INTRODUCTION
With the aging of the society, osteoarthritis (OA) has become a most common joint disease. OA is characterized by a progressive loss of cartilage. However, recent studies have demonstrated vigorous anabolism in OA cartilage, that is considered a reparative response of the chondrocytes. Since progression of the disease is determined by the balance of catabolism and anabolism, anabolism in OA has profound significance when the treatment of the disease is considered. In spite of this, only limited knowledge is currently available on OA hyperanabolism. Our preliminary study found that BMP-2 is abundantly expressed in OA cartilage. Since BMP-2 is known to have anabolic actions on articular chondrocytes, we hypothesized that BMP-2 is involved in enhanced anabolism in OA. The purpose of this study is to examine this hypothesis, together with the mechanism of BMP-2 induction in OA.

METHODS
Cartilages
Human OA cartilage was obtained from 42 knees in 40 patients (2, bilateral; age, 48-84 years) who underwent total knee replacement surgery for primary OA. Control cartilage was obtained from eight age-matched donors at the time of autopsy within 24 hours of death (age, 56-78 years).

Immunohistochemistry
BMP-2 in cartilage was detected using anti-human BMP-2 goat polyclonal antibody (Santa Cruz). Colocalization between type II procollagen C-propeptide (CPII) and BMP-2 was observed in OA chondrocytes by confocal microscopy. Cells and tissue culture
Chondrocytes were isolated from cartilage by serial digestion with Pronase (Calbiochem) and collagenase P (Rosche), and plated at the density of 2 x 10^6 cells per dish in culture, DMEM/F-12 1:1 Mixture containing 10% fetal bovine serum (Rosche), and plated at the density of 2 x 10^6 cells per dish in culture media with daily supplement of 25 µg/ml ascorbic acid. Quantitative RT-PCR
Relative amount of mRNA was determined by quantitative RT-PCR using an ABI Geneamp 5700 sequence detection system and SYBR green buffer (Perkin-Elmer), and BMP-2 was observed in OA cartilage layer, were positively stained for both antigens, thus further confirming that considerable number of OA chondrocytes, particularly the cells in the middle cartilage layer, were positively stained for both antigens, thus further confirming the involvement of BMP-2 in OA hyperanabolism.

DISCUSSION
Although accumulating evidence demonstrates enhanced matrix synthesis in OA cartilage, the mechanism for the up-regulation remains largely unknown. Some growth factors such as TGF-β or IGF-1 are assumed to enhance matrix synthesis, but several other observations suggest they have only limited roles in OA hyperanabolism. The results of this study have shown that BMP-2 could enhance matrix synthesis in OA cartilage, likely after induction by proinflammatory cytokines IL-1β and TNF-α. Thus, this study has shown a possibility that proinflammatory cytokines IL-1β and TNF-α can induce both catabolism and anabolism in OA cartilage, probably depending upon conditions including concentration in cartilage matrix. A novel mechanism shown here could be the target for development of disease modifying pharmaceutical agents in the future.

RESULTS
Expression of BMP-2 in OA cartilage
In control cartilage obtained from normal joints, weak to moderate immunostaining was observed in the middle cartilage layer. In OA cartilage, however, OA cartilage showed intense staining for BMP-2. Most cells in the middle to deep cartilage layers were positively stained where the layers were preserved, and often the staining was observed in the cartilage matrix surrounding the lacunae, suggesting abundant BMP-2 secretion.

Response of normal and OA chondrocytes to BMP-2
To determine the cellular response to BMP-2, clutured chondrocytes were treated with the graded concentrations of BMP-2, and expression of type II procollagen and aggrecan genes, and incorporation of [$\text{S}$]sulfate were determined. The results confirmed that BMP-2 has anabolic actions on articular chondrocytes, though the response with OA cells was less robust than that with normal cartilage.

The effect of growth factors and cytokines on the expression of BMP-2, BMP receptors, and antagonists
To investigate the mechanism of BMP-2 up-regulation in OA cartilage, chondrocytes were treated with relevant growth factors and cytokines in OA joints, and the expression of BMP-2 mRNA was evaluated. While TGF-β1 or IGF-1 did not alter the level of BMP-2 expression, proinflammatory cytokines IL-1β and TNF-α both strongly induce BMP-2 (Fig. 1). Western blot analysis using monoclonal antibody for human BMP-2 (a gift from Genentech Institute) revealed that the secreted BMP-2 was largely in an active form. Subsequent study showed that expression of BMP-receptors or a BMP antagonist chordin was not influenced by IL-1β or TNF-α, and therefore chondrocytes are competent to respond to BMP-2 even in the presence of these cytokines.

Time course and mechanism of BMP-2 induction
A time course study showed that BMP-2 expression reaches a plateau at around 9 hours after the addition of IL-1β or TNF-α. The up-regulation of BMP-2 expression was blocked by PDTC, a specific inhibitor for NF-κB, therefore the pathway involving NF-κB was considered to be the major mechanism for gene enhancement. The experiments with an RNA polymerase II inhibitor DRB demonstrated that both cytokines prolonged half-life of mRNA, suggesting that the enhanced gene induction resulted from increased stability of BMP-2 gene transcripts. The induction of BMP-2 mRNA was markedly enhanced by a protein synthesis inhibitor cycloheximide, indicating that protein synthesis was required for intracellular degradation of BMP-2 mRNA.

**Functional role of BMP-2 in OA cartilage**
The significance of BMP-2 in OA cartilage was evaluated in explant culture before and after TNF-α stimulation. When assessed immediately after preapration, noggin suppressed proteoglycan synthesis more than 20% (Fig. 2a, b), suggesting significant contribution of BMP-2 to cartilage anabolism in vivo. While the control explants did not show significant change up to 3 days (Fig. 2b), strong suppressive effect of noggin became greater in the explants treated with TNF-α (Fig. 2a). Immunohistochemistry was done on the explants, and strong induction of BMP-2 in TNF-α treated explants was confirmed. Autoradiography of TNF-α treated explants also confirmed suppressive effect of noggin on the incorporation of [$\text{S}$]sulfate. Lastly, the expression of BMP-2 in anabolic chondrocytes was investigated in OA cartilage. For this, colocalization between BMP-2 and CPII antigen was studied, because CPII is a reliable marker for type II collagen neo-synthesis. The result showed that considerable number of OA chondrocytes, particularly the cells in the middle cartilage layer, were positively stained for both antigens, thus further confirming the involvement of BMP-2 in OA hyperanabolism.

**Poster #0334***

IL-1β AND TNF-α STIMULATE REPAIR PROCESSES IN OSTEOARTHRITIC CARTILAGE THROUGH INDUCTION OF BMP-2

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