DECREASE OF CHONDROMODULIN-I (ChM-I), CARTILAGE SPECIFIC VASCULAR ENDOTHELIAL GROWTH INHIBITOR, ACCELERATES CARTILAGE THINNING WITH THE VASCULAR INVASION IN RAT OSTEOARTHRITIC CARTILAGE

Introduction
Cartilage, especially articular cartilage (AC), is unique among the tissues of mesenchymal origin since it is avascular and extraordinarily resistant to vascular invasion. The mechanism preserving the AC as an avascular tissue is largely unclear. Many previous reports showed that vascular invasion, at least in certain cases, plays a key role in the progression of the cartilage degradation in osteoarthritic (OA) cartilage (1). To understand the pathogenesis of OA, it is important to examine the mechanisms related to how normal AC persists in preserving hyaline cartilage throughout life and how vascular invasion occurs in the AC in OA. The decrease of chondromodulin-I (ChM-I), cartilage derived angiogenesis inhibitory factor, protein in late hypertrophic zone permit vascular invasion, subsequently occurs endochondral bone formation in embryonic epiphysial cartilage(2).

The aim of this study was to examine whether ChM-I contributes to the maintenance of the avascular condition of the AC even in postnatal life, and whether ChM-I protein decreases or not in the OA cartilage that is accompanied by vascular invasion.

Materials and Methods
Animal care and experimental procedures were conducted in accordance with institutional guidelines. <normal AC> Male Sprague-Dawley rats aged 15 days, 4, 10, and 36 weeks old were examined to evaluate the developmental changes of normal AC. <Osteoarthritis AC> Surgically-induced OA knee joints were resected on day 5 and 2, 6 and 10 weeks after surgery. Normal and OA cartilage specimens were examined by histological analysis, immunohistochemistry, and in situ hybridization (3). Immunohistochemical staining for rat ChM-I, antirat-type II collagen and antirat CD31 (for vascular endothelial cells) were performed as previously described (4). Immunohistochemical double staining for antirat CD31 and ChM-I were performed to evaluate the distribution of ChM-I protein and the vascular invasion. In situ hybridization (ISH) was performed with digoxigenin-labeled rat ChM-I, a1(II) collagen, and type I collagen riboprobes. Toluidine blue-O (TB) staining was used to estimate the content of proteoglycans (PGs). Western blot analysis was performed to compare the amount of ChM-I extracted from OA and control cartilage 2 weeks after the surgery. To examine the difference in the amount of ChM-I mRNA expression between young (4-week-old) and adult (36-week-old) rat, total RNA extracted from AC was subjected ribonuclease (RNase) protection assay. RNase protection assay was performed as reported previously with 32P-labeled antisense cRNA probes for ChM-I mRNA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA.

Results
<normal AC> The level of ChM-I mRNA was significantly lower in the AC of adult rat compared to young. In contrast, ChM-I protein was detected both in young and adult cartilage extracellular matrix. ChM-I mRNA and protein were expressed in the avascular region of the AC, especially from the middle to deep zone. <OA model> On the 5th day after the surgery, AC showed normal appearance with much ChM-I protein in the matrix. During the 2nd week after surgery, ChM-I protein decreased in the extracellular matrix of the superficial zone, while much PGs and type II collagen was detected. ChM-I protein was significantly decreased during the 2 week after the surgery by western blot analysis (Fig 1). During the 6th week after surgery, ChM-I immunostaining was significantly reduced in the extracellular matrix of all zones of the OA cartilage. In contrast, intense type II collagen staining and much PGs was observed in all zones of AC. In advanced OA, during the 10th week after surgery, extensive degeneration of the AC was detected. The surface of the AC was severely eroded and PGs content was significantly decreased. ChM-I protein could be detected only in cytoplasm and surface of the chondrocytes, not in the extracellular matrix, immediately adjacent to the margin of eroded surfaces. These chondrocytes expressed ChM-I mRNA. OA cartilage with lower content of PGs became thinner with the vascular invasion from the subchondral space (Fig 2). Immunohistochemical double staining showed that vascular invasion into OA cartilage was observed where ChM-I protein had reduced extracellular matrix.

Discussion
AC, under the healthy condition, persists as an avascular tissue throughout postnatal life. ChM-I protein is detected in the avascular zone of AC throughout postnatal life, even in adults, suggesting that ChM-I plays an important role as an angiinhibitory factor even in adult. On the other hand, ChM-I mRNA significantly decreases in AC with aging. Taken together, the function of ChM-I of stimulating chondrocyte proliferation, the significant decrease of ChM-I mRNA in adults is one of the reasons why adult AC has a remarkably poor regenerative capacity. In OA, ChM-I protein was decreased significantly in the superficial zone prior to the decline of PGs and type II collagen in the extracellular matrix. This finding suggests that the decline of ChM-I protein is an early event of cartilage degeneration. In OA, many previous reports have indicated that early changes can be observed in the superficial zone of AC. Once the superficial layer is deteriorated, PGs and other molecules withdraw from the extracellular matrix. At that time, hydrophilic mature ChM-I is easily released from the interterritorial matrix of AC. In advanced OA, withdrawal of ChM-I from entire zone of AC changes the chondrocytic switching from antiangiogenic to an angiogenic state in cartilage. The decline of ChM-I allows the vascular invasion which triggers the cartilage thinning by the reinitiation of endochondral bone formation during the progression of OA change. In conclusion, ChM-I may play an important role in the maintenance of AC as an avascular hyaline cartilage even in adults. A decrease of ChM-I protein in the extracellular matrix may be involved in the pathogenesis of cartilage degeneration.

Fig 1 ChM-I protein was decreased in OA cartilage

Fig.2 Vascular invasion into OA cartilage ChM-I could be detected only in the cytoplasm of chondrocytes not in the matrix. Vascular invasion could be detected where the ChM-I protein decreased region (arrowhead).

References
(1) Hoyland J.A. Bone and Mineral 15 151-163 1991
(3) Hatano H et al. J Path 185 204-211 1998
(4) Yamagiwa H et al Bone (in press)

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