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Research Paper
TMJ Disorders

Effects of transforming growth factor beta 1 on the plasminogen activation system, collagen and integrin synthesis, and proliferation of rabbit mandibular condylar chondrocytes

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Abstract. The objective of this study was to identify the mechanism by which mandibular condyle chondrocytes regulate the extracellular matrix. Primary rabbit condylar chondrocytes were isolated, cultured, and treated with transforming growth factor beta 1 (TGF-β1). Cells were then assayed for the following: urokinase-type plasminogen activator (uPA) and its inhibitor (PAI-1), collagen types I and II, β1 integrin expression, and proliferative activity. TGF-β1 induced synthesis of collagen type II, αVβ1 integrin, and PAI-1. TGF-β1 induced the growth of chondrocytes and suppressed the synthesis of uPA. Chondrocyte regulation of the extracellular matrix is mediated by TGF-β1. Synthesis of collagen type II, αVβ1 integrin, and PAI-1 is induced, while uPA is suppressed. Also, TGF-β1 induces cellular growth.

Key words: mandibular condyle; TGF-β1; extracellular matrix.

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Introduction

The human mandibular condyle is commonly affected either directly by aging or indirectly by functional disturbances.[1] Several reports have addressed the derangement of the temporomandibular joint (TMJ) in relation to osteoarthrosis.[2–4] Components of the TMJ are thought to retain the ability to remodel and repair the cartilaginous tissue and underlying bone after maturation and growth have ceased.[5,6] The mechanism for structural alteration of the mandibular condyle has not been completely characterized. Understanding the

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key factors mediating TMJ remodeling and repair processes will help to elucidate the mechanism of this remodeling.

Common age-related changes have been described resulting from degeneration of the condylar cartilage.[5,6] In a previous study we characterized morphological, radiological, and histological age-related changes based on 34 human condyles obtained on autopsy.[7] In that study, the frequency and severity of degenerative changes, such as erosion and ulceration of cartilaginous tissue, were found to increase with age. Additionally, the accumulation of extracellular matrix (ECM) was found to decrease with advancing age. We confirmed the decreased ECM by immunohistochemically examining the localization of cartilaginous ECM proteins (collagen types I–V, laminin, and fibronectin).[8] We also found that the expression of transforming growth factor beta 1 (TGF-β1) was enhanced in chondrocyte clusters from degenerative lesions in the younger age group (<50 years). However, TGF-β1 expression was not induced in degenerative lesions in the older age group (>70 years).

TGF-β1 influences a wide range of biological activities, including proliferation, angiogenesis, fibrosis, morphogenesis, immune regulation, nerve regeneration, and neuroblast migration.[9] One of the most potent actions of TGF-β1 is the promotion of ECM deposition via the synthesis of collagens, inhibition of ECM-degrading proteases, enhancement of integrins, and modulation of cell–ECM interactions, such as adhesion.[10]

Although we demonstrated reduced localization of TGF-β1 in chondrocyte clusters from degenerative lesions in older human tissue in the previous study, the biological action of TGF-β1 on chondrocytes could not be determined.[8] The present study examined the mechanism by which cultured rabbit chondrocytes regulate the ECM, focusing on the influence of TGF-β1.

Materials and methods

Condylar cells

All experimental protocols involving animals were reviewed and approved by the Institutional Animal Research Committee. TMJ condylar cartilage chondrocytes were isolated from 2-week-old New Zealand white rabbits (Lot No. SCXK2008-123; SEIWA, Izumo, Japan). The resected cartilage was treated with 0.25% trypsin (Sigma, St. Louis, MO, USA) for 15 min, followed by digestion with 0.2% type II collagenase (Invitrogen, San Diego, CA, USA) for 4 h, as described previously.[11] Chondrocytes were resuspended in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Grand Island, NY, USA) with 10% fetal bovine serum (FBS; HyClone, Logan, UT, USA), supplemented with 50 U penicillin/streptomycin and 2 mM L-glutamine. Primary cultures were continued for 5 days. For subsequent experiments, secondary cultures were established with 5×10^5 cells per well in 24-well plates.

Histological examination

The resected condyles were fixed in 10% neutral buffered formalin. The condyles were then decalcified in 10% formic acid and cut into 4-μm sagittal sections. The specimens were then stained with hematoxylin and eosin. Human condyles resected during oral cancer surgery from two patients aged 38 and 82 years were also examined, and no malignant cell invasion was identified histologically.

Fibrinolytic autography

Rabbit condylar chondrocytes were cultured to sub-confluency in 24-well plates with DMEM containing 10% FBS. The medium was replaced with FBS-free DMEM and the cells were cultured for 12 h. Next, the medium was replaced with FBS-free medium with either TGF-β1 (50 ng/ml) or a TGF-β1-free control. After 24 h, the culture medium was collected as conditioned medium.

The activity of plasminogen activator (PA) in the conditioned medium was examined by fibrinolytic autography. The conditioned medium was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and the gel was mounted on a fibrin plate with a mixture of 1 U of thrombin, 4 ml of 0.6% fibrinogen, and 4 ml of 2% agarose. The fibrin plate was kept at 37 °C for 12 h to allow the reaction to occur, and was then stained with Coomassie brilliant blue. Fibrinolytic areas (non-stained portions) were analyzed to determine PA activity as well as levels of its inhibitor (PAI-1).[12] The fibrinolytic activity reflects the relationship between PA and PAI-1 activities; i.e., as more PAI-1 is synthesized, the activity of PA is diminished. Thus, the relative levels of PA and PAI-1 can be determined simultaneously by fibrinolytic autography.

Immunocytochemistry

Chondrocytes were cultured to sub-confluency on glass slides with DMEM containing 10% FBS. They were then cultured under the same conditions as for analysis via fibrinolytic autography, either with or without TGF-β1 (50 ng/ml). After 24 h of cultivation, the chondrocytes were fixed with 70% ethanol for 30 min. The cells were then reacted with H_2O_2 in absolute methanol for 30 min to block endogenous peroxidase activity, followed by treatment with Super Block (ScyTek Laboratories, Inc., Logan, UT, USA) to block non-specific reactivity. Then, the cells were incubated with Rabbit-to-rabbit blocking reagent (ScyTek Laboratories), in accordance with the manufacturer's protocol. Rabbit anti-human collagen type I and II antibodies were supplied by Prof. Katsuo Sueishi, First Department of Pathology, Faculty of Medicine, Kyushu University. After application of primary antibodies overnight at 4 °C, the cells were incubated with rabbit-to-rabbit blocking reagent (ScyTek Laboratories). Then Ultra Tek Polyvalent was applied, followed by incubation with Ultra Tek HRP (ScyTek Laboratories) at room temperature. Immunoreactive proteins were visualized by application of chromogen.

Immunofluorescence

Chondrocytes were cultured under the same conditions as for immunocytochemical examination, either with or without TGF-β1 (50 ng/ml). The cells were permeated and blocked with 0.5% Triton X-100 and 5% bovine serum albumin in phosphate-buffered saline (PBS) for 30 min. The slides were incubated with primary antibody (mouse anti-rabbit αVβ1 integrin antibody; clone IBS5, Chemicon) at 4 °C overnight. This was followed by incubation with fluorescein isothiocyanate-conjugated secondary antibody for 1 h. The cells were then counterstained with Hoechst 33342 in PBS for 5 min, and the slides were mounted with 90% glycerol in PBS.

Cell growth

The incorporation of tritiated thymidine was measured to examine the effect of TGF-β1 on DNA synthesis in chondrocytes. Sub-confluent chondrocytes were incubated with tritiated thymidine (5.0 Ci/mM; Amersham, Arlington Heights, IL, USA) at a concentration of 0.5 μCi/ml in the presence of either TGF-β1 (50 ng/ml) or a TGF-β1-free control for 24 h. Isolated cells were washed and treated with cold 5% trichloroacetic acid. Salt precipitates were washed with ethanol–ether