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BREAKTHROUGHS AND VIEWS

Cartilage-Derived Growth Factor and Antitumor Factor: Past, Present, and Future Studies

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The process replacing cartilage by bone is known as endochondral bone formation. During the initial stage of this process, epiphyseal cartilage cells proliferate rapidly, becoming enlarged and hypertrophic, and increase matrix synthesis, such as cartilage-specific proteoglycans and type II, IX, X, and XI collagens. During this series of events, the metabolism of cartilage is regulated by various hormones and locally generated modulators. In 1972, Nevo and Dorfman (1) first reported that exogenous proteoglycans extracted from bovine cartilage stimulate proteoglycan biosynthesis by cultured chondrocytes. Since then many investigators have tried to identify other regulatory factors in cartilage. Klagsbrun et al. (2) purified a potent growth factor from neonatal bovine cartilage and named it cartilage-derived growth factor (CDGF). However, their CDGF was later found to be identical with the ubiquitous fibroblast growth factor-2, FGF-2 (3). Therefore, cartilage contains intrinsic FGF-2 and this factor has been suggested to be important during chondrocyte growth. In 1980-81, we found some somatomedin-like factors in fetal bovine cartilage, which we named cartilage-derived factor (CDF) (4-6). In the meantime, TGF-β and other members of its family, such as BMP (7), CDMP-1 and CDMP-2 (8), were identified in cartilage and shown to be active in chondrocyte differentiation. Then, we succeeded in identifying the structures of unique cartilage-generated modulators of both chondrogenesis and subsequent osteogenesis, and named these chondromodulin-I (ChM-I), -II (ChM-II), and -III (ChM-III) (9-11).

On the other hand, cartilage is normally avascular and resistant to invasion by both vascular endothelial cells and neoplastic cells. Because of these characteristics, Kuettner's group (12-14) and Folkman's group (15,16) tried to purify factors from cartilage that inhibit proliferation of endothelial cells *in vitro* or tumor-induced angiogenesis *in vivo.* We also reported that a high molecular weight fraction extracted from fetal bovine cartilage inhibited the growth of solid tumors and tumor-induced angiogenesis in the chick embryo chorioallantoic membrane: we named this factor cartilage-derived anti-tumor factor (CATF) (17,18). These findings show that CATF has anti-angiogenic activity, thereby inhibiting the growth of solid tumors. Then, the Harvard group partially purified a cartilage-derived collagenase inhibitor (CDI) from cartilage (19) and a cartilage-derived angiogenesis inhibitor (ChDI) from the conditioned medium of bovine scapular cartilage (20). However, we re-examined purification of old CATF preparations and found that the N-terminal amino acid sequence of the active anti-angiogenic fraction agrees with that of the ChM-I sequence (11).

CARTILAGE-DERIVED GROWTH FACTOR

Table I presents a chronological scheme of the development of our understanding of the details of cartilage-derived growth factors. Chondrocytes are highly specialized for the syntheses and secretions of cartilage matrix, chondromucoprotein (proteoglycan) and collagen. In 1975, we showed that growth-cartilage cells isolated from the ribs of rats formed differentiated colonies producing cartilage-like tissue *in vitro* (21). Furthermore, the synthesis of sulfated glycosaminoglycans by growth-cartilage cells was markedly stimulated by parathyroid hormone, calcitonin, and multiplication-stimulating activity (22,23).

Functional matrix. Besides these hormones, extracellular matrix components have long been suggested to play important roles in the control of proteoglycan synthesis. Nevo and Dorfman (1) showed that exogenous chondromucoprotein extracted from bovine nasal cartilage stimulates proteoglycan synthesis by chick embryo chondrocytes in suspension culture. Kosher et al. (24) demonstrated that extracellular proteoglycan aggregates, obtained from embryonic chick cartilage, can stimulate chondrogenic expression by chick somite

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TABLE I

Chronological Times of Results on Cartilage-Derived Growth Factor

		References
1972	Chondromucoproteins extracted from bovine nasal cartilage stimulate proteoglycan synthesis by cultured chondrocytes.	1
1973	Extracellular proteoglycan aggregates from embryonic chick cartilage stimulate chondrogenic expression by chick somite explants.	24
1973	Medium conditioned by exposure to chick embryo chondrocytes stimulates the synthesis of sulfated glycosaminoglycans by the same cell type.	25
1977	Neonatal bovine cartilage contains a potent growth factor (cartilage-derived growth factor: CDGF).	2
1980	Purified CDGF stimulates growth of chondrocytes.	26
1980	Fetal bovine cartilage contains somatomedin-like growth factors (cartilage-derived factor: CDF).	4-6
1983, 1986	CDF has synergistic effects with FGF in stimulating DNA synthesis in cultured chondrocytes	29-31
1986	CDGF is identical to the ubiquitous FGF-2.	3
1991	A new class of cartilage-specific matrix, chondromodulin-I (ChM-I) stimulates growth of cultured chondrocytes in the presence of FGF.	9
1996	Another growth-promoting component, chondromodulin-II (ChM-II), is homologous to MIM-1.	10
	Chondromodulin-III (ChM-III) was purified from cartilage and found to be identical with ribosomal protein L31.	10
1997	ChM-I stimulates colony formation of chondrocytes in agarose culture.	32
	ChM-I and ChM-II stimulate osteoblast proliferation.	36
	ChM-I is localized in the avascular zone of epiphyseal cartilage and inhibits proliferation of vascular endothelial cells as well as tube formation.	11, 42
1998	FGF-2, TGF-β, and PTHrP down-regulate the expression of ChM-I mRNA in chondrocytes.	33

explants. In addition, Solursh and Meier (25) found that medium conditioned by chick embryo chondrocytes stimulates the syntheses of sulfated glycosaminoglycans and collagen by the same cell type. Therefore, factors produced by chondrocytes or reserved in the cartilage matrix may regulate the synthesis of their own extracellular matrix by a mechanism of positive feedback. Then, we tried to isolate and characterize these active factors from fetal bovine cartilage. A protease-sensitive factor was extracted from fetal bovine cartilage with 1 M guanidine-hydrochloride and partially purified. This cartilage-derived factor (CDF) stimulated proteoglycan synthesis and DNA synthesis in a dose-dependent manner in rat and rabbit costal chondrocytes in culture (26). These findings suggest that CDF is involved in expression of the differentiated chondrocytes.

FGF. Klagsbrun et al. (2, 26) partially purified a cartilage-derived growth factor (CDGF) from neonatal bovine scapula cartilage and found that it stimulates DNA synthesis and cell division of bovine chondrocytes. However, their CDGF was later found to be identical to the ubiquitous growth factor, FGF-2 (3), which is known as the most potent angiogenic factor. Therefore, cartilage contains intrinsic FGF-2 and this factor has been suggested to be important for chondrocyte growth. In contrast, we found that 11 kDa CDF and 16 kDa CDF increase not only sulfation of glycosaminoglycans but also proliferation of rabbit chondrocytes in culture (4-6, 27, 28). These findings indicate that fetal bovine cartilage contains factors that show somatomedin-like activity in monolayer cultures of rabbit chondrocytes. Then, we found that CDF has synergistic effects with FGF or EGF in stimulating DNA synthesis in chondrocytes (29-31). The mode of the combined action of CDF with FGF or EGF in chondrocytes was studied by sequential treatments with these factors. Both FGF and EGF had synergistic effects with CDF in stimulating DNA synthesis, even when added 10 hr after exposure to CDF. Synergism was also observed in cells pretreated with CDF. These cultures were treated for 5 hr with CDF and then washed and treated with FGF or EGF (31). However, when CDF was added more than 5 hr after FGF or EGF, no synergism of effects was observed. These findings suggest that the cultured chondrocytes became activated to respond synergistically with the ubiquitous growth factors FGF and EGF for commitment to DNA synthesis when they were exposed to CDF at an early stage. Therefore, the designation "chondromodulin" was proposed to supplant the older term "cartilage-derived factor (CDF)". This term reflects the modulation of chondrocytes to respond synergistically to these ubiquitous growth factors.

Chondromodulin-I (ChM-I). As described earlier, FGF-2 in combination with some unique growth-modulating factors in cartilage may act on chondrocytes. We, therefore, searched for such factors in cartilage. In the course of the initial screening of growth-promoting components in extracts of fetal bovine cartilage, we found at least two distinct factors in terms of their affinity for heparin: one eluted from a heparin-Sepharose column with buffer containing 0.5 M NaCl and the other eluted with buffer containing 1.2

M NaCl. Finally we purified these active components that function in growth modulation of differentiated chondrocytes (9). The first component identified was a novel 25 kDa glycoprotein expressed specifically in cartilage that stimulated the proliferation of chondrocytes. In the presence of FGF-2 (0.4 ng/ml), this factor synergistically stimulated DNA synthesis 30-fold over the control. Its activity was detectable at 1 ng/ml and was maximal at about 200 ng/ml. Furthermore, it stimulated the synthesis of cartilage-proteoglycans by chondrocytes. We renamed this unique principle "chondromodulin-I (ChM-I) (9), because it modulated the growth of cultured chondrocytes, and cartilage was presumed to contain some additional modulators. ChM-I also stimulated colony formation of chondrocytes cultured in soft agar (32). ChM-I alone stimulated the formation of chondrocyte colonies weakly, but caused marked stimulation of colony formation in the presence of FGF-2. Therefore, ChM-I participates in an autocrine signaling mechanism for anchorage-independent growth of chondrocytes *in vitro* (32).

Next, we cloned cDNA of ChM-I. Mature ChM-I consists of 121 amino acids and is coded as the C-terminal part of a larger precursor which has 335 amino acids. A processing signal, RERR, is located just before the mature ChM-I sequence. Glutamic acid is the only N-terminal residue. Two O-glycosylation sites (Thr^{9} and Thr^{22}) and one N-glycosylation site (Asn^{30}) are present in the mature ChM-I sequence. The ChM-I precursor contains a single hydrophobic region and a stretch of 27 amino acid residues from Gly^{43} to Ile^{59} near its N-terminal. This region is presumed to be involved in membrane insertion. We speculate that mature ChM-I is secreted after processing of the precursor at the preceding processing signal and that the N-terminal two-thirds of the molecule reside on the cell surface of chondrocytes. Sequence similarity analysis indicated that the N-terminal two-thirds of the ChM-I precursor have significant similarity with human and rat pulmonary surfactant apoprotein C, which is specifically expressed in alveolar type II cells and contains a transmembrane domain in the N-terminal region. This sequence similarity leads us to speculate that the N-terminal part of the ChM-I precursor may also contribute to functional regulations of cartilage cells, such as possible mediation of the growth-promoting action of ChM-I. We termed this putative membrane protein "chondrosurfactant protein (Ch-SP)". Then, we expressed the ChM-I in COS cells and found that the expressed product recovered from the supernatant of COS cells gives a similar diffuse band corresponding to that of authentic ChM-I on western blot analysis. This means that the expressed product is secreted after glycosylation in a similar manner to authentic ChM-I and then processing of the precursor protein at the preceding processing signal. Ch-SP remains on the cell surface.

A single ChM-I mRNA of 1.7 kb was identified in polyadenylated bovine epiphyseal cartilage RNA. Interestingly, northern blot analysis of total RNA from various bovine tissues indicated that expression of ChM-I mRNA is highly specific to cartilage.

Next, we examined the immunolocalization of mature ChM-I and the *in situ* hybridization of ChM-I mRNA using caudal vertebral growth-plates of bovine tail. Results indicated that ChM-I protein and its mRNA are localized only in avascular zones of cartilage; i.e. the resting, proliferating, and early hypertrophic zones, and are not detectable in the outer surface of cartilage or in the late hypertrophic and calcifying zones which allow vascular invasion during endochondral bone formation (11). Immunohistochemical staining also showed that ChM-I is secreted from chondrocytes and accumulated in the inter-territorial matrix of cartilage (11). In addition, no transcript of the ChM-I gene was detected in undifferentiated ATDC5 mouse chondrogenic cells, but ChM-I transcripts were readily detectable in differentiated cultures of ATDC5 cells. ChM-I mRNA was also expressed in cultured rabbit chondrocytes, and resting cartilage of rabbit ribs, but not in osteoblastic MC3T3-E1 cells.

As described before, cartilage produces FGF-2, a very potent angiogenic factor, although cartilage is an avascular tissue that is rarely invaded by neoplasms. To study puzzling problem, we examined the immunolocalization of FGF-2 and confirmed that it was expressed not only in cartilage, but also in other connective tissues. But in contrast to ChM-I, the staining of FGF-2 was stronger in the calcified cartilage zone and in bone. Thus, ChM-I may counteract FGF-2 function.

Shukunami and Hiraki (33) isolated rabbit ChM-I precursor cDNA by RT-PCR. The deduced amino acid sequence of rabbit mature ChM-I was compared with that of its human counterpart. One amino acid deletion and 12 amino acid substitutions were found near the N-glycosylation site (Asn^{29}), although the glycosylation consensus sequence was still valid in the rabbit sequence (33). Northern blot analysis revealed that the expression of ChM-I mRNA occurred specifically in cartilage. Moreover, BMP-2 stimulated the expression of the differentiated phenotype of primary cultured chondrocytes (7) and up-regulated type II collagen mRNA in chondrocytes (33). This result was well compatible with the chondrogenic activity of BMP-2 (34). However, BMP-2 or activin only marginally affected the level of ChM-I mRNA in chondrocytes (33). In contrast, FGF-2, TGF-β_2, and PTHrP each markedly down-regulated the expression of ChM-I mRNA in chondrocytes (33). These results indicate that the expression of ChM-I is controlled by local growth and differentiation factors. A higher level of ChM-I expression was maintained in mature chondrocytes, and was markedly down-regulated on shift of the metabolic

state of chondrocytes from maturing to proliferating cells by growth stimulation with FGF-2 and/or TGF-β.

Chondromodulin-II (ChM-II). We purified another cartilage-derived modulator with a lower affinity to heparin (0.5% NaCl eluate) to homogeneity and named it chodromodulin-II (ChM-II) (10). Final purification was achieved by reversed phase HPLC. On SDS-PAGE, the active fraction gave a single band of 16 kDa. We determined the complete amino acid sequence of ChM-II and found that it consists of 133 amino acids, contains one tryptophan residue near the N-terminus, has six cysteine residues, and is rich in lysine residues. Screening of a data base indicated that ChM-II has a novel amino acid sequence. However, it has a sequence 57% identical to the repeat 1 and repeat 2 of the product of the chicken promyelocyte-specific *mim*-1 gene. The positions of six half-cystine residues in ChM-II were all conserved in these MIM-1 proteins. *mim*-1 cDNA was first reported by Ness et al. (35). The product of the chicken promyelocyte-specific *mim*-1 gene is directly transactivated by the v-Myb oncogene product. The MIM-1 protein is stored in the α-granules of normal immature granulocytes and expressed as 35 kDa and 16 kDa forms. However, its function is not yet known. Like ChM-I, purified ChM-II stimulated both DNA synthesis and proteoglycan synthesis of cultured rabbit chondrocytes dose-dependently. In the presence of FGF-2, ChM-II also synergistically stimulated DNA synthesis of chondrocytes. However, ChM-II did not inhibit DNA synthesis of cultured vascular endothelial cells of bovine carotid artery, whereas ChM-I did. Interestingly, ChM-II stimulated the differentiation of osteoclasts. The effects of ChM-II on osteoclast differentiation were evaluated using an unfractionated bone cell culture system containing mature osteoclasts from the femur and tibia of newborn mice. When mouse bone cells were cultured on dentin slices, ChM-II increased the total area and the number of resorption pits dose-dependently. Osteoclasts were represented as the number of tartarate-resistant acid phosphatase (TRAP)-positive multinucleated cells per unit area. ChM-II also dose-dependently increased the number of TRAP-positive multinucleated cells in the same concentration range. In these systems, an activated vitamin D_3 was not necessary to induce osteoclast formation. Furthermore, ChM-I and ChM-II stimulated the proliferation of clonal mouse osteoblastic MC3T3-E1 cells as well as primary mouse osteoblasts in culture (36). These findings suggest that epiphyseal growth plate cartilage may be functionally involved in the active proliferation of osteoblasts at the osteochondral junction, and hence in the longitudinal growth of long bones by sending a specific growth-promoting signal. Therefore, the cartilage-derived matricrine factors ChM-I and ChM-II are both chondormodulin/osteopoietin, and are the first characterized naturally generated, osteopoietic coupling factors of cartilage-bone growth in endochondral bone formation.

Chondromodulin-III (ChM-III). We also found a minor component with higher affinity for heparin, chondromodulin-III (ChM-III), in cartilage extracts. ChM-III stimulated DNA synthesis in chondrocytes, and its N-terminal sequence was identical with that of ribosomal protein L31 but lacked the three N-terminal amino acids (10). These findings suggest that the growth and differentiation of chondrocytes are regulated by multiple components, such as FGF-2, ChM-I, ChM-II, and ChM-III, in the cartilage matrix. Therefore, the above findings indicate that these various functional components participate sequentially in cellular growth and differentiation in endochondral bone formation in autocrine and matricrine fashions and also in punctually and spatially regulated manners.

CARTILAGE-DERIVED ANTITUMOR FACTOR

Table II shows the chronological order of progress in research on the cartilage-derived anti-tumor factor since 1973. Kuettner's group (12) first demonstrated that cartilage is avascular and resistant to invasion by neoplasms or inflammatory processes. After extraction with guanidine-hydrochloride, the tissue loses this resistance (13). These facts have led some investigators to try to extract and purify factors from cartilage that inhibit neovascularization and tumor invasion. Folkman's group (15, 16) partially purified angiogenesis inhibitors from cartilage and showed their inhibitory activity on tumor growth. Kuettner's group (14) then reported extractable protease inhibitors from bovine cartilage which inhibited tumor growth in mice. At that time, we were trying to purify cartilage-derived growth factors from fetal bovine cartilage, and used the rest of the material for study of anti-angiogenesis factors.

Cartilage-derived anti-tumor factor (CATF). Fetal calves were homogenized and the homogenate was extracted with 1 M guanidine-hydrochloride. The supernatant was fractionated with acetone. The material precipitated with 45-65% acetone was filtered through an Amicon XM300 filter, which excludes molecules of more than 3×10^5 daltons. The filtrate was concentrated on a Toyo UP20 filter, which excludes molecules of less than 2×10^4 daltons and the concentrate (UP20-XM300) was dialyzed against distilled water, and lyophilized. First, we examined the effects of the acetone fraction (45-65%) and UP20-XM300 fraction on the growth of solid sarcoma 180 inoculated subcutaneously into the loins of ICR/CRJ mice. In control mice, the tumors grew slowly in the first week, and then rapidly. When 2 mg of the acetone fraction (45-65%) was injected twice in the first week, the growth of the tumor was not inhibited. However, when 2 mg of the UP20-

TABLE II

Chronological Times of Results on Cartilage-Derived Antitumor Factor

		References
1973	Cartilage is avascular and resistant to invasion by neoplasms.	12
1975	After extraction with guanidine-HCl, cartilage loses this resistance.	13
1976–1980	Angiogenesis inhibitors extracted from cartilage inhibit tumor growth.	15, 16
1977	Protease inhibitors extracted from cartilage inhibit tumor growth.	14
1984	High molecular weight fractions extracted from fetal bovine cartilage inhibit the growth of solid sarcoma-180 (cartilage-derived anti-tumor factor: CATF).	17
1988	Partially purified CATF inhibits the proliferation of endothelial cells, the growth of B16 melanoma and B16-melanoma-induced angiogenesis.	18
1990	A clonal human chondrosarcoma cell line produces an anti-angiogenic antitumor factor.	39
	Cartilage-derived collagenase inhibitor (CDI) is an angiogenesis inhibitor.	19
1992	The N-terminal amino acid sequence of cartilage-derived angiogenesis inhibitor (ChDI) purified from the conditioned medium of bovine scapular cartilage is identical with that of the tissue inhibitor of metalloproteinase-1 (TIMP-1).	20
1995	The N-terminal amino acid sequence of the anti-angiogenic antitumor factor is identical with that of TIMP-2.	41
1997	The N-terminal amino acid sequence of endothelial cell growth inhibitor extracted from fetal bovine cartilage indicates that the inhibitor is identical with chondromodulin-I (ChM-I).	11
	ChM-I is localized in the avascular zone of epiphyseal cartilage.	11
	Recombinant ChM-I stimulates DNA synthesis and proteoglycan synthesis of chondrocytes, but inhibits proliferation of vascular endothelial cells as well as tube formation.	11, 42

XM300 fraction was injected twice in the first week, tumor growth was inhibited 60.7% and 56% after 3 and 5 weeks, respectively (17). The effect of this fraction was striking, because solid tumors shrank and stopped proliferating in 43% of the treated mice after 3 and 5 weeks. Therefore, we named this fraction cartilage-derived anti-tumor factor (CATF). In contrast, when mice were given an intraperitoneal inoculation of sarcoma 180 cells followed by intraperitoneal injection of the same doses of CATF, no difference was observed in the survival times of treated and control animals (17). Fifty percent of the mice died 17 days after tumor inoculation in both the control group and the CATF-treated group.

When CATF was added to sarcoma 180 cultures at a concentration of 200 μg/ml, it did not significantly inhibit the proliferation of the cells. This indicates that inhibition by CATF of the growth of solid sarcoma 180 is not due to its direct effect on sarcoma 180 cells. We also showed that CATF inhibits the proliferation and DNA synthesis of bovine pulmonary artery endothelial (BPAE) cells in culture (37). Next, CATF was further purified by DEAE-Sepharose CL-6B chromatography. The main peak with activity on DNA synthesis in BPAE cells inhibited the growth of B16 melanoma transplanted into the paws of C57BL/6N mice and B16 melanoma-induced angiogenesis in chick embryo chorioallantoic membranes (18). These findings suggest that CATF is an anionic macromolecule and has anti-angiogenic activity, thereby inhibiting the growth of solid tumors. Furthermore, we showed that a clonal human chondrosarcoma cell line (HCS-2/8) (38) produces an anti-angiogenic antitumor factor (39).

Cartilage-derived inhibitor (CDI) and chondrocyte-derived angiogenesis inhibitor (ChDI). Moses et al. (19) demonstrated that cartilage contains a potent inhibitor of neovascularization *in vivo* by chick embryo chorioallantoic membrane assay and rabbit corneal pocket assay. They named this inhibitor cartilage-derived inhibitor (CDI). The sequence of 28 amino acids in the N-terminal part of CDI is identical with that of the tissue inhibitor of metalloproteinase-1 (TIMP-1). Then, Moses et al. (20) reported that conditioned medium of bovine scapular chondrocytes produces an anti-angiogenic factor (chondrocyte-derived angiogenesis inhibitor: ChDI) which shows the same biological activities as CDI. On the other hand, we previously reported that TIMP-1 strongly inhibited angiogenesis in chick yolk sac membranes induced by spermine (40) and showed that the N-terminal 11 amino acid sequence of the angiogenesis inhibitor from culture medium conditioned by a human chondrosarcoma-derived cell line, HCS-2/8, is identical with that of TIMP-2 (41).

ChM-I makes a comeback at last. However, in the late 1990s, a major breakthrough occurred in the field of cartilage-derived growth factors and anti-tumor factors. As reported earlier (17, 18), CATF inhibited angiogenesis and the growth of solid tumors. We then re-examined the anti-endothelial cell growth activity in CATF preparations and found that the inhibitory activity is adsorbed to a heparin column and recovered in the 0.5-1.2 M NaCl eluate. When this fraction was characterized by gel filtration, its apparent molecular weight was decreased to 10-30 kDa. Interestingly, amino acid sequence analysis of the N-terminal part of the active fraction showed that it is identical to that of

the ChM-I sequence (11). We then found that purified and recombinant ChM-I itself inhibited both the proliferation of vascular endothelial cells and vessel formation by bovine endothelial cells cultured between collagen gels by a three-dimensional sandwich method (11, 42).

CONCLUDING REMARKS AND PERSPECTIVES

During endochondral bone formation, epiphyseal cartilage cells proliferate and increase matrix synthesis, becoming enlarged and hypertrophic. Calcification of cartilage and replacement by bone are accompanied by blood vessel invasion. To characterize the function of ChM-I *in vivo,* we examined its effect on BMP-induced ectopic bone formation. BMP was adsorbed on heparin-Sepharose beads and inoculated subcutaneously into nude mice. Endochondral bone formation with extensive vascularization occurred three weeks after BMP administration. In contrast, when heparin-Sepharose beads with adsorbed BMP and ChM-I were injected, vascularization and endochondral bone formation were markedly inhibited and extensive Alcian Blue-positive cartilage remained. These findings indicate that ChM-I may be named chondromodulin/angioinhibin. Thus ChM-I was the first clearly identified natural modulator and anti-angiogenic factor isolated from cartilage. ChM-I inhibits the invasion of vascular endothelial cells, thereby further stimulating cartilage growth and inhibiting premature replacement of cartilage by bone in the fetal stage. This is important in preventing dwarfism. These findings may offer a clue to the longstanding question of why cartilage is an avascular tissue, in spite of its abundant content of the potent angiogenic factor FGF-2. Recently, we cloned human ChM-I. Sequence similarities of the entire precursor molecule, Ch-SP, and mature ChM-I of bovine ChM-I with those of human ChM-I were 92.2%, 95.8%, and 80.0%, respectively. We confirmed that recombinant human ChM-I also stimulated the proliferation of chondrocytes, but inhibited the proliferation of vascular endothelial cells.

Solid tumors grow slowly in avascular conditions: they do not generally exceed a few millimeters in diameter because of limited supplies of oxygen and nutritients. But when new capillaries develop in the host and reach the tumors, the tumors grow rapidly. Therefore, anti-angiogenesis might be a rational approach in preventing solid tumor growth. ChM-I, recently identified as a cartilage-generated anti-angiogenic factor is one of the most promising factors for this purpose.

In the Open Forum of the US-Japan Cooperative Cancer Research Program at the Natcher Conference Center on the Bethesda NIH Campus in 1996, I presented results on ChM-I research in a session on the role of cytokines in modulating angiogenesis. ChM-I inhibits the proliferation and tube formation by endothelial cells. ChM-I counters the effect of FGF-2 and the bone forming activity of BMP. Thus, ChM-I stimulates cartilage formation and prevents its premature conversion to bone. The angiostatic properties of ChM-I may make it a nontoxic antitumor agent. In the same session, O'Reilly presented results of studies performed in Folkman's laboratory. He has found that primary tumors generate an inhibitor of angiogenesis which suppresses the growth of tumor metastases. A suppressive factor present in plasma and urine of tumor bearing mice was purified and identified as an internal fragment of plasminogen, and named angiogenesis inhibitor angiostatin (43). When given systemically, angiostatin strongly inhibited the angiogenesis induced by FGF-2 in a mouse model of corneal neovascularization. Systemic therapy with angiostatin strongly inhibited both metastatic and primary tumor growth of murine and human tumors in mice. However, plasminogen itself was inactive. Another angiostatic factor purified from a murine hemangioendothelioma is a fragment of type XVIII collagen and has been named endostatin (44). Endostatin, in combination with angiostatin, eradicated various tumors in mice without inducing resistance or obvious side effects. Cartilage may contain these types of factors.

Psoriasis is an inflammatory skin disease with dilation of capillaries as an early histological change. More developed psoriatic lesions show proliferation of blood vessels and neovascularization. Dupont et al. (45) reported that the anti-angiogenic agent AE-941, derived from shark cartilage, has anti-angiogenic and anti-inflammatory properties, and thus it is promising for treatment of psoriasis. Shark cartilage may contain a ChM-I-like factor.

Recently, Okihana and Yamada (46) prepared a good quality cDNA library from mouse growth cartilage, selected 1401 clones from this library, and examined their sequences. Among 608 clones examined, 196 were unknown and 2 were only poly A. The remaining two-thirds of the clones (410/608) coded for known sequences. Of these, 55 clones coded for type II (pro)collagen, 54 for osteonectin, and 22 for other cartilage collagens (type IX, type X, and type XI). The others encode cartilage extracellular matrix genes such as ChM-I and general cellular genes. Almost one-third of the genes were novel. Their cDNA libraries will be valuable for study of other unidentified cartilage-derived growth factors and anti-tumor factors.

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