Expression of angiotensin II receptor-1 (AT1R) in human articular chondrocytes

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INTRODUCTION
The renin-angiotensin-aldosterone system (RAS) is an essential regulator of the fluid and electrolyte balance in the human body, and it thereby controls blood pressure. The RAS system has also been implicated in atherosclerosis and cardiac hypertrophy, in which it is considered to induce cardiovascular system remodeling.

In rheumatoid arthritis (RA), human fibroblast-like synoviocytes were shown to express AT1 receptor (AT1R), and angiotensin II (Ang II) was found to protect synoviocytes from apoptosis, playing a role in synovial expansion in vitro. Furthermore, blocking of the RAS system by Ang II receptor blocker (ARB) improved RA in animal models. The anti-arthritic effect of ARB might be attributable to the suppression of the antigen-specific immune response by ARB, as reported by Sagawa et al., and Iwamoto et al. also speculated that ARB can suppress the expression of monocyte chemoattractant protein (MCP)-1 in rheumatoid synoviocytes.

Despite these findings, the precise role of the RAS system in bone and cartilage is not understood. In this study, we investigated the expression of the angiotensin II receptors in articular chondrocytes from arthritic patients in order to clarify the role of RAS in arthritis.

MATERIALS and METHODS
Human articular chondrocytes were obtained from patients with OA (n = 14; from 12 knee and 2 hip joints), RA (n = 14; from 14 knee joints), and traumatic fractures (n = 13; from 13 hip joints), who underwent joint arthroplasty at St. Marianna University School of Medicine Hospital or Nippon Medical School Hospital. All patients were examined by a certified rheumatologist or orthopedic surgeon and diagnosed on the basis of the criteria developed by the American College of Rheumatology (http://www.rheumatology.org/practice/qmc/criteria_old.asp). The fracture patients had no history of joint disease and thus served as a control. Written informed consent was obtained from all patients, and the protocol was approved by the institution’s ethics committee. The study was performed in compliance with the Declaration of Helsinki proposed by the World Medical Association in 1964. This experiment was approved by the institutional review board and informed consent.

RESULTS

Human articular chondrocytes express angiotensin receptors
We used RT-PCR to investigate the expression of angiotensin receptor mRNA by chondrocytes. The results are shown in Figure 1. As demonstrated, both AT1R and AT2R were expressed in all samples tested, although there was considerable variation in the expression levels among the samples.

Since AT1R is the dominant Ang II receptor, we further confirmed the expression of this receptor at the protein level. The chondrocytes in the cartilage obtained from OA, RA, and fracture patients were stained positively for AT1R (Fig. 2, A,B). No significant pattern was apparent in the distribution of AT1R-expressing chondrocytes within the cartilage.

IL-1 up-regulates AT1R mRNA but not AT1R protein in chondrocytes
To clarify the role of inflammatory mediators in AT1R expression, we stimulated chondrocytes with a representative proinflammatory cytokine, IL-1, and analyzed them for receptor expression.

As shown in Figure 3, IL-1-stimulated chondrocytes tended to exhibit higher expression levels of both AT1R and AT2R mRNA than non-stimulated ones. This result was observed with OA, RA, and control (fracture) samples, but it was most significant with the OA samples (Fig. 3B).

Next, we investigated whether the IL-1 upregulates AT1R expression at the protein level. The western blot analysis, however, showed that the difference in AT1R protein expression between the IL-1-stimulated and non-stimulated chondrocytes was not significant in most samples, although some samples showed that it was slightly greater in the stimulated cells (Fig. 4). We further tested the cell surface expression of AT1R by using flow cytometry. The results confirmed the cell surface expression of AT1R in a subset of chondrocytes (Fig. 4B), but the level of AT1R expressed did not change with IL-1 stimulation.

It is noteworthy that in the western blotting, AT1R was detected as a band at approximately 60 kD when 2 different AT1R-specific Abs were used (data not shown), suggesting a posttranslational modification as reported previously.

DISCUSSION
In conclusion, we demonstrated the expression of angiotensin receptors in human articular chondrocytes. Understanding the involvement of AT1R-mediated signaling in arthritis may open up a new avenue for establishing novel anti-degenerative strategies focusing on the RAS system in articular joints.