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Matrix metalloproteinases and their inhibitors in bone: an overview of regulation and functions

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1. ABSTRACT

Matrix metalloproteinases (MMPs), a family of Zn^{2+}-dependent endopeptidases, mediate different physiological processes by digesting components of the extracellular matrix. Nevertheless, overexpression of MMPs is implicated in the pathogenesis of various diseases. Different MMPs and tissue inhibitors of MMPs (TIMPs) are expressed in bone cells, and their biosynthesis is regulated by local and systemic hormones and factors. The levels of enzymatically active MMPs in bone are further controlled by secretion, activation of proenzymes, inhibition by TIMPs, cellular uptake and degradation. Members of the cysteine and serine family of proteinases also coordinate some of the biological activities of MMPs in bone. The functions of MMPs and TIMPs in bone include regulation of processes, such as degradation of collagen and other components of the bone matrix, migration and survival of bone cells, endochondral bone formation and bone resorption. Abnormal expression of MMPs may lead to pathological conditions affecting bone and cartilage. Various pharmacological agents can inhibit MMPs, and some of these inhibitors may be potential therapeutic agents for certain bone diseases. This review briefly describes the regulation and functions of different MMPs and TIMPs in bone, and provides an insight into the role of MMPs in bone development, remodeling and pathology.

2. INTRODUCTION

Matrix metalloproteinases (MMPs) are a family of Zn^{2+}-dependent proteolytic enzymes that can collectively degrade all the components of the extra-cellular matrix (1-4). About twenty five MMPs have been identified so far (2). Based on substrate specificity, MMPs can be categorized into collagenases, gelatinases, stromelysins and others. Collagenases can cleave intact collagen fibrils at physiological pH, whereas gelatinases can digest denatured collagens. Stromelysins are capable of breaking down a variety of extra-cellular components including gelatins. Although each MMP may have a preferred substrate, there is considerable overlap in substrate specificities of various MMPs. Most MMPs are secreted into the extra-cellular matrix (ECM), however, a few membrane-bound MMPs, designated as membrane-type MMPs (MT-MMPs), have been described. The major physiological functions of MMPs include modulation of embryonic development, angiogenesis, growth plate remodeling, wound healing, uterine involution and ovulation. However, MMPs have been implicated in the pathogenesis of diseases, such as neuroinflammation, atherosclerosis, cancer, arthritis, periodontal disease and others (5-12).

MMP expression occurs in diverse cell types, and it is not limited to matrix-producing cells. MMP levels are regulated by multiple mechanisms and at multiple levels:

tissue specific expression, regulation by systemic and local hormones and factors, enzymatic activation of zymogens into active enzymes, cell and matrix localization, and inhibition. MMPs are synthesized as a proenzyme and subsequently activated by proteolytic cleavage. Serine proteases, such as plasminogen activator (PA), are shown to be potent activators of MMPs; however, some members of the MMP family can also activate other MMPs. In addition to enzymatic activators of MMPs, tissue inhibitors of MMPs (TIMPs) are critical mediators of MMP activity (13). Four TIMPs have been identified, and they inhibit MMP activity by binding mostly the active forms of MMPs. Expression of certain TIMPs is also modified by hormones and growth factors, thereby contributing to the complexity of MMP action.

Bone is a dynamic tissue undergoing continuous remodeling. Primarily, there are two types of bone cells: osteoblastic cells responsible for bone formation by synthesizing the components of the bone matrix and osteoclastic cells facilitating bone resorption by degrading bone matrix. Under normal physiological conditions of skeletal remodeling, the activities of osteoclasts and osteoblasts are coordinated such that bone resorption is balanced by bone formation. However, in pathological situations, such as osteoporosis or osteopetrosis, this balance between bone formation and resorption is shifted resulting in bone loss or excessive bone formation. Synthesis of different MMPs and TIMPs by bone cells is modulated by factors controlling remodeling, and several studies have suggested that MMPs may play a critical role in mediating skeletal development and remodeling (14, 15). Although normal expression of MMPs and their regulation by bone remodeling agents are essential for maintaining bone mass, overexpression of MMPs may be linked to pathologic bone loss.

3. COLLAGENASES

Collagenase was identified in the skeletal tissue of numerous species in 1960s and 70s (16-19). Later on, it was observed that isolated osteoblastic cells could synthesize and secrete collagenase in response to various bone resorbing stimuli (20-22). Whether collagenase is produced by osteoclasts remains controversial (23, 24). There are three types of collagenases, collagenase-1 (MMP-1), -2 (MMP-8) and -3 (MMP-13), and all of them can be present in the bone microenvironment. Collagenase-1 is also known as fibroblast collagenase or interstitial collagenase. The collagenase indicated in studies using human osteoblasts or osteosarcoma cell lines by Meikle *et al.*, 1992, Rifas *et al.*, 1994, Panagakos and Kumar, 1995, and DeBart *et al.*, 1995, is likely to be collagenase-1 (25-28). Collagenase-2, also known as polymorphonuclear collagenase, is synthesized by osteoblasts during bone formation (29, 30). A novel collagenase, collagenase-3, was first identified in human breast carcinomas in 1994 (31). The collagenase, described as interstitial collagenase or collagenase-1 in some of the earlier studies, especially in rodent cells, is in fact collagenase-3. To date, collagenase-1 expression has not been detected in rat or mouse cells. During human fetal bone development, collagenase-3 expression can be detected in chondrocytes, osteoblasts and periosteal cells (32). This collagenase is present during intramembranous and endochondral ossification during gestation (32, 33). Collagenase-3 is now recognized as the major form of collagenase synthesized by osteoblasts of different species, including humans (31-34).

3.1. Regulation

Collagenase-1 expression has been reported to be stimulated by parathyroid hormone (PTH), tumor necrosis factor (TNF)-alpha and interleukin (IL)-1 alpha and inhibited by 17 beta-estradiol in human osteoblasts or osteosarcoma cells (25-28, 35). Although the mechanisms regulating the collagenase-1 gene in fibroblasts have been extensively studied, data regarding the molecular basis of collagenase-1 regulation in bone cells are limited. Collagenase-2 expression is shown to be differentially regulated during skeletal development; however, there is little information pertaining to the regulation of collagenase-2 by physiological agents in bone (29, 30).

Most studies on regulation of collagenase in osteoblasts have been carried out using rodent cells, which express the collagenase-3 gene. Collagenase-3 level in bone is regulated by different mechanisms (Figure 1). Collagenase-3 is induced in osteoblasts by stimulators of bone resorption, including PTH, steroids, cytokines and various growth factors (Table 1). In contrast, stimulators of bone formation, such as insulin-like growth factors (IGFs) and members of the transforming growth factor (TGF)-beta superfamily, suppress collagenase-3 expression in osteoblasts (Table 1). Cells of the osteoblastic lineage synthesize collagenase-3 when exposed to mechanical strain, a major regulator of bone cell function (72). It seems that estrogen doesn't affect collagenase-3 expression directly; however, increased collagenase-3 expression occurs in bone in ovariectomized rats suggesting that estrogen deficiency may promote collagenase-3 production *in vivo* indirectly (73). In addition to systemic or local physiological factors, collagenase-3 expression is affected by a variety of pharmacologic agents, including bisphosphonates and tetracyclines (regulation of various MMPs by different pharmacological agents is discussed in section 5 below) (74, 75). Expression of collagenase-3 also appears to be related to osteoblast differentiation, as there is a progressive increase in collagenase-3 levels in osteoblasts undergoing differentiation *in vitro* (76, 77). In addition, it seems that there are mechanistic differences in the regulation of collagenase-3 in primary and malignant osteoblasts. For instance, in primary osteoblastic cells, TGF-beta suppresses collagenase-3 expression, whereas, in an osteosarcoma cell line, UMR106, TGF-beta enhances collagenase-3 (64).

The collagenase-3 gene is regulated by both transcriptional and post-transcriptional mechanisms. The collagenase-3 gene promoter contains binding sites for different nuclear transcription factors, such as Runx2, AP-1, Ets-1 and others (36, 54, 78, 79). Runx2 is a critical transcription factor mediating the formation of osteoblasts from their precursors (80, 81). Targeted disruption of the

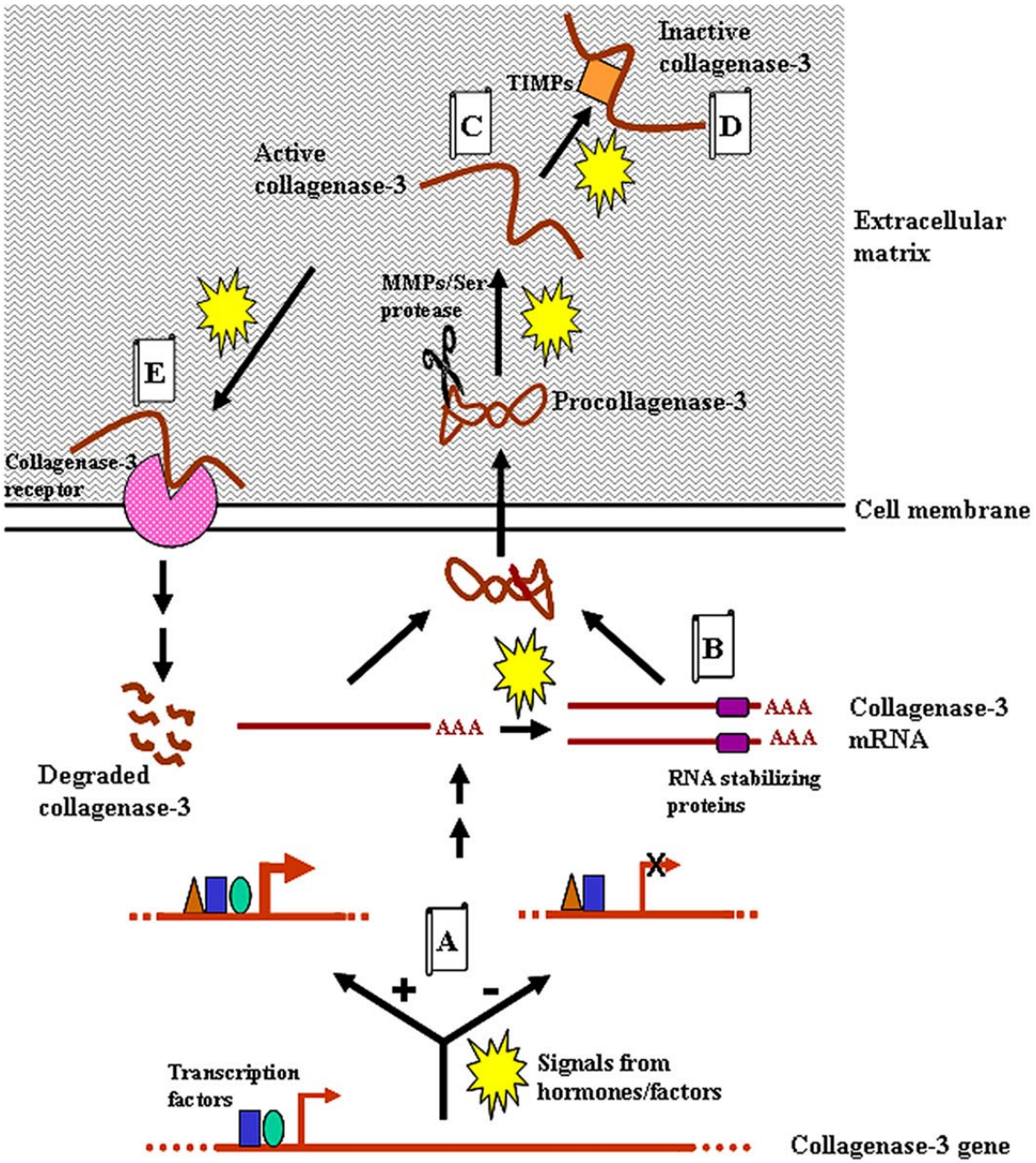

Figure 1. A schematic representation of various mechanisms of collagenase-3 regulation in bone: A, Transcriptional positive (+) or negative (-) regulation; B, post-transcriptional regulation by stabilization of mRNA; C, cleavage of procollagenase into active collagenase by MMPs/serine proteases; D, inhibition of collagenase by TIMPs; and E, cellular uptake of collagenase by a scavenger receptor.

Runx2 gene results in a complete lack of bone formation (82, 83). Expression of Runx2 is found to be a prerequisite for collagenase-3 synthesis by osteoblasts (84). In addition, Runx2 is reported to be a mediator of collagenase-3 induction by PTH (54). Interestingly, the suppression of the collagenase-3 gene by bone morphogenetic protein (BMP)-2 is also found to be mediated through the Runx2 binding site (67). Therefore, it appears that Runx2 may serve as a molecular switch, critical for both positive and negative regulation. Protein modifications of Runx2 may impact collagenase-3 gene regulation; recent studies have shown that Runx2 can undergo phosphorylation in response to stimulation by various physiological agents, including PTH and BMPs (85-87). In addition, Runx2 may interact directly or indirectly with other transcription factors, such as the AP-1 complex, CREB and Smads, in regulating collagenase-3 gene expression (36, 54, 55, 64). Enhanced binding of the Jun and Fos, components of the AP-1 complex, to the collagenase-3 proximal promoter occurs during the activation of collagenase-3 gene transcription by

Table 1. Regulation of collagenase-3 expression in bone

Regulating agent	Tissue/Cell type	Reference	Mechanism of gene expression	Reference
Stimulators				
Cyclic AMP	Rat osteosarcoma cell line UMR 106	36, 37	Transcriptional activation, mediated through an AP-1 site	36
1, 25-Dihydroxyvitamin D_3	Mouse calvaria and primary osteoblasts, mouse osteoblast-like cell line MC3T3, rat primary osteoblasts	21, 38-41	Transcriptional activation, possibly mediated through an AP-1 site	40
Epidermal growth factor	Rat primary osteoblasts, UMR 106	39, 42		
Fibroblast growth factor-2	Rat primary osteoblasts, MC3T3	43-45	Transcriptional activation, mediated through an AP-1 site	44
Glucocorticoids	Rat primary osteoblasts	37, 46	mRNA stabilization , mediated through AU-rich regions in the 3'-UTR	37, 46
Heparin	Mouse calvaria	47		
Interleukin-1	Mouse calvaria, mouse and rat primary osteoblasts, MC3T3	38, 39, 41, 48, 49	Transcriptional activation	49
Interleukin-6	Rat primary osteoblasts	48, 50	Transcriptional activation	50
Leukemia inhibitory factor	Rat primary osteoblasts	51	Transcriptional and post-transcriptional regulation	51
Macrophage migration inhibitory factor	Rat primary osteoblasts	52	Mediated by Src-related Tyr kinase-, Ras-, ERK1/2- and AP-1-dependent pathway	52
Oncostatin M	Rat primary osteoblasts	51	Transcriptional and post-transcriptional regulation	51
Parathyroid hormone	Mouse calvaria, mouse and rat primary osteoblasts, MC3T3 and UMR 106	20, 21, 36, 38, 39, 41, 42, 53-56	Transcriptional activation, mediated via AP-1, Runx2 and Ets-1 binding sites	36, 53-56
Phorbol esters	Rat primary osteoblasts, UMR 106 and ROS 17/2.8	37, 55, 57	Transcriptional activation	37, 55
Platelet-derived growth factor BB	Rat primary osteoblasts	58, 59	Transcriptional and post-transcriptional mechanisms; transcription mediated through an AP-1 site	58, 59
Prostaglandins (PG)	Mouse calvaria, mouse and rat primary osteoblasts, MC3T3 and UMR 106	21, 38, 39, 41, 42, 60	PGE_2 stimulates transcription	60
Retinoids	Mouse calvaria, rat primary osteoblasts, UMR106	38, 61, 62	Transcriptional activation	62
Thyroid hormone	MC3T3	63	Transcriptional activation	63
Transforming growth factor-beta	UMR 106	64	Transcriptional activation, needs Smad and MAP kinase signaling pathways and Runx2	64
Tumor necrosis factor-alpha	Mouse calvaria, mouse and rat primary osteoblasts, MC3T3	38, 39, 41		
Inhibitors				
Bone morphogenetic proteins (BMPs)	Rat primary osteoblasts	65-67	Inhibition of transcription, effect of BMP-2 is mediated through a Runx2 site	65-67
Insulin-like growth factor I & II	Rat primary osteoblasts	68, 69	Transcriptional inhibition	69
Interferon-gamma	Rat primary osteoblasts	39		
Transforming growth factor-beta	Rat primary osteoblasts	67, 70, 71	Transcriptional and post-transcriptional mechanisms	67, 71

PTH, fibroblast growth factor (FGF) -2 and platelet-derived growth factor BB (44, 59, 88). The DNA response elements mediating collagenase-3 gene expression by many other bone regulatory agents, particularly steroids and cytokines, are not yet defined. Further, a number of factors regulate collagenase-3 expression at the post-transcriptional level, by altering mRNA stability (Table 1). Glucocorticoids increase collagenase-3 by post-transcriptional mechanisms, and studies revealed that the AU-rich elements in the 3'-UTR mediate the stability of collagenase-3 mRNA (46). Basal collagenase-3 levels and their regulation by several physiological agents are also found to be dependent on *de novo* protein synthesis suggesting that collagenase-3 expression is mediated by cellular factors of short half-life (37, 43, 58, 61, 63, 68, 89). The identity of these labile factors, most likely transcription factors or RNA binding proteins, remains to be established.

Newly synthesized procollagenases are secreted into the bone matrix. Procollagenases are converted into active enzymes by cleavage of the prodomain by serine proteinases and other MMPs, which in turn are regulated by different hormones and growth factors (90-92). The major activators of procollagenase-3 in bone may be other MMPs, such as stromelysin-1, 72 kDa gelatinase and membrane-type 1-MMP (MT1-MMP), present in the bone microenvironment (92).

Collagenase-3 activity in bone can be inhibited by TIMPs. TIMP-1,-2 and -3 are present in bone, and the biosynthesis of TIMPs, particularly that of TIMP-1 and-3, is stimulated by different bone remodeling agents, including growth factors and cytokines (27, 28, 43, 51, 65, 70, 71). Consequently, the availability of active collagenase-3 to degrade the bone matrix is further modulated by different hormones and factors that can alter the MMP:TIMP ratio. Although several inducers of collagenase-3 also stimulate TIMP expression, the potent stimulators of bone formation, such as BMPs and TGF-beta, differentially regulate the expression of collagenase-3, and TIMP-1 and -3; it suggests that a decrease in collagenase-3 along with an increase in TIMPs may promote bone formation (65, 70, 71).

In addition to the regulation of collagenase-3 at the level of biosynthesis, activation and complex formation with TIMPs, recent studies have shown that collagenase-3 levels in the matrix are also regulated by receptor-mediated uptake and intracellular degradation by endosomes and lysosomes (93-95). Collagenase-3 binds to a specific cell surface receptor and then internalized in participation with the low density lipoprotein receptor-related protein (95). The abundance and function of the collagenase-3 receptor, and the intracellular degradation of collagenase-3 are regulated by PTH, and possibly by other physiological agents (94).

3.2. Functions

Collagenases can initiate the degradation of interstitial collagens, type I, II and III collagens, at physiological pH. All collagenases cleave collagen fibrils primarily at a unique helical locus three-fourth of the way from the N-terminus generating one-quarter and three-quarter length fragments. However, collagenase-3 has been shown to cleave type I and II helical collagens at additional loci (96-98). The cleavage of collagen triple helix is critical for the initiation of fibrilar collagen degradation. The non-helical collagen fragments, generated by the initial collagenase cleavage, are further digested by other MMPs, particularly gelatinases. Among collagenases, collagenase-3 also possesses high gelatinase activity, and therefore, it may also contribute to further degradation of denatured collagen fragments (96).

Collagenase activity is critical for endochondral bone formation. Collagenase-3 is expressed at high levels in chondrocytes, and it is regarded as the major enzyme involved in the degradation of type II collagen, the predominant form of collagen in cartilage (32, 98, 99). In fact, collagenase-3 cleaves type II collagen more efficiently than type I collagen (96, 98). During endochondral bone formation, cartilage matrix is degraded and then replaced with bone. Therefore, this enzyme appears to be a major player in collagen turnover during endochondral bone formation. Collagenase-3 may also participate in the ECM remodeling during intramembraneous ossification (100).

In addition to enabling collagen breakdown during bone formation, collagenases regulate collagen turnover during bone remodeling. Type I collagen, the major organic component of the bone matrix, is the primary target of collagenases in bone. Involvement of collagenases, induced by bone remodeling agents, in the breakdown of bone collagen has been implicated by the inhibition of collagen degradation following the addition of recombinant TIMPs into bone cultures (91). Furthermore, stimulation of collagenase by bone remodeling agents has been demonstrated in several studies by measuring the degradation of radiolabeled-type I collagen fibrils or by assaying for hydroxyproline, an end product of collagen breakdown, in bone cell and explant cultures (21, 38, 39).

Bone collagen breakdown by collagenases is linked to the survival of cells of osteoblastic lineage. In transgenic mice resistant to cleavage of type I helical collagen, there is altered skeletal remodeling with a significant increase in apoptosis of osteoblasts and osteocytes (101). Surprisingly, these animals appear to have developed normally; it has been suggested that the cleavage of collagen at a non-helical N-telopeptide site is sufficient to support collagen degradation associated with bone formation (102). Also, mice resistant to cleavage of helical collagen have increased bone deposition, caused by the activation of bone-forming surfaces. It has been shown that collagen degradation products may generate antiapoptotic signals via integrin-dependent anchorage to the cell surface (103)

Collagenase activity is associated with bone resorption. Most inducers of collagenase-3 are also stimulators of bone resorption (15). It has been hypothesized that collagenase is responsible for the removal of osteoid, a thin layer of non-mineralized matrix on bone surface, so that osteoclastic cells can be recruited to the mineralized bone surface to resorb bone (104). However, an *in vitro* study by Holliday *et al.* has shown that there is no significant difference in the attachment of osteoclasts to bone slices in the presence or absence of MMP inhibitors (105). This study has also demonstrated that the inhibition of collagenase-3 activity, using specific antibody against this enzyme, in bone cultures, blocked osteoclastic bone resorption. Therefore, it has been suggested that collagen fragments, generated by collagenases, stimulate osteoclastic cells to resorb bone. The role of collagenase activity in bone resorption has been further illustrated by *in vivo* studies by Zhao *et al.*, showing that PTH-stimulated bone resorption, as measured by Ca^{2+} release into serum, is diminished in transgenic mice engineered to synthesize collagenase-resistant type I collagen (102). Furthermore, a study by Chiusaroli *et al.* has showed that collagen degradation by activated collagenase is necessary for basal and PTH-receptor induced osteoclast activation and consequently, bone resorption (106).

In addition to interstitial collagens, collagenase-3 may target other ECM proteins in bone and cartilage. In different *in vitro* studies, collagenase-3 has been shown to degrade a variety of ECM components including type IV, IX, X, and XIV collagens, aggrecan, tenascin C, fibronection and fibrilin (107). Further, collagenase-3 can activate latent TGF-beta 3, an anabolic agent for bone (108). Collagenase-3 may also contribute to increased availability of active IGFs, potent stimulators of bone growth, by degrading IGF binding protein (IGFBP) -5 in bone (109). Importantly, degradation of bone matrix components by collagenase-3 may lead to the release of growth factors stored in the matrix, including IGFs, TGF-beta and FGF-2. Thus, collagenase-3 activation may directly or indirectly cause an increase in the levels of active growth factors in the bone microenvironment. Increased bioavailability of growth factors may serve as a mechanism to signal bone formation following bone matrix degradation, a mechanism for coupling bone formation to resorption.

Recently, the skeletal phenotype of the collagenase-3 null mice has been characterized. Studies by

Stickens *et al.* in collagenase-3 deficient mice revealed that this proteinase is essential for normal endochondral bone development (110). Absence of collagenase-3 leads to alterations in growth plate architecture and an increase in trabecular bone during endochondral bone formation. Nevertheless, cortical bone is unaffected by collagenase-3 deficiency, suggesting that other MMPs may contribute to cortical bone remodeling. A parallel study by Inada *et al.* has also identified defects in the development of growth plate cartilage and endochondral ossification in animals with collagenase-3 deficiency (111). Conditional inactivation of collagenase-3 in bone has resulted in an increase in trabecular bone volume, however, maintained a normal zone of hypertropic cartilage (110). Adult mice with collagenase-3 deficiency have developed a form of chondrodysplasia, characterized by increased length of growth plates, and increased number and distorted alignment of chondrocytes (111). In humans, a chondrodysplasia with similar characteristics, the Missouri variant of spondyloepimetaphyseal dysplasia, is caused by a mutation in the collagenase-3 gene (112).

4. MMPs OTHER THAN COLLAGENASES

In addition to collagenases, bone cells also express other MMPs including gelatinases, stromelysins and MT-MMPs. These MMPs contribute to different aspects of bone development and remodeling. Because bone matrix has a very complex biochemical and structural composition, concerted actions of different MMPs are required to fully degrade all the components of the bone matrix during bone modeling and remodeling.

4.1. Gelatinases

Gelatinase A or 72 kDa gelatinase (MMP-2) and gelatinase B or 92 kDa gelatinase (MMP-9) are synthesized by osteoblasts (25, 27, 48, 50, 63, 70, 113, 114). Expression of gelatinase A is stimulated by IL-1, IL-6, TGF-beta and TNF-alpha in osteoblasts (27, 48, 50, 70, 113). Gelatinase B expression is stimulated in cells of osteoblastic lineage by PTH, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), IL-1, TNF-alpha and thyroid hormone (25, 48, 63, 113). Expression of gelatinase B is also observed in osteoclasts, and it is enhanced by the chemokine, CXCL12, and receptor activator of NF-kappa B ligand (RANKL), a major stimulator of osteoclast formation and function (115-119).

The primary substrate of gelatinases is denatured collagens (4, 90), and therefore, collagen fragments, generated by collagenases in bone, are thought to be further degraded by gelatinases. In addition, gelatinases may have other important functions in bone; for example, gelatinase A may activate procollagenase-3, and gelatinase B may regulate migration of osteoclasts (120, 121). Gelatinase A and B have been shown to degrade IGFBP-3 and -1, respectively, in other cell types; it is possible that these proteinases may also affect the availability of IGFs in bone by processing these IGFBPs (122, 123). Gelatinase A null mice develop without major bone abnormalities (124). However, a human mutation, resulting in the absence of active gelatinase A, leads to an autosomal recessive form of multicentric osteolysis, a rare disease leading to destruction and resorption of the affected bones (125). Based on the function of gelatinase A in degrading denatured collagen, an osteopetrotic phenotype can be expected from gelatinase A deficiency. However, the osteolytic phenotype in humans may have resulted from an imbalance in coupling between bone resorption and formation, a possible outcome of incomplete matrix degradation in the absence of gelatinase A. Mice with null mutation in gelatinase B gene develop an abnormal pattern of skeletal growth plate vascularization and ossification suggesting that gelatinase B activity is necessary for normal bone development (126). In addition, mice lacking gelatinase B exhibit a delay in osteoclast recruitment into developing long bones (127).

4.2. Stromelysins

Stromelysin-1 (MMP-3) is synthesized by cells of osteoblastic lineage (25, 49, 90, 128, 129). Stromelysin-1 expression is augmented in estrogen deficiency (129). Active stromelysin-1 is observed in osteocytes and in matrix surrounding osteocytic lacunae, while stromelysin-2 (MMP-10) is expressed at high levels in osteoclasts (128). Stromelysin-3 (MMP-11) is expressed in osteoblastic cells, and its expression is regulated by FGF-2 and TGF-beta (130, 131). Although the specific functions of stromelysins in bone are poorly understood, it is likely that they act in concert with other MMPs to promote activation of proenzymes and the degradation of non-collagenous matrix components, such as proteoglycans (96, 132). Stromelysin-1 may enhance the bioavailability of IGFs in bone by degrading IGFBP-1 and -3, as shown in other cell types (122, 123, 133).

4.3. Membrane-type MMPs

Six MT-MMPs have been described (4). MT1-MMP (MMP-14) is synthesized by mature osteoblasts and osteoclasts (134, 135). It has been demonstrated that the expression of MT1-MMP in osteoblastic cells is stimulated by estrogen, progesterone and $1,25(OH)_2D_3$ (136-138). However, PTH has been shown to inhibit MT1-MMP expression in osteoblast-like cells (139). MT1-MMP expression varies during osteoblast differentiation, and it peaks in mature nodule forming osteoblasts (135). There is limited information regarding the expression of other MT-MMPs, known as MT2-MMP (MMP-15), MT3-MMP (MMP-16), MT4-MMP (MMP-17), MT5-MMP (MMP-24) and MT6-MMP (MMP-25), in bone.

MT1-MMP activates procollagenase-3 and progelatinase A, and therefore, it may coordinate actions of other MMPs in the skeletal tissue regulating collagen degradation (120, 140). MT1-MMP deficiency impairs collagenolytic activity and osteogenic potential of osteoblastic cells and disrupts both intramembranous and endochondral ossification (141). MT1-MMP is an activator of the latent form of TGF-beta, a regulator of osteoblast survival during transdifferntiation into osteocytes (142). Lack of MT1-MMP causes increased apoptosis of osteocytes (141, 143). In addition, disruption of collagen cleavage in MT1-MMP null mice interferes with normal development and maintenance of osteocyte processes (144). Mice lacking MT1-MMP develop severe connective

tissue growth and remodeling abnormalities, such as dwarfism, osteopenia, fibrosis of soft tissue, arthritis and skeletal dysplasia (141). Adult MT1-MMP deficient mice have increased bone resorption leading to progressive osteopenia. These observations prove that MT1-MMP is an essential enzyme for normal bone development and remodeling.

4.4. Other MMPs

Elastase (MMP-12) has been found to be expressed in osteoclasts (145). Elastase can cleave osteopontin and bone sialoprotein, two important bone matrix proteins, and therefore, it may participate in bone matrix degradation along with other MMPs expressed in bone. There is little information about the expression of other MMPs, such as matrilysin 1 (MMP-7), matrilysin 2 (MMP-26), enamelysin (MM-20), epilysin (MMP-28), MMP-19 and MMP-23, in bone cells.

5. INHIBITORS OF MMPs

A variety of natural and synthetic inhibitors of MMPs are known. As indicated earlier, the major peptide inhibitors of MMPs are TIMPs. MMPs are also inhibited by pharmacological agents, such as bisphosphonates and derivatives of tetracycline. In addition, a number of synthetic drugs that compete for the binding of MMPs to collagens have been developed and tested by different pharmaceutical companies for the treatment of diseases associated with an increase in MMP activity.

5.1. TIMPs

Four TIMPs, TIMP-1, -2, -3 and -4, have been identified in vertebrates. TIMP-1, -2 and -3 are synthesized by osteoblasts and osteocytes (30, 43). In osteoblasts, expression of TIMP-1 is enhanced by physiological stimuli, including IL-1, TNF-alpha, FGF-2, BMP-2, TGF-beta, $1,25(OH)_2D_3$, thyroid hormone and retinoic acid (27, 28, 43, 51, 63, 65, 70, 71, 146-149). TIMP-3 expression in osteoblasts is shown to be stimulated by IL-1, TNF-alpha, FGF-2, TGF-beta and BMP-2 (43, 65, 71, 146). Unlike TIMP-1 and -3, expression of TIMP-2 is not regulated by most bone remodeling agents, however, a modest stimulation by PTH and TGF-beta has been observed (71, 150). In addition to regulation by endocrine and paracrine agents, TIMP-1 and -2 levels depend on the maturation state of osteoblasts, and they may vary during different stages of osteogenesis (135). TIMP-1 is also found to be produced by osteoclastic cells, and its expression in osteoclasts is inhibited by RANKL as expected for cells that are stimulated to resorb bone matrix (120, 151-153).

The major function of TIMPs in bone appears to be the modulation of MMP activities. Collagen degradation, mediated by $1,25(OH)_2D_3$, is inhibited by treatment of bone cultures with inducers of TIMP expression (148). When recombinant TIMPs are added to cultures of bone explants, PTH- and $1,25(OH)_2D_3$-induced bone resorption is blunted (117, 154). Recently, transgenic mice over-expressing TIMP-1 in osteoblasts are shown to have decreased bone resorption and increased bone mineral density (155). It is likely that a higher concentration of TIMPs, relative to active MMPs, is partly responsible for minimizing bone matrix breakdown and resorption in bone segments not undergoing active remodeling. However, the balance between active MMPs and TIMPs seems to be shifted by increased production and activation of MMPs in the presence of bone resorbing agents leading to matrix degradation and resorption during remodeling. TIMPs have differential binding affinities toward individual MMPs; thus, they exhibit varying degrees of MMP binding specificity. TIMPs inhibit MMPs by complexing with them in a 1:1 stoichiometry. TIMP-1 is known to bind and inactivate most MMPs; however, it is a relatively poor inhibitor of MT-MMPs, particularly MT1-MMP (4, 156, 157). TIMP-2 appears to be the natural inhibitor of MT1-MMP (158). In fact, TIMP-2 and MT1-MMP complex acts as a receptor for progelatinase A, allowing the procollagenase to localize and become activated in the pericellular region. Unlike TIMP-1 and -2, TIMP-3 can inhibit some members of the ADAM family of metalloproteinases, a novel family of membrane proteins containing a disintegrin and metalloprotease domain, along with MMPs (157).

Apart from inhibiting metalloproteinases, TIMPs have additional functions which include regulation of cell growth and apoptosis (157). In bone cells, TIMP-1 and -2 at low doses can directly stimulate bone resorbing activity independent of their ability to inhibit MMPs (159). Some of these unique functions of TIMPs, unrelated to the inhibition of MMPs, may also contribute to regulation of bone formation and remodeling. Although mice with null mutations in TIMP-1, -2 and -3 have been described, no major bone defects have been reported. It is likely that the biological functions of TIMPs in the skeletal tissue are redundant so that the lack of one of the TIMPs in transgenic animals may be compensated by others. Alternatively, a remodeling stress or challenge in addition to the depletion of TIMP is necessary to uncover the bone phenotype.

5.2. Bisphosphonates

A number of recent studies have indicated that bisphosphates are potent inhibitors of MMPs. Bisphosphonates, analogues of inorganic pyrophosphates, are extensively used for the treatment of different bone disorders, particularly osteoporosis (160, 161). These drugs inhibit bone resorption by suppressing osteoclast formation and activity, and promoting osteoclast apoptosis (162-164). They also interfere with the formation of calcium phosphate crystals and mineralization (165, 166). Bisphosphonates, such as alendronate, clodronate, pamidronate, tiludronate and zolendronate, inhibit the enzymatic activity of different purified MMPs (Table 2). The inhibitory effect of bisphosphonates on MMP activity is likely to be caused by their ability to chelate Zn^{2+} (179). Some of the bisphosphonates have also been shown to directly inhibit or stimulate the biosynthesis and secretion of certain MMPs (Table 2). Despite the stimulation of MMPs in some cases, the net effect of bisphosphonates on MMPs appears to be a reduction in active MMP levels in tissues or circulation (180-182). Because of their ability to inhibit MMPs, bisphosphonates have been used to prevent

Table 2. Regulation of MMPs by bisphosphonates

Bisphosphonate	Regulation	Reference
Alendronate	Inhibits enzymatic activity of MMP-1, -2, -3, -8, -9, -12, -13 and -20 Inhibits MMP-2 synthesis and secretion Inhibits MMP-2 and -9 secretion Stimulates MMP-13 synthesis	167, 168 169 170, 171 74
Clodronate	Inhibits enzymatic activity of MMP-1, -2, -8, -9, -13 and -14 Inhibits MMP-14 synthesis	167, 168, 172, 173 174
Pamidronate	Inhibits enzymatic activity of MMP-1, -2, -3, -8, -9, -12, -13 and -20 Stimulates MMP-2 and 9 secretion	167, 168 175, 176
Tiludronate	Inhibits MMP-1 and -3 activity	167, 177
Zolendronate	Inhibits enzymatic activity of MMP-3, -12, -13, and -20 Inhibits MMP-9 synthesis and activity Stimulates MMP-2 secretion	167, 168 178 176

tumor cell metastasis to bone (179, 183). The effectiveness of bisphosphonates in the treatment of osteoporosis and other bone disorders may also be partly due to their ability to inhibit MMP activity in bone. Lately, bisphosphonates are used not only to treat bone disorders, but also considered for the treatment of different non-bone diseases, including periodontal disease, dental caries and arthritis (184-187).

5.3. Tetracyclines

Tetracycline derivatives inhibit the production and activity of different MMPs, including collagenases, gelatinases and MT1-MMP (75, 188-190). These therapeutic agents include the classic antimicrobial tetracyclines, such as tetracycline, doxycycline and minocycline, and the chemically modified tetracycline derivatives that lack the antimicrobial properties (188). Tetracycline derivatives are potential therapeutic agents for cancer metastasis and different forms of arthritis, because of their inhibitory effects on MMPs (188-193). Tetracycline derivatives have been used for the treatment of periodontal disease, and the antimicrobial and MMP inhibitory properties of these drugs have contributed to their efficacy (194, 195). To a lesser extent, tetracyclines have been useful in treating rheumatoid arthritis (196). Consistent with the ability to inhibit various MMPs, tetracycline derivatives modify bone resorption and reduce bone loss in different disease models; for example, tetracyclines inhibit bone loss associated with periodontal disease (197, 198), diabetics (199) and ovariectomy (200, 201).

5.4. Other MMP inhibitors

In addition to TIMPs, a few proteins with modest inhibitory effects on MMPs are known; however, their involvement in regulating MMP activity in bone remains to be established. Tissue factor pathway inhibitor 2, thrombospondin-1 and -2, and alpha 2-macroglobulin can interact with and inhibit MMPs (4, 157). Proteins containing some sequence similarity with the N-terminal sequence of TIMPs, such as netrins, secreted frizzled-related proteins and type I collagen C-proteinase enhancer protein, may act as MMP inhibitors (184). Reversion-inducing cysteine-rich protein with Kazal motifs, also known as RECK, is a membrane-anchored glycoprotein that can negatively regulate MMP functions, and inhibit tumor invasion and angiogenesis (187, 202). It is possible that some of these natural MMP inhibitors are functional in bone.

Different MMPs are inhibited by aspirin, neovastat (an extract from shark cartilage), and catechins and theaflavins found in tea (203-205); some of these agents are also found to be useful for inhibiting angiogenesis and tumor invasion. A number of collagen peptidomimetic and nonpeptidomimetic inhibitors of MMPs have been developed recently by different pharmaceutical companies and tested for their potential therapeutic use in diseases, such as cancer, arthritis, periodontal disease, atherosclerosis, chronic obstructive pulmonary disease, and inflammation (191, 206-208). The collagen peptidomimetic inhibitors are designed to mimic the structure of collagen at the MMP binding site, and the nonpeptidic MMP inhibitors have been engineered on the basis of structural conformation of MMP active sites. Although some of these compounds have been effective in preventing various diseases in experimental models, there has been limited success in identifying appropriate candidates for treating human diseases because of various adverse effects, including less selectivity, metabolic instability, reduced bioavailability, lack of efficacy, and toxicity, observed during clinical trials. These problems can possibly be resolved in future by developing and testing new classes of MMP inhibitors. To date, most of the clinical studies using synthetic MMP inhibitors have focused on diseases other than bone disorders, and the potential use of synthetic MMP inhibitors in treating bone disorders remains to be evaluated.

6. MMPs AND OTHER PROTEINASES

The biological functions of MMPs in bone are closely linked to other proteolytic enzymes, particularly members of the cysteine and serine proteinases. Cathepsins, a group of cysteine proteinases, and members of the PA/plasmin family of serine proteinases participate with MMPs to degrade bone matrix.

6.1. Cathepsins

Cathepsins mediate matrix degradation by breaking down collagen and other matrix components most effectively at acidic pH. Cathepsin K is the most abundant form of cathepsin synthesized by osteoclasts, and it is considered to be the major enzymatic tool used by osteoclasts to degrade bone collagen and other matrix components within the resorption hemivacuole, the unit of osteoclastic resorption (209, 210). Targeted disruption of cathepsin K gene in mice leads to osteopetrosis (211), and mutations in the cathepsin K gene cause pycnodysostosis, an autosomal recessive disease characterized by

osteopetrosis and short stature (211, 213). Other cathepsins, such as cathepsin B, H, L and S, may also be involved in the degradation of bone matrix, as suggested by their expression in osteoclasts and osteoblasts during the remodeling of bone fractures (214). MMPs and cathepsins play different coordinated roles in matrix degradation and bone resorption. Complete degradation of bone collagen leads to the formation of modified collagen peptide fragments, ICTP and CTX, which can be measured in serum or urine as markers of bone resorption. Recent studies have shown that MMPs are responsible for producing ICTP, whereas cathepsins, primarily cathepsin K, are generating CTX (215); nevertheless, inhibition of MMPs blunts the release of both ICTP and CTX fragments consistent with the idea that MMPs, rather than cathepsins, are instrumental in initiating resorption. MMPs and cathepsins may co-operate in degrading some of matrix macromolecules in a step-by-step manner, as suggested by the observation that complete degradation of type X collagen requires initial digestion by collagenase followed by subsequent degradation by cathepsin B (216). Site specific functional differences, based on MMP and cathepsin activities, seem to exist in osteoclasts; for instance, osteoclastic resorption of the calvarial bones is dependent on MMPs and cathepsins whereas that of long bones is primarily dependent on cathepsins (217). It is possible that cathepsins may regulate the enzymatic activity of MMPs in bone by altering TIMP levels, as demonstrated by the ability of cathepsin B to degrade TIMP-1 and -2 and consequently, inhibit angiogenesis by elevating active MMP levels (218). Thus, cathepsins can participate with MMPs in regulating bone matrix turnover by multiple mechanisms.

6.2. PA/plasmin family of proteinases

PA/plasmin family of serine proteinases mediates different functions in bone (219). PAs convert plasminogen into plasmin, a serine proteinase that can degrade a number of matrix components. Urokinase-type PA (uPA), tissue-type PA (tPA), PA inhibitor types 1 and 2, and type 1 receptor for uPA are expressed in osteoblasts and osteoclasts (220, 221). Moreover, biosynthesis of the PAs is augmented in osteoblasts by stimulators of bone resorption suggesting that these enzymes may play a critical role in bone matrix degradation (222). Using cells from uPA and tPA null mice, it has been demonstrated that the lack of PAs reduces the degradation of non-collagenous components in the bone matrix (223). Although inhibition of serine proteinases in calvarial explants diminishes calcium release, PA/plasmin system may not directly affect osteoclastic bone resorption (220, 223). In addition to mediating matrix degradation, serine proteinases may stimulate bone cell proliferation and migration (224, 225). As indicated earlier, serine proteinases can convert different pro-MMPs into active forms; activation of MMPs is regarded as the major mechanism by which these proteinases mediate MMP actions in bone. Apart from activating MMPs, serine proteinases may affect MMP activity in bone indirectly via mediating the release of growth factor regulators of MMPs, such as TGF-beta and IGF-1, from the bone matix (226, 227). Serine proteinases may also regulate the levels of some MMPs by inactivation; free MMP-2, not complexed with TIMP-2, has been shown to be degraded into inactive fragments by plasmin (228). Therefore, like cathepsins, serine proteinases can mediate MMP actions in bone.

7. PERSPECTIVE

Various studies described above, particularly the observations made in MMP deficient animal models, support the conclusion that MMPs are critical for normal bone formation and remodeling. During bone formation, MMPs, particularly collagenase-3 derived from chondrocytes, degrade cartilage matrix, allowing osteoblasts to lay down bone matrix. MMPs produced by osteoblasts and osteoclasts play a role in all aspects of bone remodeling, from initiation to resorption and the coupling of resorption to formation.

Although there is considerable information pertaining to the regulation and functions of some of the MMPs, particularly of collagenase-3, our current knowledge of the role of other MMPs and their regulation in skeleton remains limited. Additional studies, focusing on the regulation and functions of MMPs other than collagenases in bone cells, are needed to generate a complete understanding of the role of this family of proteinases in bone. It is not known how different MMPs interact and orchestrate the matrix degradation process in a regulated manner. MMPs are interdependent proteinases - some MMPs require others for activation and to complete the degradation of large substrates like collagen fibrils. Therefore, it appears that there is a cascade of MMP-mediated proteolytic events occurring during bone formation or remodeling in response to various physiological stimuli. Further, non-MMP proteolytic enzymes, such as serine proteases and cathepsins, and their regulators, also affect matrix breakdown during bone formation and remodeling. Thus, appropriate matrix degradation is an enormously complex process requiring temporal and spatial coordination of various molecular events mediated by numerous proteolytic enzymes and their inhibitors. This complexity reflects the molecular complexity of the bone matrix itself. In future, studies should be designed to elucidate the complex interactions among the various participants involved in ECM turnover.

Although major substrates of MMPs in bone are presumed to be structural components of the ECM, MMPs may also process different types of non-structural proteins (229). Based on studies in non-bone cells, MMPs are capable of regulating cell proliferation, migration and signaling by altering the bioavailability of different growth factors, cytokines and chemokines. As indicated earlier, MMPs may regulate the availability of potent skeletal growth factors, such as IGFs and TGF-beta, in bone. Other cytokines and growth factors affected directly or indirectly by MMPs in different tissues include interferon-beta, connective tissue growth factor, vascular endothelial factor, epidermal growth factors, FGFs, TNF-alpha and IL-1 beta (229-231). In addition, MMPs modulate the levels of different chemokines, such as monocyte chemoattractant protein-1, -2, -3 and -4, and CXCL1, -5, -8 and -11 (230,

232). MMPs may also be involved in mediating cell signaling through integrins, cell surface receptors of collagens and other ECM molecules (233). At present, there is limited information pertaining to the possible MMP-modulation of the bioavailability and signaling of different cytokines and chemokines in bone. The skeletal system is influenced by changes in the immune system, particularly inflammation, and future studies to uncover the role of MMPs in regulating cytokines, chemokines and their signal transduction in bone may contribute to our understanding of bone loss associated with inflammatory diseases.

Are MMPs involved in the pathogenesis of common bone diseases, such as osteoporosis? Osteoporosis results from an imbalance between bone formation and resorption; a disruption in the balance between MMPs and their inhibitors may play a role in the pathophysiology of osteoporosis. Several lines of evidence suggest this possibility: first, various stimulators of bone resorption increase the levels of active collagenase-3 in bone; secondly, bone resorption is inhibited by TIMPs and other MMP inhibitors; thirdly, bone resorption is reduced in transgenic animals resistant to collagen degradation by collagenases; and finally, the targeted disruption different MMP genes in mice cause bone defects. Thus, collagenase-3 and other MMPs can be potential targets for the therapeutic intervention of bone loss. A number of chemical and peptide inhibitors of MMPs are currently available, and the possibility of preventing or treating osteopenia should be explored.

Increased biosynthesis of MMP family members is also associated with diseases that affect bone indirectly. Induction of collagenase-1 and -3 by proinflammatory cytokines and prostaglandins in synovial fibroblasts and chondrocytes has been implicated in the pathogenesis of arthritis and localized osteopenia associated with arthritic lesions (7, 11). Overexpression of MMPs in tumor cells has been considered to be a major factor in the migration and bone metastasis of tumor cells (234, 235). Skeletal growth factors, such as TGF-beta, released by the degradation of bone matrix by MMPs, can promote the proliferation and activity of tumor cells in bone (236). Thus, MMPs impact the health of the skeletal tissue in multiple ways, and developing therapeutic strategies to control the expression of MMPs in different tissues may also help to prevent diseases that may indirectly affect bone health.

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