

Joint Diseases and Matrix Metalloproteinases: A Role for MMP-13

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Abstract: The role of matrix metalloproteinases in disease has been investigated over the last two decades. A focus on this family of proteases is particularly emphasized in two major arthritides in humans, osteoarthritis and rheumatoid arthritis. Early work described the presence of multiple MMP family members in the joint of the disease state and recent advances in the development of new knockout mice and disease models have allowed investigators to directly test the role of the MMP proteases in arthritis. MMP-13 is expressed by chondrocytes and synovial cells in human OA and RA and is thought to play a critical role in cartilage destruction. The recent development of an MMP-13 knockout mouse has documented the important role for this enzyme in cartilage formation and further studies under disease conditions promise to reveal the function of this enzyme in disease pathology. This review describes a body of research that supports the development of novel selective MMP-13 inhibitors with the hope of developing these compounds in clinical trials for the treatment of arthritis.

INTRODUCTION

There are two major arthritides in humans, i.e. osteoarthritis and rheumatoid arthritis. Osteoarthritis is a primary disease of the cartilage characterized by deterioration of the articular cartilage caused by deleterious proteinases released from articular chondrocytes. In the synovium, inflammation is not prominent during the early stages of the disease, although in the advanced stages of the disease synovitis may exert its effect through the production of proteinases and modulation of chondrocyte function [1]. On the other hand, rheumatoid arthritis is characterized by a chronic proliferative synovitis, which is caused by abnormal immunological reactions and exhibits hyperplasia of the synovial lining cells, inflammatory cell infiltration and angiogenesis in the sublining cell layer. Synovitis plays a key role in the destruction of articular cartilage and bone in rheumatoid arthritis [1]. Although these arthritides are different in the origin of the diseases, they share the destruction mechanisms of the articular cartilage by proteinases [1].

Articular cartilage has a simple avascular structure composed of a large amount of extracellular matrix (ECM) and a small number of chondrocytes and is the major target tissue for destruction in both rheumatoid arthritis and osteoarthritis. In these arthritides, excessive degradation of cartilage ECM components by proteinases is key to the destructive process. Cartilage ECM is composed mainly of proteoglycans including the major proteoglycan, aggrecan, and other minor proteoglycans (decorin, fibromodulin, lumican and biglycan etc) and collagens such as fibrillar type II collagen and other minor collagens (type IX, XI and VI collagens). Depletion of proteoglycans from articular cartilage (degradation of proteoglycans) is a common initial change in these joint diseases with subsequent degradation of the collagen fibrils.

After the initiation of collagen fibril fibrillation and laceration of the articular cartilage begins and is a result of the destruction of the arcade structures of the collagen fibrils in the articular cartilage, which is composed of the superficial, transitional, radial, and calcified zones.

Aggrecan is a glycosaminoglycan-containing molecule with three globular (G1, G2 and G3) domains. The G1 domain binds to hyaluronan chains with the aid of a link-protein. A number of proteinases including matrix metalloproteinases (MMPs), ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) species, neutrophil elastase, cathepsin G, and cathepsin B are capable of degrading aggrecan. After cleavage of the interglobular G1-G2 domain, the major aggrecan fragments detach and are released from the aggrecan-hyaluronan network. Many MMPs, including MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13 and MT1-MMP preferentially cleave the Asn³⁴¹-Phe³⁴² bond (the MMP site) of aggrecan [2] and ADAMTS1 [3], ADAMTS4 [4], and ADAMTS5 [5] clip the Glu³⁷³-Ala³⁷⁴ bond (the aggrecanase site) in addition to other sites in the G2-G3 domains. Studies indicate that the two major aggrecan fragments with the N-terminal sequences starting from Phe³⁴² or Ala³⁷⁴ of the core protein are detected in the joint fluid from patients with various inflammatory arthritides and osteoarthritis [6]. Thus, members of both the MMP and ADAMTS families are considered to play a central role in aggrecan degradation in arthritides [6].

Type II collagen, a major fibrillar interstitial collagen in cartilage, is extremely resistant to most proteinases because of its triple-helical structure. Thus, only MMPs including the classical collagenases (MMP-1, MMP-8, and MMP-13) and MT1-MMP can degrade fibrillar collagens including type I, II and III collagens, although the specific activity of MT1-MMP for type II collagen is extremely weak [7]. Once the collagen molecules are cleaved, the helical structure is unwound at body temperature and becomes denatured into gelatin. Gelatin is then digested into smaller peptides by ge-

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latinases (MMP-2 and MMP-9) [8, 9]. Type IX and XI collagens are degraded by MMP-3 [10] and MMP-2 [11].

EXPRESSION OF MMPs IN HUMAN ARTHRITIDES

In human osteoarthritis, many MMPs including MMP-1 [12], MMP-2 [13, 14], MMP-3 [15], MMP-7 [16], MMP-8 [12], MMP-9 [14], MMP-13 [12, 17] and MT1-MMP [13] are expressed in articular cartilage. The immunohistochemical expression levels of MMPs such as MMP-3, MMP-7 and MT1-MMP in chondrocytes are reported to correlate directly with the histological destruction score of the articular cartilage [13, 15, 16]. Among the classical collagenases (MMP-1, MMP-8 and MMP-13), MMP-13 is thought to be most important for degradation of collagen within the cartilage due to its preferential digestion of type II collagen over type I and III collagens [17, 18]. Since MT1-MMP efficiently activates proMMP-2 and proMMP-13 within the osteoarthritic cartilage [13, 19], MT1-MMP may be another key MMP involved in cartilage degradation through the activation of proMMP-2 and proMMP-13 and its own proteolytic activity against cartilage ECM. In addition, since MMP-3 not only digests many cartilage ECM components such as aggrecan, type IX collagen and link protein but also activates proMMP-1, proMMP-7, proMMP-8, proMMP-9 and proMMP-13, MMP-3 is also important to the osteoarthritic cartilage destruction. Human cultured chondrocytes express ADAMTS1, ADAMTS4 and ADAMTS5. However, information about their expression and regulation of the activities in human osteoarthritic cartilage is limited.

In rheumatoid arthritis, degradation of articular cartilage is caused by proteinases derived from both the inflamed synovium and stimulated chondrocytes. Synovial lining cells in rheumatoid arthritis overproduce MMP-1 [20], MMP-3 [21], MMP-9 [22] and MT1-MMP [23] as well as TIMP-1 [20] and TIMP-3 [24]. The sublining fibroblasts produce MMP-2 and TIMP-2 [25]. Polymorphonuclear leukocytes infiltrating the synovium and joint cavity secrete MMP-8 and MMP-9, and macrophages produce MMP-1, MMP-9, TIMP-1 and TIMP-2. Synovial cells are also known to produce ADAMTS4 [26]. These MMPs and ADAMTS4 are secreted into the synovial cavity and may attack the surface of the cartilage immersed with synovial fluid. Actually, MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, TIMP-1 and TIMP-2 are contained within rheumatoid synovial fluid, and the molar ratios of MMPs to TIMPs correlate with metalloproteinase activity, which is detectable in rheumatoid synovial fluids [27]. Among these, MMP-3 levels in rheumatoid synovial fluid are particularly higher than other MMPs [27]. The MMP-3 level in serum is useful for predicting joint destruction [28] and monitoring of therapies such as anti-tumor necrosis factor- α antibody treatment and arthroplasty [29]. Destruction of articular cartilage especially at the margins of the articular surface may also occur through direct contact of proteolytic synovium and/or pannus tissue with the cartilage. Actually, rheumatoid synovial lining cells exhibit strong gelatinolytic activity, which is probably generated through activation of proMMP-2 by the action of MT1-MMP [23]. On the other hand, in rheumatoid arthritis, MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-13 and MT1-MMP are immunolocalized to chondrocytes, suggesting that these

MMPs derived from chondrocytes are also involved in accelerated degradation of cartilage ECM components in rheumatoid arthritis.

This series of accumulated evidence supports the likelihood that several MMPs including MMP-13, MMP-2/MT1-MMP and MMP-3 play key roles in cartilage destruction of osteoarthritis and rheumatoid arthritis through degradation of aggrecan and collagens. However, since investigation using human tissue samples is limited, experimental arthritis models are essential for a better understanding of the molecular mechanism of joint destruction.

MMPs IN EXPERIMENTAL MODELS FOR OA AND ARTHRITIDES

Risk factors (age, occupation and gender) of OA are closely related to the mechanical loading of the joints and it is assumed that a large part of OA is induced by accumulated mechanical stress. Animal models of OA induced by producing instability of joints through surgical intervention have therefore been developed in rabbits, guinea pigs and rats, to clarify the mechanism by which this stress leads to OA development. Due to the availability of transgenic (TG) and knockout (KO) mice they are now the preferred species for the study of the genetic determinants of the physiological and pathological components of the disease. There are several spontaneous mouse models exhibiting OA with aging such as the STR/ort mouse [30]. However, since most of these models exhibit other cartilage disorders such as chondrodysplasia due to a genetic mutation of the cartilage matrix components, even in the absence of mechanical stress, they are inadequate to study the mechanism of the stress-induced OA.

Kamekura *et al.* [31] have recently established experimental mouse OA models using a microsurgical technique to produce instability in the knee joints. These models show severe, moderate, and mild types of OA depending on the severity and direction of joint instability imposed by combinations of ligament transection and meniscectomy and exhibit cellular and molecular changes similar to those seen in the human OA. In the experimental OA models, the expression pattern of MMP-13 correlates with the presence of pathological chondrocytes that undergo hypertrophic differentiation in the early stage of OA development [31]. MMP-2 and MMP-9 are localized solely in the calcified cartilage and the subchondral bone area, while MMP-3 localization is visible above the tidemark. In contrast, the immunostaining of MMP-13 is enhanced in the chondrocytes located in the superficial and middle zones of the OA cartilage, which also express type X collagen, a marker of hypertrophic chondrocytes [31]. In the severe model, co-localization of MMP-13 and type X collagen in the area adjacent to the destructive lesion of the OA cartilage is also identified, confirming the close connection between the hypertrophic differentiation and MMP-13 production by chondrocytes. TG mice expressing constitutively active MMP-13 exhibit OA changes under physiological conditions, suggesting a close relationship between MMP-13 and cartilage destruction in OA [32]. In addition, the link between chondrocyte hypertrophy and MMP-13 is suggested from studies on the transcriptional activator, Runx2, which is known to positively regulate both

MMP-13 and chondrocyte hypertrophy [33]. An experimental mouse OA model using heterozygous Runx2-deficient mice demonstrates a decrease in cartilage destruction and osteophyte formation, along with reduced type X collagen and MMP-13 expression, as compared with wild-type mice after induction of knee joint instability [34]. Thus, the data from these experimental OA models suggest that MMP-13 plays a key role in cartilage destruction in OA.

There are several studies examining arthritis models using MMP or ADAMTS KO mice. However, despite the prominent role of MMP-13 in osteoarthritis, to date there are no studies examining the OA model in the MMP-13 knockout mice. In antibody-induced arthritis models using MMP-2 KO or MMP-9 KO mice, the MMP-2 KO mice are reported to exhibit severe clinical and histological arthritis compared to wild-type mice and MMP-9 KO mice [35]. In contrast, the collagen-induced arthritis model on the MMP-3 KO background exhibits no significant difference in articular cartilage destruction and proteoglycan staining as compared to wild-type mice [36]. Moreover, no detectable difference is observed in the expression or distribution of aggrecanase-specific and MMP-specific aggrecan cleavage neo-epitopes between the MMP-3 KO and wild-type mice. Among aggrecanases (ADAMTS1, 4, 5, 8, 9 and 15), ADAMTS1 KO mice are not protected from cartilage destruction in the experimental arthritis model [37]. However, recent studies demonstrate that cartilage aggrecan degradation is inhibited in ADAMTS5 KO mice, but not in ADAMTS4 KO mice, suggesting that ADAMTS5 is a major aggrecanase in mouse cartilage [38, 39].

GENERATION OF MMP-13 KO MICE AND THEIR PHENOTYPE

MMP-13 preferentially digests type II collagen among interstitial collagens [40] and is expressed by chondrocytes and synovial cells in human OA [17] and RA [41]. In the experimental OA models, MMP-13 is expressed by chondrocytes with the cellular phenotype and its distribution is similar to that observed in the human OA chondrocyte [31]. Moreover, overexpression of active MMP-13 in chondrocytes actually induces OA changes [32]. All these circumstantial findings strongly suggest a role for MMP-13 in cartilage destruction in arthritides, and experimental studies using MMP-13 KO mice will be important for a better understanding of the actual involvement of this proteinase in cartilage destruction. Thus, similar to others, we have developed MMP-13 KO mice for the study of arthritis.

For the development of the MMP-13 KO mice, we isolated genomic mouse MMP-13 cDNA by screening a BAC library prepared from 129Svev mouse genomic DNA. A targeting vector was generated by replacing 12 kb of the MMP-13 gene, spanning exons 1-10 with a dEGFP and a PGK-neo cassette (Fig 1A). The targeting construct was introduced into 129Sv/Ev ES cell lines by electroporation, followed by G418 selection. ES clones carrying a MMP-13 allele disrupted by homologous recombination were identified by a combination of PCR and genomic Southern blot analyses. Targeted clones were injected into blastocysts of C57BL6 mice, three of these transmitted to the germ-line transmission. Homozygous null mice were generated by het-

erozygote matings, and mice were crossed back to the C57BL6 strain. A stable phenotype has been observed in mice backcrossed for five generations. Southern blotting analysis of genomic DNA identified fragments of 8.9 and 4.0 kb for wild-type mice and disrupted alleles in MMP-13 KO mice (Fig. 1B). Northern blotting analysis demonstrated no expression of MMP-13 mRNA in the 15.5 dpc embryo tissues from MMP-13 KO mice, while a 2.8-kb transcript was obtained in the wild-type mouse embryos (Fig. 1C). In addition, although protein bands of both proMMP-13 and activated MMP-13 were detected in the wild-type embryonic fibroblasts by immunoblotting, no such bands were present in the MMP-13 KO mouse embryonic cells (Fig. 1D). These data confirm the successful generation of the MMP-13 KO mice.

MMP-13 KO mice exhibited a normal lifespan with sufficient fertility and did not exhibit gross phenotypic abnormalities. However, microscopic analyses of the whole skeletal system demonstrated profound defects in the growth plate cartilage with a marked increase in the hypertrophic chondrocyte zone and a delay in primary ossification (Figs. 2 and 3). During our preparation of the MMP-13 KO mice, two other groups have also described the generation of the MMP-13-null mice by homologous recombination [42] or Cre/Lox recombination methods [43]. The phenotype exhibited in our MMP-13 KO mice is principally similar to those of the MMP-13 knockout mice described in the literature. However, there are some differences among these three mouse lines. As shown in Fig. (2), the development of the primary ossification center in the skeletal system of our knockout mice is delayed, supporting the finding reported by Inada *et al.* [42], although Stickens *et al.* [43] did not identify such a delay.

On the other hand, all three mouse lines demonstrated marked defects in the growth plate cartilage, showing marked elongation of the hypertrophic chondrocyte zones (Figs. 3 and 4). The expansion of the hypertrophic chondrocyte zone appears to generally increase until 2 weeks of age and the phenotype normalized by 7 weeks of age (Figs. 3 and 4). The published studies did not describe changes in the secondary ossification center [42, 43]. However, in our MMP-13 KO mice, we also detected a delay in the secondary ossification of the long bones, which was ameliorated by 8 weeks of age as shown in Fig. (5). Thus, our MMP-13 KO mice exhibit a delay in the primary, growth plate and secondary ossification center, and as reported by Inada *et al.* [42], defective vascular invasion of the cartilage is associated with the developmental phenotype (Fig. 2).

Altogether, these data obtained from the MMP-13 KO mice suggest that MMP-13 secreted from hypertrophic chondrocytes plays an important role in facilitating endothelial cell and chondroclast invasion of the cartilaginous primordium probably through modulation of the ECM and/or growth factors such as vascular endothelial growth factor. Since MMP-13 KO mice exhibit no abnormality in the development and structure of articular cartilage, they are considered to be suitable for the analyses of MMP-13 function in articular cartilage destruction by inducing experimental OA and arthritides. Such studies are now under way in our laboratories.

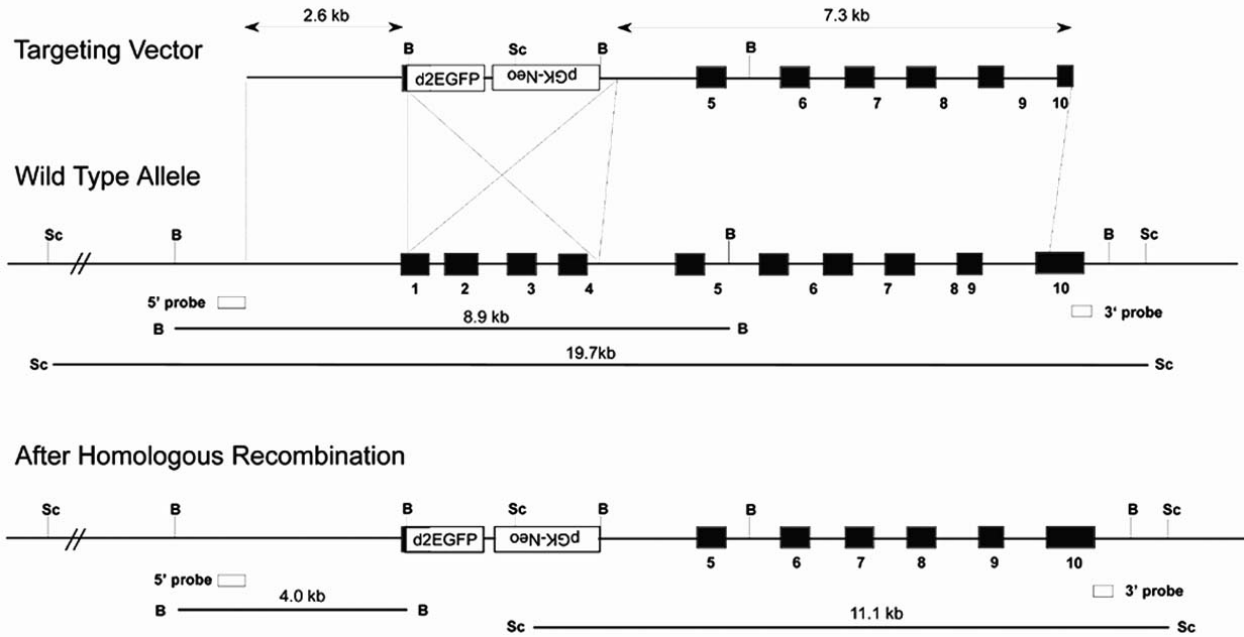


Fig. (1). Targeting of MMP-13 gene. (A) Schematic representation of the genomic organization of the wild-type and targeted alleles after homologous recombination. A 1.8 kb fragment (exons 1-4) containing the part of catalytic domain was excised from a genomic MMP-13 clone and replaced by d2EGFP and a neomycin cassette with the pgk promoter. The location of the fragment used as a probe for Southern blotting analysis is shown. Restriction sites are BamHI (B). (B) Southern blotting analysis. Genomic DNA was isolated from MMP-13 (+/+), MMP-13 (+/-) and MMP-13 (-/-) mice, digested with BamHI and analyzed by Southern blotting. Wild-type (8.9 kb) and mutant (4.0 kb) bands are indicated. (C) Northern blot analysis of MMP-13 gene expression in 15.5 dpc embryos of wild-type (+/+) and MMP-13 KO (-/-) mice (upper panel). 18S ribosomal RNA is shown as a loading control (lower panel). (D) Immunoblotting analysis of MMP-13 protein using supernatants from cultured embryonic fibroblasts from wild-type and MMP-13 KO mice.

INVOLVEMENT OF MMPs IN BONE RESORPTION

In the early stages of rheumatoid arthritis osteoclasts are responsible for bone resorption. Resorption is commonly observed at the bare zone, where pannus-like granulation tissue invades the bone marrow and destroys the subchondral bone. Bone resorption is a tightly regulated process involving bone-resorbing osteoclasts and bone-forming osteoblasts [44]. Osteoclasts are derived from hematopoietic stem cells expressing RANK and require RANKL signals from osteoblasts for their proliferation, differentiation and bone-resorbing activity [45]. They are specifically differentiated for bone resorption and attach to osteopontin on the bone surface through avb3 integrin. The cells then become highly polarized to form a tight ring-like sealing zone of adhesion and the subosteoclastic compartment is formed. ECM degradation of the mineralized bone is possible only after demineralization of the bone matrix, since proteinases cannot permeate the mineralized tissue. Thus, osteoclasts secrete H^+ into the compartment to decalcify the bone minerals prior to ECM degradation, and then the ECM is degraded under acidic (pH 4 to 5) and hypercalcemic (40 to 50 mM Ca^{++}) conditions [46].

Osteoclasts are known to synthesize several proteolytic enzymes such as cathepsin K and MMP-9, which are transported toward the apical side of the cells, secreted into the sealed compartment and degrade bone ECM components mainly type I collagen. Cathepsin K exhibits strong collagenolytic activity with a broad pH optimum and is selec-

tively expressed by osteoclasts and giant cell tumors [47]. In addition, cathepsin-K KO mice exhibit osteopetrosis and a short stature [48], a similar phenotype of autosomal recessive osteochondrodysplasia caused by a mutation in cathepsin K [49]. Thus, cathepsin K is considered to be critical for bone resorption. However, MMP-9 is also highly expressed by osteoclasts in normal and rheumatoid bones [50]. In addition, osteoclasts are known to express MT1-MMP [51] and MMP-7 [52] under pathological conditions. Since osteoclastic bone resorption is equally inhibited by cysteine proteinase inhibitors and metalloproteinase inhibitors [46], these MMPs as well as cathepsin K play a key role in osteoclastic bone resorption. Importantly, MMP-13 is expressed by osteoblasts and may be involved in bone resorption by degrading collagens present within the non-mineralized bone matrix (i.e. osteoid), since removal of osteoid on the mineralized bone surface is the essential process for osteoclasts to attach to the bone matrix for initiating the process of bone resorption. Studies on the role of MMPs such as MMP-9 and MMP-13 in bone resorption under experimental OA and arthritis models are limited. Future studies in the respective KO mice will likely provide better insight into the role of these proteases in this pathological process.

Multiple studies have demonstrated a prominent role for MMP-13 in tissue injury and repair. Therefore, further studies utilizing MMP-13 KO mice need to examine the particular function of this protease under disease conditions. For example, we have recently demonstrated that loss of MMP-13 protects mice from the development of fibrosis post bile

duct ligation injury [53]. In addition, unpublished studies from our laboratory demonstrate that mice lacking MMP-13 have a greater susceptibility to cardiac rupture post myocardial infarction (data not shown). These studies reveals a role for MMP-13 in tissue injury and repair and the findings will likely be recapitulated in other disease states utilizing the MMP-13 KO mice.

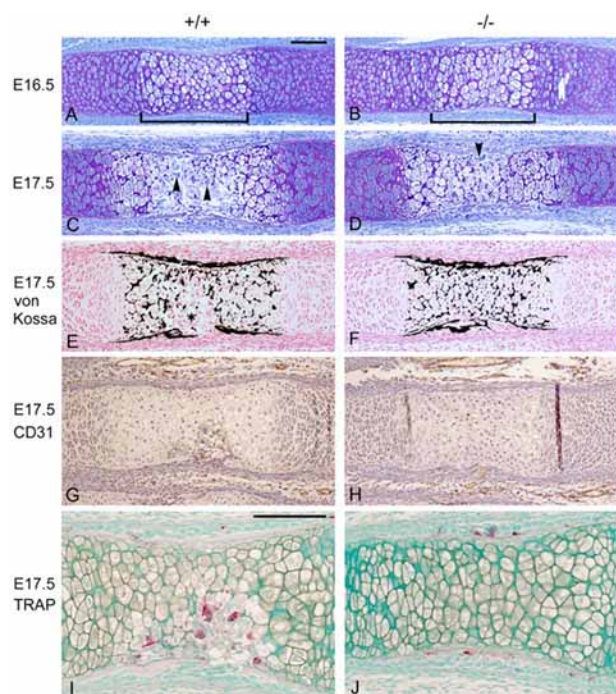


Fig. (2). Primary ossification process of metatarsals in wild-type and MMP-13 KO mice. Feet were obtained from wild-type (+/+) and MMP-13 KO (-/-) embryos at 16.5 (A and B) and 17.5 dpc (C-J) and metatarsals were histologically examined by staining with toluidine blue, von Kossa staining for mineralization (E and F), CD31 immunohistochemistry for vascularity (G and H) and TRAP staining for detection of osteoclasts (I and J) were also carried out. Note that primary ossification is delayed in MMP-13 KO mice (C and D) and that invasion of vascularity and TRAP-positive osteoclasts is decreased in MMP-13 KO mice (G-J).

THERAPEUTIC STRATEGIES USING MMP INHIBITORS

MMP activity within the tissue is controlled at several steps, which include gene expression of MMPs, production (transcription and secretion) of MMPs, activation of proMMPs and inhibition of MMPs activities by natural inhibitors (TIMPs and α 2-macroglobulin). Information about factors that modulate MMP gene expression (induction and suppression) and MMP secretion after intracytoplasmic synthesis is available [54, 55]. In addition, the activation processes of proMMPs have been intensively studied and the accumulated data indicate that proMMPs are activated by MMP species-specific steps, i.e. intracytoplasmic (e.g. MMP-11 and MT-MMPs), pericellular (e.g. MMP-2 and MMP-7) and extracellular (e.g. MMP-1 and MMP-3) activation pathways [1]. The numerous molecules involved in these pathways are also known. Thus, these are theoretically

targets for regulation of MMPs in joint tissues and the potential of signal transduction inhibitors for arthritis treatment has been described [56, 57]. However, inhibition by synthetic low molecular weight inhibitors to MMPs is more realistic for the treatment of arthritides.

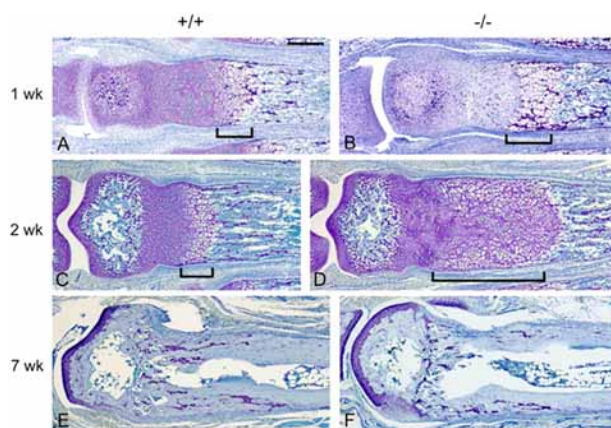


Fig. (3). Endochondral ossification of metatarsals in wild-type and MMP-13 KO mice. Feet were obtained from wild-type (+/+) and MMP-13 KO (-/-) mice at 1, 2 and 7 weeks and metatarsals were histologically examined. Note marked elongation of the hypertrophic chondrocyte zone in MMP-13 KO mice at 1 and 2 weeks.

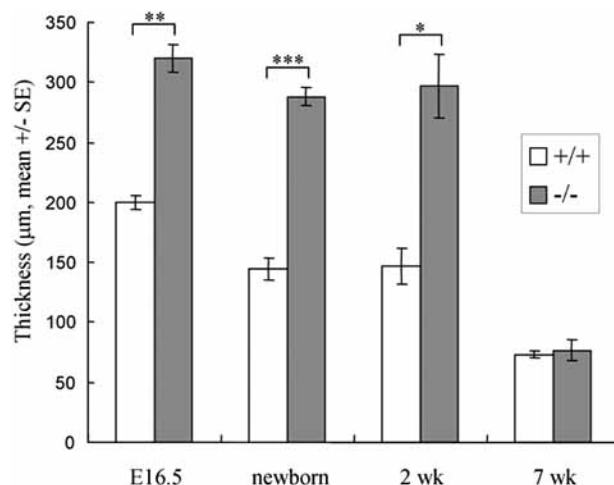


Fig. (4). Length of the hypertrophic chondrocyte zone in the tibiae. Note that the length is increased in MMP-13 KO (-/-) mice at 16.5 dpc, newborn and 2 weeks (n=3). *, p<0.05.

For the past 30 years, over 56 matrix metalloproteinase (MMP) inhibitors have been pursued as clinical candidates in various therapeutic areas, mainly for targeting cancer, arthritis, or cardiovascular diseases [58]. Despite these efforts, the majority of these have failed in clinical trials for various reasons including musculo-skeletal problems such as arthralgia, myalgia and tendonitis and other negative side effects of the nonspecific MMP inhibitors [59, 60]. Thus, no MMP inhibitor compound has been licensed thus far [61]. The most challenging aspect of developing MMP inhibitors is in finding candidates having acceptable pharmacological parameters

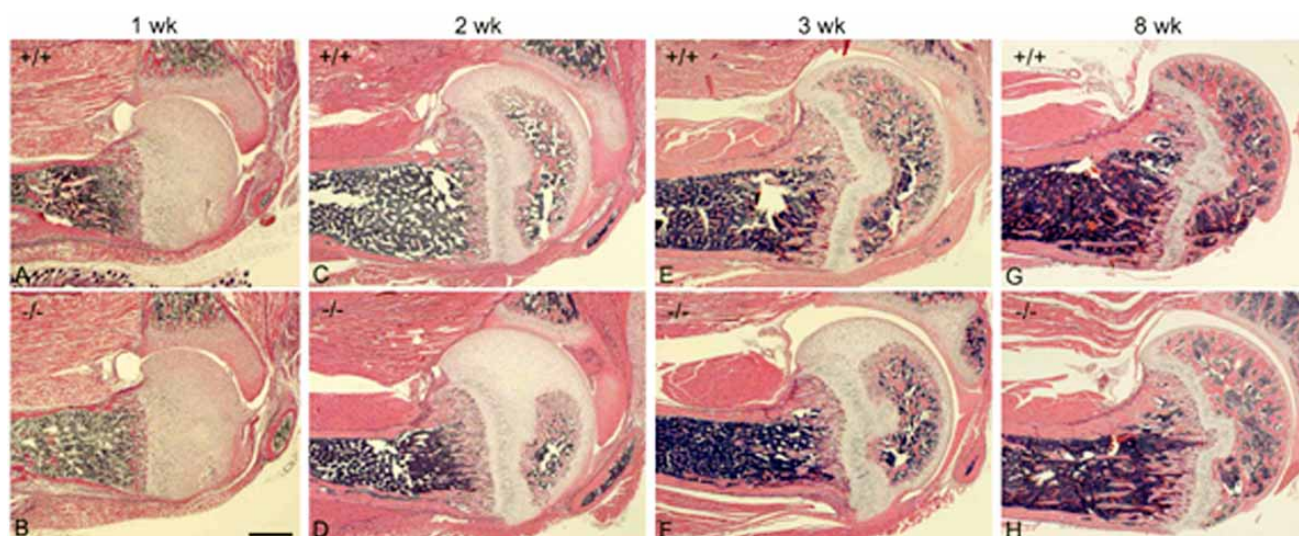


Fig. (5). Process of secondary ossification center formation in long bone of wild-type and MMP-13 KO mice. Humerus was obtained from 1-, 2-, 3- and 8-week-old wild (+/+) and MMP-13 KO (-/-) mice and histologically examined. Note that formation and development of secondary ossification are delayed in MMP-13 KO mice.

including PK and selectivity profiles [62]. Because of the availability of crystal structures for many MMPs [63], the second-stage MMP inhibitors with more selectivity to MMP species have been developed, and they appear to be less toxic. Furthermore, it appears that not one, but several MMP species such as MMP-13, MMP-3 and MT1-MMP as well as aggrecanases (ADAMTS-4 and ADAMTS-5) play a role in cartilage ECM degradation [64]. Further studies using experimental animal models are needed to develop effective strategies for arthritis treatment. MMP inhibitors specific to a single critical MMP or combined inhibitors that block both MMP and ADAMTS activities will likely be required in order to prevent joint destruction in arthritis patients [65, 66].

Early studies demonstrate that broad-spectrum MMP inhibitors have dose limiting toxicity in the form of musculoskeletal side effects including joint stiffness and inflammation [67], identified as the musculoskeletal syndrome (MSS). The cause of MSS is not clear however, to overcome this side effect it has become necessary to design more selective MMP inhibitors. In targeting MMP-13, a major goal is to identify compounds targeting MMP-13 but not other collagenases such as MMP-1 and MMP-14.

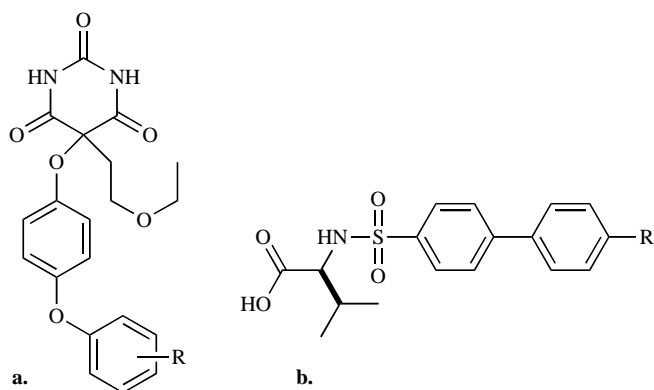


Fig. (6). Selective MMP-13 Inhibitors. **a.** Pyrimidinetrione analogs [68]. **b.** benzofuran mimetics [69].

Blagg *et al.* recently report the development of a series of pyrimidinetrione-derived orally available MMP-13 inhibitors that were highly selective for MMP-13 (Fig. 6a) [68]. This compound although highly selective for MMP-13 as compared to MMP-1 and 14, is not particularly selective against MMP-2, 8 or 12 [68]. Alternatively, Wu *et al.* have identified a series of selective MMP-13 inhibitors that has selectivity against not only MMP-14 but also MMP-2, 7, and 9 (Fig. 6b) [69]. The efficacy of selective MMP-13 inhibitors in osteoarthritis and osteoporosis should be examined in future clinical trials given the prominent role of MMP-13 in these disease processes.

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Received: April 10, 2006

Accepted: October 25, 2006