Upregulation of proangiogenic factors expression in the synovium of temporomandibular joint condylar hyperplasia

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Objective. Condylar hyperplasia (CH) is a complex disorder of the temporomandibular joint. Many studies have focused on cartilage proliferation, but the behavior of the synovium in CH is poorly understood. The aim of the present study was to investigate the expression of angiogenic-associated factors in the synovium and to discuss the possible role of the synovium in CH progression.

Study Design. CH condylar tissues were stained by hematoxylin and eosin staining, and proliferative activity was confirmed by single-photon emission computed tomography. Synovial cells isolated from the temporomandibular joint of patients with CH were collected, and flow cytometric analysis was used to examine the expression of CD34 and CD44. The gene expression of FGF-2, MMP1, MMP3, and MMP13 in synovial cells was examined by quantitative real-time polymerase chain reaction. Western blotting was used to detect the protein expression of VEGF, FGF-2, ANG1, DKK1, TSP1, MMP1, MMP3, MMP13, TIMP1, and TIMP3.

Results. The typical hyperplastic area and activity were observed in condylar tissues. The expression of VEGF, FGF-2, ANG1, DKK1, TIMP1, TIMP3, and CD34 was significantly increased in the synovial cells of CH, but TSP1, MMP1, MMP3, and MMP13 expression was decreased.

Conclusions. This study exhibited a potential role for proangiogenic factors in the pathogenesis of CH. (Oral Surg Oral Med Oral Pathol Oral Radiol 2016;121:e65-e71)

Condylar hyperplasia (CH) of the temporomandibular joint (TMJ) is a complex proliferative disorder that leads to occlusal disturbance, facial asymmetry, joint pain, and limitation of mouth opening.[1,2] CH is characterized by the presence of hypertrophic cartilage, islands of chondrocytes in the subchondral trabecular bone, and an uninterrupted layer of undifferentiated, germinating mesenchymal cells[1,3]; however, the pathogenesis of CH is only partially understood. Infection, trauma, genetics and some growth factor changes were believed to be the possible causes of CH.[4] Most previous fundamental studies of CH only focused on the condylar cartilage and shed light on the changes of chondrocytes during the pathologic process.[5,6] Our recent study revealed that the chondrocytes of CH had enhanced proliferation capacity,[7] and the expression of insulin-like growth factor-1, bone morphogenetic protein-2, and transforming growth factor-β1 was upregulated in the cartilage of CH.[5]

The synovium, together with condylar cartilage, constitutes the inner environment of the TMJ, but the role of the synovium in the TMJ CH is unclear. Synovial fluid produced by the synovium contributes to the protection of the surface of articular cartilage.[8] During the pathologic process in the TMJ, the composition of the synovial fluid changed, and the profile of angiogenic-associated factors in the TMJ synovium showed characteristic changes. Enhanced expression of vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2) has been detected in synovial tissue with internal derangement.[9,10] In the synovium in osteoarthritis, VEGF was increasingly expressed under hypoxia.[11] The expression of *VEGF* and *FGF-2* mRNA was also significantly enhanced in the synovium of TMJ synovial chondromatosis.[12] In addition, mRNA expression of matrix metalloproteinase proteins (MMP-1, MMP-3, MMP-9) increased in human synovial cells after excessive compression.[13] However,

Statement of Clinical Relevance

This study contributes to understanding the role of the synovium in the progression of temporomandibular joint condylar hyperplasia. Proangiogenic factors were found to be upregulated in the synovium and might play a role in the pathogenesis of condylar hyperplasia.

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Table I. CH synovial cells samples used for analysis

Case	Gender	Age (years)	Affected side	SPECT
1	Female	18	Left	+
2	Female	24	Right	+
3	Male	27	Right	+
4	Female	24	Left	+
5	Female	23	Right	+
6	Male	19	Left	+

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

angiogenic-associated factors are responsible for the growth of the mandibular condyle. For example, abundant VEGF expression was detected throughout all layers of the condylar cartilage[14] and disk.[15] The synovium might be another source of angiogenic-associated factors in the CH. Therefore, in the present study, we investigated the expression of angiogenic-associated factors in the synovium and discussed the possible role of the synovium in CH progression.

MATERIALS AND METHODS

Sample collection and cell culture

The synovial membrane specimens of CH were obtained from six patients (aged 18 to 27 years; Table I) who were undergoing condylectomy and arthroplasty. The growth activity of the affected TMJ had been confirmed by single-photon emission computed tomography beforehand.[16] The synovial tissues of three patients with condylar fracture and without accelerated growth activity were harvested as the control group (Table II). Informed consent was obtained from all patients, and the experimental protocol was approved by the Human Research Ethics Committee, School and Hospital of Stomatology, Wuhan University, Wuhan, China.

The synovial tissues were cut into 1-mm^3 explants and cultured in Dulbecco's modified Eagle's medium (Hyclone, Logan, UT) containing 15% fetal bovine serum (Sigma, St. Louis, MO), streptomycin (100 μg/mL; Hyclone) and penicillin (100 units/mL; Hyclone) at 37°C in a humidified atmosphere containing 5% CO_2, as described previously.[17] When the cells grew out of the explants and became confluent, they were digested with 0.25% trypsin (Hyclone). Then, the synovial cells were plated in a 10-cm^2 dish and cultivated to confluence with Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Subsequently, the cells at the third passage, during which time they were more similar to cells in a pathologic state, were used for experiments.

Histologic observation

Condylar tissues from patients with CH were fixed in fresh 4% paraformaldehyde and treated with 10% ethylene diaminetetraacetic acid for 6 to 12 months. After a series of routine procedures, the 4-μm-thick paraffin-embedded sections were stained with hematoxylin and eosin (H&E) staining.

Table II. Synovial cells of control samples

Case	Gender	Age (years)	Affected side
1	Female	15	Left
2	Male	50	Right
3	Male	28	Left

Flow cytometric analysis

The cells were digested with 0.25% trypsin/diaminetetraacetic acid, pelleted at 1000 rpm for 10 minutes and resuspended in phosphate-buffered saline (PBS) at a concentration of 2×10^6 cells/mL. Then, the cells were incubated with allophycocyanin--labeled mouse antihuman CD34 (560940, Becton Dickinson, Mountain View, CA) and fluorescein isothiocyanate–labeled mouse antihuman CD44 (560977, Becton Dickinson) on ice for 30 minutes. Equal amounts of isotype standards without primary antibody were used as negative controls. After being washed three times, the cells were resuspended in 800 μL PBS and analyzed on the flow cytometer (Becton Dickinson) in combination with flow cytometry software (FCS Express software; De Novo Software, Glendale, CA).

Western blotting

The cells were cultured at a density of 2×10^5 cells/well in six-well culture dishes. When they became confluent, the cells were washed three times in cold PBS and incubated with 30 μL of cold lysis buffer. The resulting extract was incubated on ice for 30 minutes and centrifuged at 12,000 g for 10 minutes at 4°C. The protein concentrations of the supernatants were measured by a bicinchoninic acid protein assay kit (Thermo Scientific, Rockford, IL). Twenty micrograms of protein per lane was run on a 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gel and then transferred onto 0.45 μm polyvinylidene difluoride membrane (Millipore, Bedford, MA) for 1 to 2 hours at 4°C. After blocking with 5% skim milk in tris-buffered saline and Tween 20 for 60 minutes at room temperature, the membranes were treated with the primary antibodies at 4°C overnight at the following dilutions: Rabbit anti-VEGF polyclonal antibody (dilution 1:1000, 19003-1-AP, Protein Tech, Wuhan, China), goat anti-FGF-2 polyclonal antibody (dilution 1:300, sc-1390, Santa Cruz Biotech, Santa Cruz, CA), rabbit anti-thrombospondin-1 (TSP1) polyclonal antibody