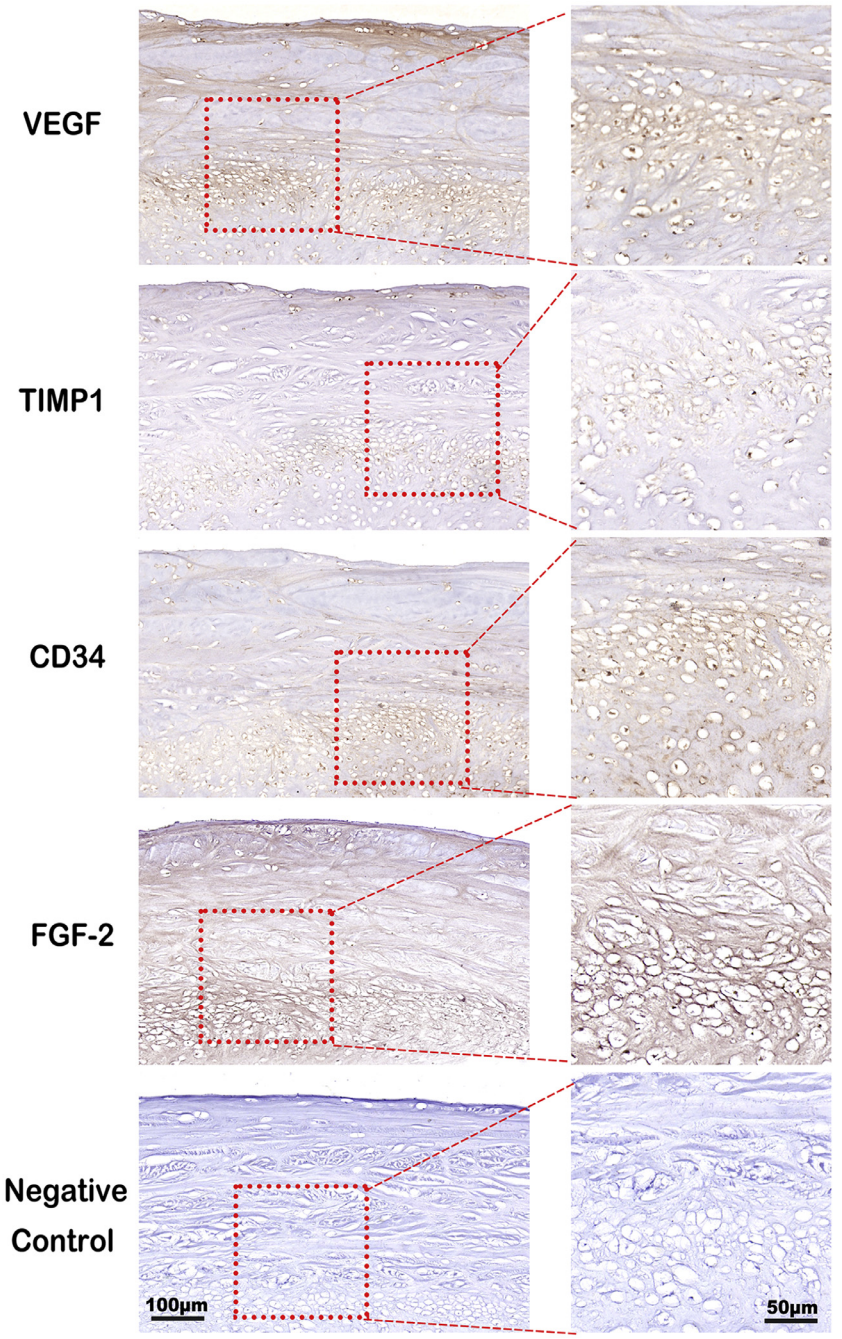

Fig. 2. The expression of factors related to angiogenesis and matrix degradation in condylar hyperplasia (CH) tissues. The tissues exhibited a thickened hypertrophic cartilage layer, and vascular endothelial growth factor (VEGF), tissue inhibitor of metalloproteinase 1 (TIMP1), cluster of differentiation 34 (CD34), and fibroblast growth factor 2 (FGF-2) were expressed in the tissues of CH.

synovial fibroblasts of CH exhibited a homogeneous, characteristic spindle morphology and gradually connected with each other (Figure 3B). In contrast, as a result of enzymatic digestion, most of the primary chondrocytes from CH tissues were heterogeneous in shape, with irregular polygon-like morphologies (Figures 3C and 3D).

Expression of factors related to angiogenesis and matrix degradation in co-cultured synovial fibroblasts

To address how secretory mediators originating from the chondrocytes of CH affect synovial fibroblasts, we used a transwell apparatus with a pore size of 0.4 μm to create a co-culture system that allowed for the

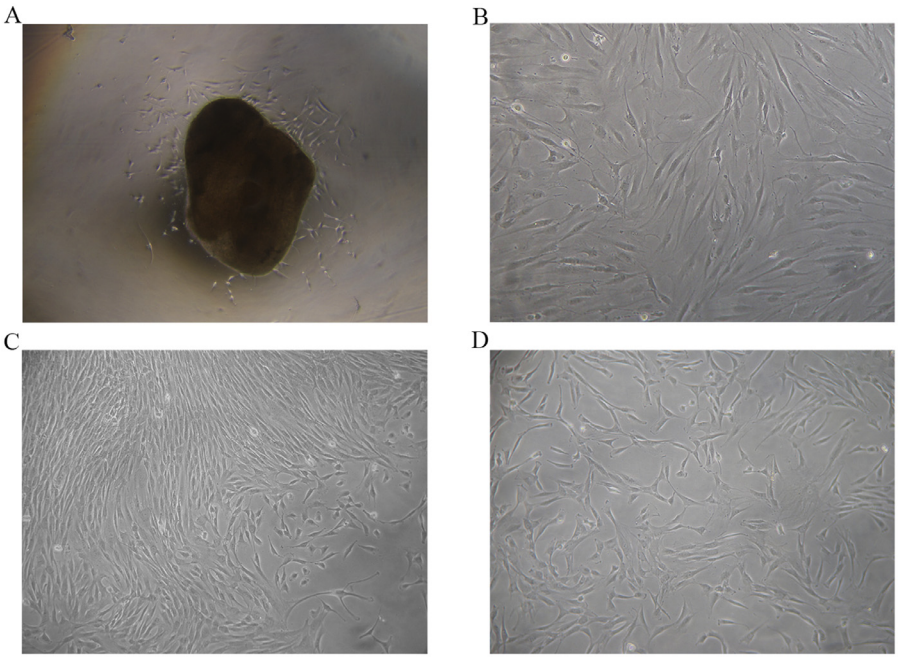

Fig. 3. The morphology of synovial fibroblasts and chondrocytes in condylar hyperplasia (CH). **A**, Primary synovial fibroblasts migrated outward from the synovial tissue blocks of CH (objective magnification: × 4). **B**, Synovial fibroblasts of CH presented a homogeneous spindle shape after passage (objective magnification: × 10). **C and D**, The majority of CH chondrocytes cultured in monolayer were round or polygonal (objective magnification: × 10).

transfer of secreted factors but not cells. The synovial fibroblasts from the patients with condyle fracture (the control group) were seeded on the bottom of the plates, and the chondrocytes of CH were cultured on the membrane of the insert. In accordance with the results for the synovial fibroblasts of CH, the expressions of VEGF, FGF-2, and TIMP1 were significantly increased in the co-cultured synovial fibroblasts relative to the control cells. Furthermore, TSP1 and MMP3 expressions were decreased in the co-culture group compared with the control group, exhibiting trends similar to those observed in the synovial fibroblasts of CH (Figure 4A and 4B).

DISCUSSION

One of the most distinct clinical properties of CH is facial asymmetry, which is the main reason patients seek medical attention. Specifically, patients with unilateral TMJ CH exhibit fullness of the lower third of the face on the ipsilateral side, flatness of the contralateral side, and deviation of the chin away from the affected side.[16] In the present study, the diagnosis of CH was made on the basis of clinical findings and was supported by CBCT imaging and SPECT investigation, which is currently the gold standard for CH diagnosis. In this study, in addition to observing the above clinical and imaging features, we found positive staining for several angiogenic-associated factors in CH condylar tissue and observed increased expression of these factors in synovial fibroblasts co-cultured with CH chondrocytes. These findings could reveal previously unknown pathologic changes in the progression of CH.

The regenerative capacity of articular cartilage is poor because of the low mitotic ability of chondrocytes and the avascular nature of the cartilage; however, the cartilage showed proliferative characteristics in CH. Immunohistochemical staining has confirmed the presence of some growth factors, including IGF-1, BMP-2, and TGF-β1, in the condyle of CH.[3,17] Angiogenesis is a crucial step in bone and cartilage formation, and VEGF and FGF-2 are 2 angiogenic growth factors involved in the process. VEGF is an essential coordinator of chondrocyte death, bone formation, and extracellular matrix remodeling in the growth plate. VEGF has been demonstrated to be expressed in the upper hypertrophic layer in rat condyles during natural growth.[18] FGF-2 is a chondrocyte mitogen that promotes the synthesis of cartilaginous matrix, chondrocyte differentiation, and osteoblast proliferation. Our previous findings showed that a high level of FGF-2 was responsible for the pathogenesis of synovial chondromatosis, another proliferative disease of the TMJ.[19] In addition, it has been reported that VEGF and FGF-2 can upregulate the expression of BMP-2.[20,21] Furthermore, CD34 has been considered a marker of vessel formation in many studies because it is expressed by hematopoietic stem cells and progenitor cells.[22] Mesenchymal stem cells consistently exhibit an increase in proliferation

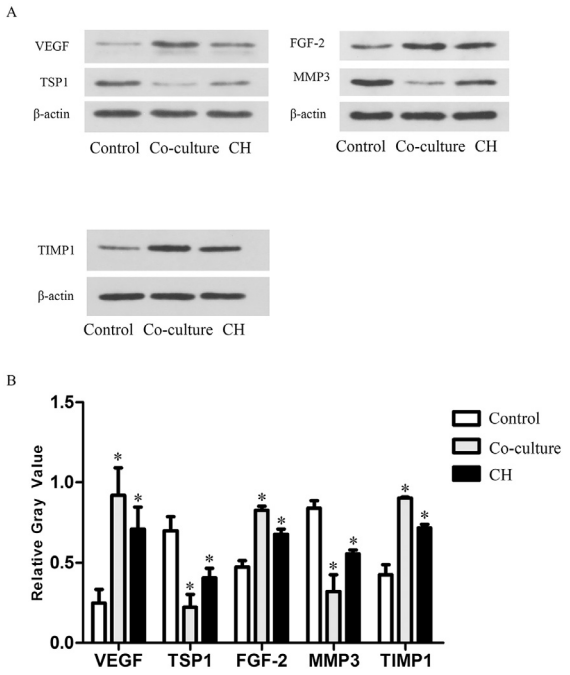

Fig. 4. Protein expression of vascular endothelial growth factor (VEGF), thrombospondin 1 (TSP1), fibroblast growth factor 2 (FGF-2), matrix metalloproteinase 3 (MMP3), and tissue inhibitor of metalloproteinase 1 (TIMP1) in the co-cultured and CH synovial fibroblasts. **A**, The Western blotting results of the factors. **B**, The gray value of the Western blotting results. Data were normalized relative to the expression of β-actin and are presented as mean ± standard deviation (SD). * $P < .05$.

rate under upregulated CD34 expression.[23,24] In the present study, VEGF, FGF-2, and CD34 were highly expressed in the condyle in CH, and this may be associated with the high proliferative state during CH.

In this study, we found that the factors VEGF, FGF-2, TSP1, MMP3, and TIMP1 in synovial fibroblasts co-cultured with CH chondrocytes showed similar trends as those observed in synovial fibroblasts in CH. This result suggests interactions between the condylar cartilage and synovial tissues during the onset and development of CH. Many studies have been conducted on the molecular interactions between different types of cells. For instance, the cross-talk between the articular cartilage and the subchondral bone is considered a main feature of OA onset, and changes in subchondral bone microarchitecture occur before articular cartilage degeneration during OA progression.[25] Biologic cross-talk between the cartilage and the synovium is also significant in OA and rheumatoid arthritis.[14,26,27] Co-culture of synovium-derived mesenchymal stem cells with sodium nitroprusside −stimulated chondrocytes was found to inhibit the secretion of inflammatory factors by stem cells, increase the concentration of IGF-1 in the culture medium, and promote the proliferation of chondrocytes.[28] According to our research findings, chondrocyte-induced increases in angiogenic factors in synovial fibroblasts cannot be negligible during CH development. In contrast to VEGF and FGF-2 expressions, which were upregulated, the expression of TSP1, an inhibitor of angiogenesis, was significantly decreased in the co-cultured synovial fibroblasts. Thus, the loss of the normal avascular state of the articular cartilage in CH may be attributed to decreases in antiangiogenic factors, such as TSP1.[29]

CH not only has the characteristic of abnormal cartilage proliferation but also maintains a noninflammatory status and causes pronounced condylar thickenig.[30] In this study, only a few sites of TIMP1-positive staining were detected in the condylar tissue in CH. However, in co-cultured synovial fibroblasts, MMP3 was strongly downregulated, and TIMP1 was extensively upregulated. MMPs facilitate the loss of collagen and the degradation of extracellular matrix, and their activity could be inhibited by TIMPs.[31] We speculate that increased TIMP1 expression and decreased MMP3 production may promote the deposition of cartilage matrix in CH.

On the basis of previous reports in the literature and the histopathologic characteristics of CH, condylar cartilage and subchondral bone were considered the starting sites of this disease. Our data indicate that the chondrocytes of CH may induce a series of reactions in synovial fibroblasts. However, whether changes in the synovium or the synovial fluid can affect the cartilage remains unknown. It has been demonstrated that secreted factors from synovial fibroblasts could regulate gene expression in chondrocytes[32] and that co-incubation of the cartilage with the synovium plus the joint capsule or co-culture of chondrocytes with synovial fibroblasts could lead to changes of the cartilage or the chondrocytes of the knee joint.[14,27] Therefore, further studies are needed to explore the effects of synovial fibroblasts on chondrocytes during CH progression.

CONCLUSIONS

We demonstrated positive staining for some angiogenic factors in the condylar tissue in CH and similar trends in angiogenic factors, as well as matrix degradation-related factors in synovial fibroblasts co-cultured with CH chondrocytes, as those observed in CH synovial fibroblasts. Our data suggest that angiogenesis and matrix synthesis may be involved in the process of CH and that cross-talk may occur between synovial fibroblasts and chondrocytes and may provide a novel way to understand the pathogenesis of CH.

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PRESENTATION

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