Expression of Activin and Activin Receptors in Synovial Tissues in Rheumatoid Arthritis - Role of Activin in Angiogenesis

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Introduction
Synovial proliferation with excessive angiogenesis is observed prior to bone and joint destruction in rheumatoid arthritis (RA). Angiogenesis has been believed to be an essential process for pannus formation, which cause bone and joint destruction in RA, although little evidence for that has been demonstrated. We recently found that an angiogenesis inhibitor suppresses joint inflammation and subsequent bone and joint destruction in adjuvant arthritis (AA), an animal model similar to RA. Various angiogenic factors and their inhibitors may be involved in angiogenesis of RA. Activin, a member of TGF-beta superfamily, has wide range of biological activities, such as regulation of cell differentiation and proliferation. The activin-binding protein, follistatin, which can neutralize the activin, regulates endothelial cell proliferation and induces angiogenesis in vivo, suggesting that activin is an angiogenesis inhibitor. High concentrations of activin is detected in joint fluid in patients with RA. We hypothesized that activin negatively regulates angiogenesis in synovial tissues, thereby inhibiting not only synovial hyperplasia, but also subsequent joint destruction. In this study, we firstly examined the presence of activin and activin in synovial tissue in RA, and activin receptors, and secondary we localized them around the joint tissue in RA and in AA.

Methods
Rat adjuvant arthritis > Male Lewis rats, 8-weeks of age, were received a single intradermal injection of 0.1ml of a 10 mg/ml emulsion of freeze dried Mycobacterium butyricum in mineral oil in the base of the tail. About six to 10 days after injection, arthritis were observed. Six weeks after the injection, the rats were sacrificed and X-ray picture were prepared and the ankles were dissected and fixed in 4% paraformaldehyde for immunohistochemical examination and TRAP staining. Tissues of patients with RA > Synovial tissues and eroded bones obtained were collected from patients with RA undergoing arthroplasty. A part of the collected tissues were immediately frozen in liquid nitrogen for RNA extraction of and the reminding samples were fixed with 4% paraformaldehyde for immunohistochemical examination and TRAP staining.

Synovial tissues with eroded bone were decalcified with 10% EDTA 2Na and 9% EDTA 4Na in phosphate buffer solution. The tissues were dehydrated with graded ethanol and embedded in paraffin. Five micrometer sections were prepared for histological examinations. Immunostaining was conducted using Vectastain ABC kit. Anti activin receptor type I, II, and IIB and activin subunits antibodies and anti-Von Willebrand factor antibody for the detection of endothelial cells were used for immunohistochemical staining.

Results
In all of the synovial tissues examined, mRNAs of activin receptor type I, type II and type IIB were detected by RT-PCR. Immunohistochemically, synovial lining cells and vascular endothelial cells were positive for type I, type II, and type IIB activin receptors, and activin subunits. Multinucleated osteoclasts on eroded surface in destructed bone were not only positive for TRAP staining but also positive for type II activin receptor. In ankle joint of AA rat, the cells on bone destruction front were also positive for type II activin receptor.

Discussion
Although the concentrations of activin in joint fluid is reported to be increased in patients with RA, the source of the activin present in arthritic joint has not been known. In the present study, we demonstrated that activin beta subunit and activin receptor are present in synovial tissues in patients with RA at mRNA and protein level. Co-localization of the beta A subunit and the activin receptor type I and type II in vascular endothelial cells in the synovial tissues suggests that activin exerts the biological effects through the type I and type II receptor complex in an autocrine/paracrine fashion in articular joints.

Activin is induced by bone-resorbing cytokines such as IL-1 and TNF-alpha. Interestingly, the activin can antagonize the other bone-resorbing cytokines, IL-6 and IL-11, thereby potentially inhibiting bone resorption. The activin induced in arthritic joint might negatively regulate the enhanced bone resorption. Activin stimulates bone formation in vivo and in vitro. We previously reported that activin receptors are abundantly expressed on osteoblasts at site of bone formation. Activin might protect the articular joint from destruction by stimulating bone formation as well as by regulating bone resorption.

Activin-binding protein, follistatin, is induced by FGF, one of an angiogenic factor, which is highly present in synovial tissues in RA. Excessive expression of activin might overcome the follistatin activity regarding angiogenesis. In the present study, we found that the presence of activin receptors in vascular endothelial cells and synovial lining cells in articular joints, which prompts us to examine the effect of administered activin on synovial inflammation and bone and joint destruction in an animal model of RA.

Table 1. List of primers:

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<thead>
<tr>
<th>mRNA segment</th>
<th>Sequence of primers</th>
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<tr>
<td>activin receptor type I and II</td>
<td>Forward primer: 131-148 (5'-GTG CTT ATC ATG ATT GCT) Reverse primer: 526-509 (5'-CAG GCA GCC TAA AAG ACA)</td>
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<tr>
<td>activin receptor type II</td>
<td>Forward primer: 402-418 (5'-GGT TGT TGG CTG GAT GA) Reverse primer: 832-813 (5'-ATT TTG AGC GCC ACA TAT TC)</td>
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References