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Review

Unraveling metalloproteinase function in skeletal biology and disease using genetically altered mice

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ABSTRACT

The metalloproteinase family includes MMP, ADAM and ADAMTS proteases. Mice deficient in individual or pairs of metalloproteinases have been generated, and a number of these genetic models spontaneously develop skeletal abnormalities. Here we review metalloproteinase function in endochondral and intramembranous ossification, as well as in postnatal bone remodeling. We highlight how metalloproteinases enable interactions between distinct bone cell types and how this communication contributes to the skeletal phenotypes observed in knockout mice. In addition to the physiological actions of metalloproteinases in the skeletal system, the experimental manipulation of metalloproteinase-deficient mice has revealed substantial roles for these enzymes in osteoarthritis and rheumatoid arthritis. MMP, ADAM and ADAMTS proteases thus emerge as key players in the development and homeostasis of the skeletal system.

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1. Introduction

The extracellular matrix (ECM) is a highly heterogeneous mix of collagens, other proteins and proteoglycans that is essential for shaping multi-cellular organisms and imparting key characteristics to tissues. In vertebrates, the calcified bone matrix provides the structural plan for the body, protects organs, anchors the musculature, houses the bone marrow and acts as a calcium reservoir. Bone is a dynamic organ primarily composed of three cell types. At the bone surface are osteoclasts which degrade bone as well as osteoblasts which deposit new bone matrix and play a role in its mineralization. As bone is being formed, some osteoblasts become encased in their ECM and transition into osteocytes. These cells are the mechanosensors of bone and have unique structures and functions that will be described further in this review. Cartilage, the second major component of the skeleton, is avascular, rich in proteoglycans and type II collagen, and is constituted by chondrocytes. The extracellular matrix of growth plate cartilage is synthesized and maintained by chondrocytes of progressive maturity and contributes to the longitudinal growth of bone. Articular cartilage at joint surfaces has a different structure from that of growth plate cartilage and contributes to both efficient weight-bearing and the reduction of friction during joint movement.

Metalloproteinases, which were first identified as collagen cleaving enzymes, have come to fulfill novel functions in biology. Here we describe the growing body of evidence that metalloproteinases impact not only ECM remodeling but also cellular interactions in the matrix-rich skeleton. Metalloproteinases are named for their dependence on a zinc ion at the catalytic site, which is coordinated by a water molecule and 3 conserved histidine residues [1]. Matrix metalloproteinases (MMPs) are soluble proteases, with the exception of the 6 membrane type MMPs (MT-MMPs), and are primarily involved in cleaving specific ECM substrates along with cytokines and growth factors. Cell surface-bound proteinases termed ADAMs (A disintegrin and metalloproteinase[s]) have the capacity to shed growth factors, cytokines and their receptors from cell membranes. ADAMs with thrombospondin domains (ADAMTSs) are also soluble proteases principally involved in collagen and proteoglycan processing. These enzymes are regulated transcriptionally, through the activation of their zymogen forms and by their endogenous inhibitors, called tissue inhibitors of metalloproteinases (TIMPs) [2]. Fig. 1 illustrates the metalloproteinases and TIMPs localized to specific skeletal cell types by *in situ* hybridization. Evidence of metalloproteinase expression by primary bone cell cultures is not included. Of the MMPs expressed in bone, MMP2 and MMP13 have been linked to inherited human skeletal disorders. *MMP2* mutations were identified in several multicentric osteolysis syndromes, while the Missouri variant of spondyloepimetaphyseal dysplasia ($SEMD_{MO}$) is caused by a missense mutation in *MMP13* (reviewed by Fanjul-Fernándes et al. in this issue). Genetic mouse models have informed the study of these diseases and, conversely, the recognition of these syndromes has led to more specific analysis of the models.

This review focuses on data derived from the study of mice lacking metalloproteinases, either singly or in pairs, and of knock-in mice

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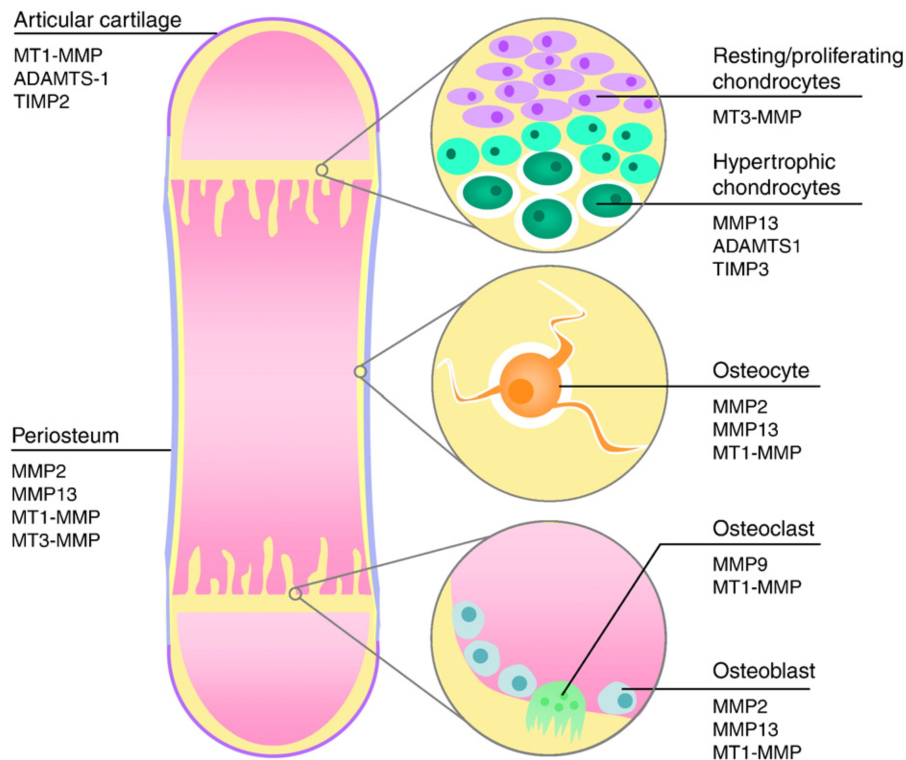

Fig. 1. Expression of metalloproteinases and their inhibitors in murine bone. A typical long bone is depicted with enlargements showing specific cell types. MT1-MMP is widely expressed in articular cartilage [116], the periosteum [7,69,116], osteocytes [69], osteoblasts [117] and osteoclasts [116,118]. MMP13 is specifically expressed in hypertrophic chondrocytes [23] as well as in the periosteum [69], osteocytes [69] and osteoblasts [23,35]. MMP2 is similarly found in the periosteum [116], osteocytes [33] and osteoblasts [117]. MMP9 is uniquely expressed by osteoclasts [16,22,119,120]. MT3-MMP is detected in the periosteum and in resting and proliferating chondrocytes [7]. ADAM-TS1 is expressed in articular cartilage and by hypertrophic chondrocytes [95]. Of the TIMPs, TIMP2 is expressed in articular cartilage [116] and osteoblasts [117] while TIMP3 is expressed in hypertrophic chondrocytes [121]. Only *in situ* hybridization studies which indicate the cell types responsible for metalloproteinase or TIMP expression are included.

where ECM components are mutated to prevent cleavage by metalloproteinase subsets. Most single metalloproteinase-deficient mice are born at the expected Mendelian ratios and have normal life spans, although certain ADAMs and MMPs are indispensable for proper embryonic development and postnatal survival. *Adam10* null mice die at E9.5 [3], while mice with catalytically inactive *Adam17*/*Tace* (TNF-alpha converting enzyme) die between E17.5 and one day after birth [4]. Of the single MMP deficient mice generated to date, only *Mt1-Mmp* nulls display reduced viability by weaning age [5,6]. The combined loss of *Mt1-Mmp* and *Mt3-Mmp* leads to lethality one day after birth due to clefting of the palate [7], while that of *Mt1-Mmp* and *Mmp2* results in a failure to initiate breathing and death within minutes of birth [8]. We integrate the information acquired through the study of the skeletons of these and other mouse models to highlight the commonalities and differences between metalloproteinase function in skeletal development, the regulation of bone remodeling and the progression of joint disease.

2. Metalloproteinases in skeletal development

Formation of the skeleton has long been known to require the progression of two major ossification processes. Endochondral ossification leads to the development of most bones in the body, while intramembranous ossification is essential for the establishment of flat bones. More recently, through the study of MMP deficient mice, a localized remodeling process was revealed to be important at specific sites throughout the skeleton. An excellent review by Krane and Inada comprehensively describes the skeletal phenotypes in MMP deficient mice [9]. Here, we present these developmental changes in the context of steps in the ossification process and additionally include relevant ADAM and ADAMTS skeletal phenotypes.

2.1. *Metalloproteinases in endochondral bone development*

2.1.1. *Mesenchymal cell condensation*

The first step in the development of the mammalian skeleton is the condensation of mesenchymal cells derived from the somatic layer of lateral plate mesoderm [10]. The physical compaction of mesenchymal cells has two phases, which involve changes in the ECM and the establishment of cell–cell contact. Increased hyaluronidase activity degrades hyaluronan while the fibronectin content of the matrix increases. Subsequently, cell–cell interactions are created, specifically through neural-cadherin (N-cadherin) and neural cell adhesion molecule (N-CAM) (reviewed in [11]). The remaining undifferentiated mesenchymal cells surround the cartilage core and form the perichondrium which later gives rise to osteoblasts (Fig. 2A). The role of metalloproteinases in the condensation and differentiation of mesenchymal cells to cartilage has not been well studied. N-cadherin is shed by the sequential activities of ADAM10 and the gamma-secretase complex in neuronal cells [12,13] and it has been recently demonstrated that N-cadherin can be shed by MMP9 and MMP12 in vascular smooth muscle cells [14]. N-CAM, a less well-established metalloproteinase target, can be cleaved *in vitro* by *Xenopus* MMP28 [15], but the efficiency of the human and murine MMP28 orthologs in N-CAM cleavage has not been described. Thus far, evidence from mouse models suggests that individual metalloproteinases do not play essential roles in mesenchymal condensation as the metalloproteinase-deficient mice generated to date have proper skeletal templates.

2.1.2. *Chondrocyte proliferation*

Once mesenchymal cells compact, chondrocyte differentiation is initiated. The former mesenchymal cells acquire the typical rounded appearance of chondrocytes and begin synthesizing a matrix high in

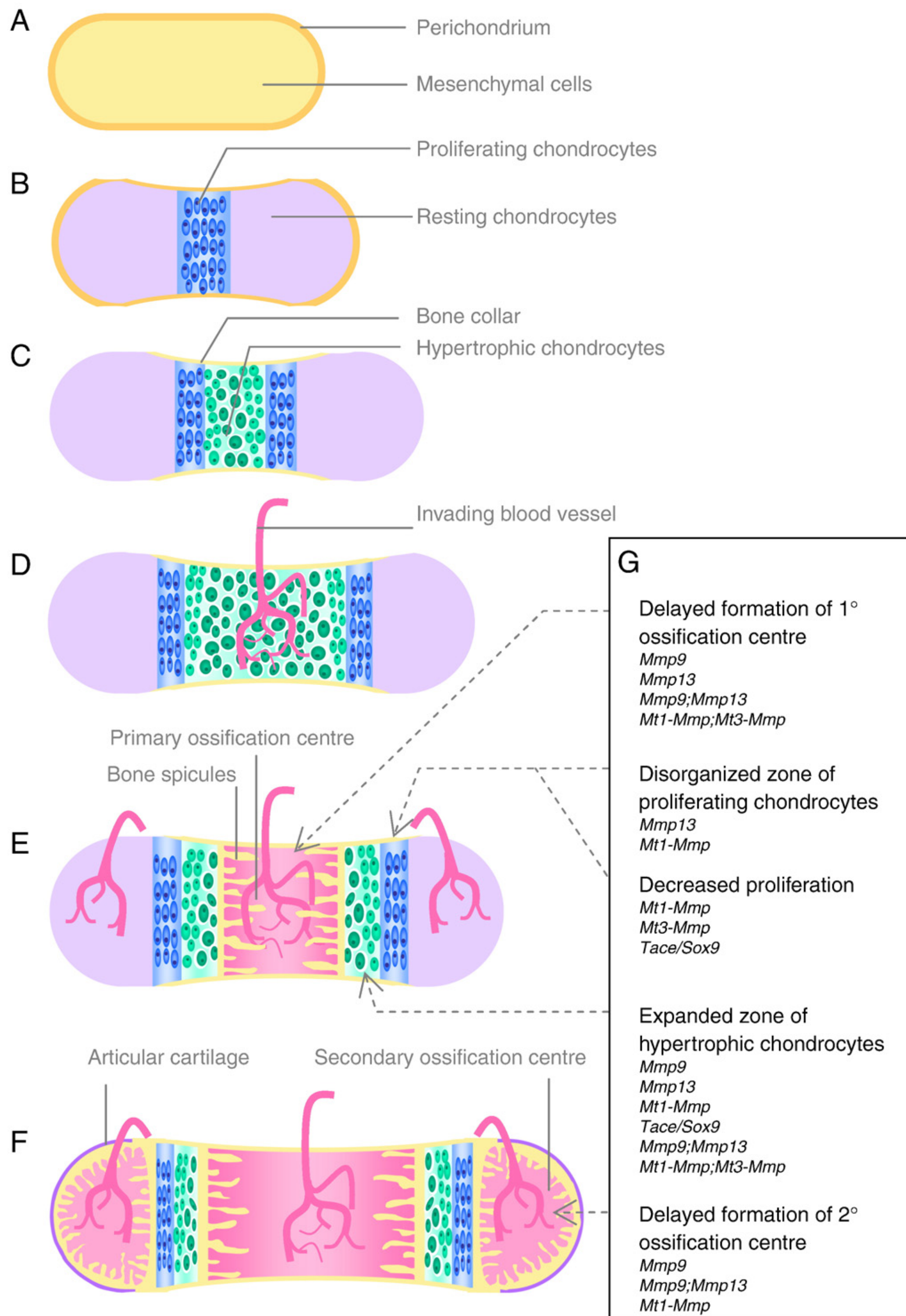

Fig. 2. Endochondral bone development is impacted by metalloproteinase loss in genetic models. (A) Endochondral ossification begins during embryogenesis when mesenchymal tissue condenses. (B) Mesenchymal cells differentiate into chondrocytes, and the most centrally located of these begin to proliferate. Remaining mesenchymal cells surrounding the cartilage core form the perichondrium. (C) Chondrocytes withdraw from the cell cycle, enter the prehypertrophic stage and progress to hypertrophy, enlarging and mineralizing the surrounding matrix. Hypertrophic chondrocytes induce cells within the perichondrium to differentiate into osteoblasts and form a bone collar. (D) The primary ossification centre is formed when the scaffold of mineralized cartilage is invaded by blood vessels carrying hematopoietic cells that populate the marrow cavity and give rise to osteoblasts and osteoclasts. (E) Osteoclasts/chondroclasts degrade the transverse bridges that previously surrounded the hypertrophic chondrocytes, leaving behind spicules of cartilage that form the basis of the trabecular bone spicules. Osteoblasts deposit their own matrix on these calcified cartilage templates. Concurrently, chondrocytes in the bone epiphyses have been maturing and are invaded by blood vessels. (F) The secondary ossification centres form leaving articular cartilage to cushion joint surfaces and growth plate cartilage separating the diaphysis of the bone from the epiphyses. (G) The phenotypes observed in metalloproteinase null mice, with arrows indicating the stage of development impacted. (;) = double deficient mice, (/) = conditional knockout.

collagen type IIa and aggrecan. These chondrocytes proliferate and form organized columns of cells that result in the longitudinal extension of the cartilage template (Fig. 2B). Disorganized columns of chondrocytes are observed in the developing bones of *Mmp13*$^{-/-}$ and *Mt1-Mmp*$^{-/-}$ mice [5,6,16]. In both cases these changes are not due to alterations in the expression of the essential cartilage signaling molecules, Indian hedgehog (Ihh) and its receptor patched (Ptc), or the PTH/PTHrP (parathyroid hormone/parathyroid hormone-related peptide) receptor (PPR) [5,16]. Additionally, in *Mt1-Mmp*$^{-/-}$ mice, decreased chondrocyte proliferation is observed

in the growth plate cartilage of 5.5-week-old mice [6]. The zone of proliferating chondrocytes in *Mt3-Mmp*$^{-/-}$ mice is also characterized by decreased proliferation [7]. However, the combined loss of *Mt1-Mmp* and *Mt3-Mmp* does not further decrease proliferation when compared to the levels in *Mt1-Mmp*$^{-/-}$ mice, indicating a common function of MT1-MMP and MT3-MMP in the maintenance of proliferating chondrocytes [7]. Recently, using a *Sox9-Cre* promoter, *Adam17* was conditionally deleted in cells of the osteoblast and chondrocyte lineages (*Tace*/*Sox9* mice) [17]. This loss of TACE activity caused a shortening of the zone of proliferating chondrocytes and a decrease in the overall level of chondrocyte proliferation [17]. Primary knockout and wild type chondrocyte cultures proliferated at the same rate, suggesting that cell-extrinsic mechanisms are responsible for the *in vivo* phenotype [17].

2.1.3. Chondrocyte hypertrophy

The transition of proliferating chondrocytes to the prehypertrophic stage is marked by a cessation of proliferation and a shift in the balance of collagen production from type II to type X [18]. Prehypertrophic chondrocytes secrete Ihh, which increases the proliferation of the less mature chondrocyte population and, by upregulating perichondrial PTHrP secretion, delays the progression of prehypertrophic cells to hypertrophy [19]. When chondrocytes enter the hypertrophic stage they expand in size 5–10 fold, secrete type X collagen, deposit vascular endothelial growth factor (VEGF) into the matrix [19,20], as well as produce alkaline phosphatase and calcify their ECM [21]. Terminally hypertrophic chondrocytes additionally express *Mmp13* prior to undergoing apoptosis, and leave behind empty lacunae surrounded by a calcified matrix that is subsequently remodeled into bone. During this process, Ihh secreted by prehypertrophic chondrocytes also induces mesenchymal cells within the perichondrium to differentiate into osteoblasts and form a collar of bone around the cartilage template in a PTHrP dependent manner [19] (Fig. 2C). Given that the expression of *Ihh* has not been found to be altered in MMP deficient bones by *in situ* hybridization, it is not surprising that no defects are reported in bone collar formation in these mice.

Metalloproteinase loss affects the hypertrophic stage of cartilage development more strongly than the proliferative stage. Expansion of the hypertrophic zone has been noted in several single and double metalloproteinase knockout mice with varied severity. Prenatally, the hypertrophic zones in the long bones of E16.5 *Mt1-Mmp*$^{-/-}$ embryos are expanded 3–4 times compared to those in wild type embryos but the severity of this phenotype decreases with age [5]. Mice doubly deficient for *Mt1-Mmp* and *Mt3-Mmp*, similarly have an expanded hypertrophic zone at birth [7]. At this stage, the hypertrophic zone in *Mmp9*$^{-/-}$ mice is approximately twice as long as in wild type mice and increases 6–8 times by 3 weeks of age [22]. While the hypertrophic chondrocytes in these mice are morphologically normal and able to calcify the matrix, their death by apoptosis is delayed [22]. Increased hypertrophic zones are also noted in a variety of bones in *Mmp13*$^{-/-}$ mice, but with different severities and timelines. The population of most terminally differentiated hypertrophic chondrocytes, which normally express *Mmp13* and osteopontin, is specifically expanded [16,23]. Chondrocyte-specific deletion of *Mmp13* through the use of *Cre* recombinase expressed under the type II collagen promoter in chondrocytes did not rescue this phenotype, while osteoblast-specific deletion using the type I collagen promoter resulted in a normal growth plate, demonstrating that chondrocyte produced MMP13 is essential for the proper remodeling of hypertrophic cartilage early in development [23]. However, such increases are transient as the hypertrophic zones of *Mmp9*$^{-/-}$ and *Mmp13*$^{-/-}$ mice are comparable to those of wild type mice by 8 and 12 weeks of age, respectively [22,23]. Similarly, the metatarsals of *Tace*/*Sox9* mice display a transient expansion of the hypertrophic zone at 2–3 weeks of age that resolves by 8 weeks of age [17]. It should be noted that in another model of *Mmp13*$^{-/-}$ mice a mild increase in the growth plate width persists at least until 16 weeks of age [16]. Together, these studies indicate that in the absence of a critical MMP, alternate means of cartilage turnover are employed. The compensatory roles of MMP9 and MMP13 in hypertrophic cartilage remodeling are suggested in double *Mmp9*$^{-/-}$*Mmp13*$^{-/-}$ knockout mice which have overpopulated, dramatically expanded zones of hypertrophic cartilage with disrupted architecture at 5 weeks of age. Notably, even this phenotype resolves by 5 months of age [23].

Growth plate defects do not occur in mice deficient in *Adamts1* [24], *Adamts4* [25] or *Adamts5* [26]. Consistent with this, transgenic mice expressing mutated aggrecan that cannot be cleaved by either MMPs [27] or ADAMTSs [28] fail to show growth plate or overall bone defects. Of the ADAMTSs, ADAMTS4 is the primary aggrecanase expressed in the growth plate. The lack of aggrecan accumulation in *Adamts4*$^{-/-}$, *Adamts5*$^{-/-}$ or *Adamts4*$^{-/-}$*Adamts5*$^{-/-}$ mice, and the absence of detectable compensatory aggrecan cleavage probed through neo-epitope antibodies suggests that growth plate aggrecan is cleared by mechanisms other than metalloproteinase-mediated activity [25,29].

2.1.4. Vascular invasion and ossification centre formation

The progression of endochondral ossification requires that bone building cells, osteoblasts, and bone degrading cells, osteoclasts, have access to the cartilage. This occurs via blood vessel invasion into the hypertrophic zone (Fig. 2D), which is initiated by hypertrophic chondrocyte secretion of VEGF, and delivers osteoblasts and hematopoietic cells, including osteoclast precursors. The degradation of the cartilage matrix is suggested to occur specifically through the actions of cells called chondroclasts [22], although the precise nature of these cells and their relation to osteoclasts is unclear. Regardless, once differentiated, osteoclasts/chondroclasts begin degrading the calcified cartilage matrix, carving out a space termed the primary ossification centre in the middle of the bone. At the same time, osteoblasts begin laying down a bone matrix on the remaining spicules of cartilage matrix (Fig. 2E). With time and as the primary ossification centre expands, these spicules are completely replaced with bone matrix. Soon after birth, this process is repeated in the epiphyses (ends) of the bone. Here, vascular invasion through uncalcified cartilage creates the secondary ossification centres leaving articular cartilage at the joint surfaces and growth plate cartilage separating the primary and secondary ossification centres (Fig. 2F).

Proper remodeling of the ossification centres is impacted by the loss of individual or pairs of MMPs. *Mmp9*$^{-/-}$ mice display markedly delayed enlargement of the primary ossification centre [22]. The abnormally expanded zone of hypertrophic cartilage is eventually remodeled ectopically following aberrant chondrocyte apoptosis [22]. *Ex vivo* growth plate cultures allowed the study of blood vessel formation and indicated that reduced angiogenesis delays growth plate remodeling in *Mmp9*$^{-/-}$ mice [22]. Impaired blood vessel invasion is also noted in *Mt1-Mmp*$^{-/-}$ mice where the primary ossification centre forms appropriately, but the creation of the secondary ossification centres is delayed and occurs in an unusual manner [5]; once the cartilage adjacent to the bone calcifies, the ossification front follows osteoclast migration from the periphery towards the middle, as opposed to progressing outwards from the middle following vascular invasion [5,6]. The effect of *Mmp13* loss on the formation of the primary ossification centre is less clear. One study reported no difference in the initiation of the primary centre nor in the distribution of angiogenic markers [23] while another group reported a delay in centre formation from E15.5 to E17.5 [16]. However, both studies are in agreement in observing a lack of staining for collagen neo-epitopes at the last transverse septae (horizontal bridge of matrix closest to the ossification front) of the growth plates of *Mmp13*$^{-/-}$ mice, suggesting that MMP13 is the primary collagenase at this site [16,23]. The completion of the primary ossification centre formation is

more delayed in *Mmp9*$^{-/-}$*Mmp13*$^{-/-}$ mice than in the single knockouts, and these double knockouts also exhibit delayed development of the secondary ossification centres [23]. Further, the neo-epitope generated by MMP cleavage of aggrecan is absent at their ossification front, although it is seen in *Mmp9*$^{-/-}$, *Mmp13*$^{-/-}$ and wild type mice indicating the involvement of both of these MMPs in the cleavage of aggrecan [23]. Recently, antibodies raised against the neo-epitopes exposed when MMP2, 9 and 13 lose their pro-domains were used to localize active MMPs during the invasion of vascular canals that create the secondary ossification centre [30]. In line with the genetic data described above, Lee et al. found that MT1-MMP is cell-bound at the invasion front, while active MMP13 and MMP9, but not MMP2, are detected in the ECM and are associated with type II collagen degradation [30]. Analysis of another double knockout combination, *Mt1-Mmp*$^{-/-}$*Mt3-Mmp*$^{-/-}$, revealed a prominent phenotype where islands of intact hypertrophic cartilage remain within the primary ossification centre of long bones in newborn mice [7]. This demonstrates a role of these membrane anchored metalloproteinases in efficient cartilage remodeling. Further analysis of primary and secondary ossification centre formation was prevented by the early death of these double mutants. Lastly, a brief report noted a delay in osteoclast invasion into the hypertrophic cartilage of the metatarsals of E18.5 *Tace*$^{zn/zn}$ mice where TACE is catalytically inactive due to the deletion of the zinc binding domain [31]. However, the progression of ossification centre formation was not described, nor was the distribution of VEGF or endothelial cells. These features have not yet been described in *Tace*/*Sox9* conditional knockout mice [17]. Overall, these murine studies show that MMP activity is important, but individually not essential, for the ultimate formation of the bone marrow cavity.

2.2. Intramembranous ossification

While the majority of the skeleton develops through endochondral ossification, the flat bones of the skull, the mandible and part of the clavicles arise by intramembranous ossification. This process begins with the condensation of mesenchymal cells derived from the neural crest and paraxial mesoderm rather than from the lateral plate mesoderm as in endochondral ossification [10]. During intramembranous ossification mesenchymal cells differentiate into osteoblasts, not chondrocytes, and directly calcify the matrix.

Mmp2 knockout mice were originally reported to develop normally with only a slight growth delay [32]. However, identification of the human nodulosis, arthropathy, osteolysis (NOA) familial syndrome caused by an *MMP2* mutation prompted closer inspection of these mice and several deformities were subsequently reported. Snout, jaw and overall skull length are decreased, the cranial vault is dome-shaped and the sutures are sclerotic in *Mmp2*$^{-/-}$ mice [33,34]. *Col1a1*$^{r/r}$ knock-in mice, which harbour an engineered mutation in *Col1a1* that results in type I collagen which is resistant to collagenase activity, also exhibit changes in the skull starting at 6 weeks and progressing with age [35]. Combining these two mutant strains (*Mmp2*$^{-/-}$*Col1a1*$^{r/r}$) causes severe runting and a decreased lifespan, with more pronounced skull defects than those seen in either mutant alone. The calvarial bones are not fully formed even at 5 months of age, sclerotic membranes persist instead of bone in the calvaria, and sutures that do form are thin and porous in these double mutant mice [36]. Similarly, *Mt1-Mmp*$^{-/-}$ mice have prominent sutures, shortened mandibular and maxillary bones, dome-shaped cranial vaults, and have incomplete suture closure between the parietal and interparietal bones [5,6]. Severe membranous bone deformities arise in *Mt1-Mmp* and *Mt3-Mmp* double knockout mice. At birth these mice display shortened, domed skulls, poorly formed bones, decreased bone mineralization and, fatally, cleft palates [7]. The contributions of osteocyte activity to the skull phenotypes in *Col1a1*$^{r/r}$, *Mmp2*$^{-/-}$ and *Mt1-Mmp*$^{-/-}$ mice will be described later (Section 3.3).

2.3. Non-endochondral cartilage remodeling

A distinct process in bone development that involves the remodeling of specific cartilage elements was identified through the observation of “ghost cartilage” remnants in the skulls of *Mt1-Mmp* deficient mice, and termed metamorphic remodeling. This observation prompted Holmbeck et al. to identify focal sites other than the calvaria where this process occurs: i) Merckel's cartilage, the cartilage precursor for the mandible, malleus of the inner ear and spheno-mandibular ligament, ii) the insertion sites of ligaments into cartilage and bone, and iii) the groove of Ranvier in long bones [6,37]. While the remodeling of cartilage during endochondral development is dependent on the actions of osteoblasts and osteoclasts, it has been proposed that metamorphic remodeling is carried out by chondrocytes themselves. *Mt1-Mmp* expression by non-hypertrophic chondrocytes leads to degradation of the unmineralized cartilage matrix and chondrocyte apoptosis [37]. Such non-endochondral cartilage remodeling is limited to specific tissues and is a previously unappreciated, yet essential part of skeletal development at these sites.

3. Metalloproteinases facilitate bone cell interactions during skeletal remodeling

Bone remodeling occurs throughout adult life and is essential for maintaining bone health and circulating calcium levels (reviewed in [38]). An imbalance between resorption and deposition underlies diseases where bone density is altered including osteopetrosis, and more commonly, osteopenia and osteoporosis. Several metalloproteinase-deficient mice have abnormal bone remodeling as summarized in Table 1. Given the established role of metalloproteinases in matrix degradation, one might expect that they contribute directly to bone resorption. However, metalloproteinases function at physiological pH and the acidic environment of the osteoclast resorptive pit is not conducive to their activity. Rather, the cathepsins, largely belonging to the cysteine protease family, are responsible for osteoclastic bone resorption, with cathepsin K being the primary effector [39–41].

We describe below the varied roles of metalloproteinases in bone remodeling, which include i) regulating the bioavailability of critical factors, ii) operating as effectors upon hormonal stimulation, and iii) causing structural changes to enable the development of the osteocyte canalicular network. This physical network allows osteocyte processes to form gap junctions that make possible the transfer of secreted proteins between other osteocytes as well as osteoblasts [42]. In each case metalloproteinase activity is important for regulating or establishing communication between bone cells — osteoblasts, osteoclasts and osteocytes. When these means of communication are perturbed by the absence of a specific metalloproteinase activity, the balance of bone remodeling is altered and changes in the bone structure result.

3.1. Metalloproteinases regulate osteoclast activation via the RANK/RANKL/OPG axis

As mentioned, both bone resorption by osteoclasts and bone deposition by osteoblasts are necessary for appropriate bone growth and remodeling. Until a decade ago, the factors regulating osteoclastogenesis were unknown, although it was hypothesized that stromal cells and osteoblasts played a role in osteoclast differentiation. The discovery of the secreted receptor osteoprotegrin (OPG) led to the identification of its ligand, originally named OPGL, which was found to be identical to TNF-related activation induced cytokine (TRANCE) expressed on activated T-cells, and is most commonly referred to as receptor activator of NFκB ligand (RANKL) due to its association with the receptor RANK (reviewed in [43]). The importance of the OPG/RANK/RANKL triad in bone biology was demonstrated in mice deficient in these molecules.

Table 1
Long bone remodeling in genetic mouse models with impaired metalloproteinase activity.

	Osteoblasts	Osteoclasts	Osteocytes	Trabecular bone	Cortical bone
Mmp2$^{-/-}$ [33,34]	Transiently decreased numbers (4d), decreased ability to differentiate *in vitro*	Transiently decreased numbers (4d), decreased ability to differentiate *in vitro*	Mildly disrupted canalicular network and no difference in viability (5w onwards)	Decreased mineralization (5w onwards), transiently decreased volume (4d)	Decreased mineralization (5w onwards), ragged appearance (4d)
Mmp9$^{-/-}$ [22]				Transiently decreased (3w), then increased (4w), then normal (8w)	No difference
Mmp13$^{-/-}$ [23]	No difference in proliferation	No difference in number, localization		Increased (3w-6m), resolved by 1y	No difference
Mt1-Mmp$^{-/-}$ [6,69]	Decreased activity and viability (40d onwards)	Increased activity and numbers (40d onwards)	Severely disrupted canalicular network but no change in viability (20d onwards)	Decreased (40d onwards)	Decreased (40d onwards)
Tace$^{-/-}$/*Sox9* [17]	Increased activity (8w)	Increased numbers, activity (8w)		Decreased (8w onwards)	Decreased (8w onwards)
Col1a1$^{r/r}$ [33,35,61]	Decreased viability, activity (4w)	Increased numbers, decreased activity (4w)	Severely disrupted canalicular network and fewer viable osteocytes (3w onwards)	Increased (4w onwards)	No change (4w), increased (10m)

d = day; w = week; m = month; y = year.

Both RANK and RANKL null mice are osteopetrotic due to a lack of mature osteoclasts, whereas mice lacking the decoy receptor OPG have excessive osteoclast activity and develop osteoporosis [44–46]. Specifically, RANKL expressed by both stromal cells and mature osteoblasts interacts with its receptor RANK on osteoclast precursors (Fig. 3A), triggering multiple signal transduction pathways that drive osteoclast maturation and activation [47].

Soon after the discovery of the OPG/RANK/RANKL triad, it was shown that RANKL can be shed from the cell surface and that normally this shedding downregulates osteoclast activation [48,49]. Several metalloproteinases have been investigated as putative RANKL sheddases (reviewed in [50]). While TACE and ADAM19 are able to cleave RANKL peptides *in vitro*, TACE is unable to cleave full length RANKL [51] and ADAM19 only does so when over-expressed [52]. ADAM19 does not generate the same cleavage products as detected in primary osteoblast cultures [53]. On the other hand, MT1-MMP shedding of RANKL has been observed in primary osteoblast cultures and ADAM10 also produces cleavage products identical to those generated in primary osteoblasts. Thus these two proteases are likely the physiologically relevant RANKL sheddases (Fig. 3A) [53]. The *in vivo* effect of ADAM10-mediated RANKL shedding is unknown as *Adam10* null mice die at E9.5 before the formation of the skeleton [3], and to date no skeletal-specific deletion of *Adam10* has been reported. The excessive osteoclast activity and early onset osteopenia in *Mt1-Mmp*$^{-/-}$ mice [6] could possibly arise from an increased bioavailability of cell-anchored RANKL. It should be noted that changes in osteoblast characteristics also likely contribute to osteopenia in *Mt1-Mmp* null mice; osteoblast activity and proliferation are decreased, osteoblasts lining the periosteal bone surface are displaced into the fibrotic periosteum and they have a decreased capacity to cleave collagen substrates [6]. The molecular mechanism(s) by which MT1-MMP loss causes these changes in osteoblasts has yet to be elucidated.

The activity of soluble RANKL and the metalloproteinases responsible for its shedding appear to be altered in pathological settings. While the shedding of RANKL at physiological levels downregulates osteoclast activation, high levels of soluble RANKL, either globally or focally, are able to efficiently activate osteoclasts. Overexpression of a soluble RANKL construct in mice caused osteoporosis [54]. In a model of prostate cancer metastasis to bone, MMP7 sheds RANKL leading to osteoclast activation and osteolysis [55] (Fig. 3A). *Mmp7*$^{-/-}$ mice have been described as phenotypically normal, although a detailed examination of their skeletons has not been reported [56]. Overall, metalloproteinases are important in regulating physiological and pathological osteoclastogenesis, as the ability to shed RANKL provides stromal cells and osteoblasts with an additional level of control in mediating osteoclast maturation and activation.

3.2. Osteoblastic MMP13 mediates PTH catabolic signals

Parathyroid hormone (PTH) is produced by the parathyroid glands to maintain calcium homeostasis. Intermittent exposure of bone to low levels of PTH results in an overall anabolic effect and is a current therapy for osteoporotic patients, but high PTH levels promote catabolic activity, releasing calcium into the blood [57]. Although osteoclasts are the ultimate target of PTH stimulation, it is osteoblasts that normally express its receptor PPR and mediate PTH signals (reviewed in [58]). PPR activation in osteoblasts leads to protein kinase A signaling which triggers changes in gene expression, including downregulation of type I collagen, osteonectin and alkaline phosphatase, and upregulation of RANKL and MMP13 (Fig. 3B) (reviewed in [59]). MMP13 is the only reported MMP to be induced in response to catabolic PTH stimulation.

Striking evidence of the importance of MMP activity in the catabolic response to PTH is observed in *Col1a1*$^{r/r}$ mice. Typically, repeated supra-calvarial injection of PTH leads to increased bone resorption as seen by enlarged marrow spaces and prominent osteoclasts in wild type mice. In *Col1a1*$^{r/r}$ mutants, however, few osteoclasts are observed and bone resorption is minimal even though the osteoblastic response to PTH and MMP13 production are intact [60]. In line with this data, when mice expressing a constitutively activated PPR in osteoblasts are crossed with *Col1a1*$^{r/r}$ mice, osteoclast activity is diminished [61]. The effect of exogenous PTH has not been determined in *Mmp13*$^{-/-}$ mice, although this MMP is critical in bone remodeling as *Mmp13*$^{-/-}$ mice uniquely exhibit osteopetrotic changes in trabecular bone; other MMP knockout mice show either no change or osteopenia as outlined in Table 1 [16,23]. Elegant genetic experiments by Stickens et al. show that this increase in trabecular bone is due to the specific loss of *Mmp13* in osteoblast and not in chondrocyte populations [23]. These studies suggest that the inability of osteoblasts to transmit PTH signals to osteoclasts may contribute to the osteopetrosis in *Mmp13*$^{-/-}$ mice.

As an established target of PTH-mediated gene regulation, MMP13 is considered to be the principal metalloproteinase involved in generating signals that recruit or activate osteoclasts, with the postulated mechanisms illustrated in Fig. 3B. Early *in vitro* experiments showed that interstitial collagenase can prime bone for osteoclast resorption by clearing surface collagen and exposing the underlying mineralized bone [62,63]. Additionally, collagenase digested collagen fragments can stimulate osteoclast activity [62,63]. Furthermore, it is possible that collagen cleavage reveals cryptic sites important for osteoclast survival [60]. Survival signaling is likely mediated through interaction with $\alpha v\beta 3$ integrin, which is highly expressed by osteoclasts and involved in bone resorption [64–66], although, direct evidence to support the role of cryptic peptides in osteoclast survival is lacking.

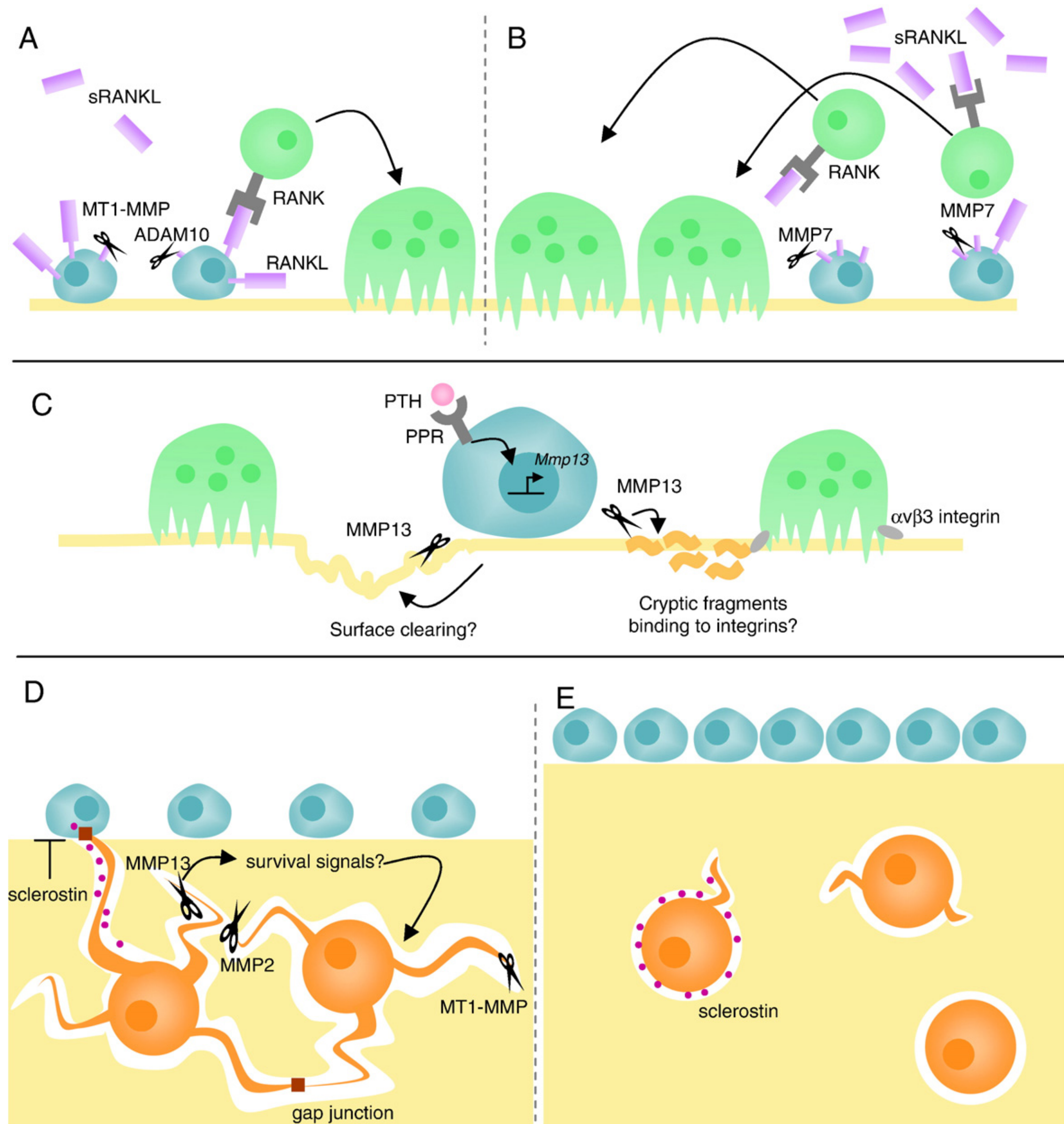

Fig. 3. Metalloproteinases enable communication between bone cells. (A) RANKL shedding from stromal cells or osteoblasts at physiological levels decreases the amount of biologically active RANKL and downregulates osteoclastogenesis. (B) In pathological settings, excessive RANKL shedding creates a local pool of soluble RANKL that activates osteoclast precursors and upregulates osteoclastogenesis. (C) PTH stimulation of osteoblasts leads to the production of the collagenolytic MMP13. The cleavage of collagen type I by MMP13 contributes through unknown mechanisms to osteoclast activation, potentially by pre-clearing surface collagen and facilitating osteoclast attachment and/or by generating cryptic collagen fragments that interact with osteoclast αvβ3 integrin receptors to promote osteoclast survival. (D) MMP2, MMP13 and MT1-MMP create and maintain the osteocyte canalicular network allowing communication between osteocytes as well as osteoblasts at the bone surface. The osteocyte-secreted protein, sclerostin, downregulates osteoblast activity at the bone surface. MMP2 and MMP13 activity is also important for osteocyte survival, through unknown mechanisms. (E) In the absence of MMP2, MMP13 or MT1-MMP the canalicular network is disrupted and calvarial bone thickens, likely due to the absence of sclerostin inhibitory activity on osteoblasts. Empty lacunae and osteocytes undergoing apoptosis are common in MMP2 and MMP13 deficient calvaria.

3.3. Metalloproteinases maintain the canalicular network enabling cell–cell communication

Osteocytes are cells of osteoblastic origin that reside in lacunae within the bone matrix. By sending cellular processes through an extensive network of canaliculi they establish gap junctions and maintain physical connections with osteoblasts at the surface and other osteocytes [42]. Osteocytes make up 90–95% of the total cells in adult bone and are believed to be the primary, if not sole, sensors of mechanical strain on the bone by detecting shear stress caused by fluid movement within the canalicular network (reviewed in [67]). The ability of osteocytes to sense decreased bone loading and effect changes in osteoblast and osteoclast activity were demonstrated *in vivo* through the specific ablation of approximately 70–80% of the osteocyte population in adult mouse bones [68]. These mice were resistant to unloading-induced bone loss following tail suspension [68], and highlight the importance of an intact osteocyte network for communicating signals to cells present at the bone surface.

The presence of a proper canalicular network is integral to osteocyte communication and metalloproteinase activity is necessary to establish and maintain this network. The first evidence of this was shown by Zhao et al. in 2000. *Col1a1*$^{r/r}$ mice have an increased number of empty lacunae, as well as increased apoptosis of the remaining osteocytes at 1 month of age, that persists at 12 months of age in both the calvaria and long bones [35]. This finding is associated with increased bone deposition at both sites in the aged mice [35]. The

calvaria of aged *Mmp2*$^{-/-}$ mice show an almost identical phenotype of thickened bones containing a higher number of empty osteocyte lacunae and increased osteocyte apoptosis [33]. However, unlike in the *Col1a1*$^{r/r}$ mice, the long bones of *Mmp2*$^{-/-}$ mice do not show the same defects and have deficient mineralization [33,34]. When measured, the canalicular network was moderately decreased in *Mmp2*$^{-/-}$ long bones but more severely affected in *Mmp2*$^{-/-}$ calvaria, while both calvaria and long bones in *Col1a1*$^{r/r}$ mice showed a severe disruption [33]. A compromised network of osteocyte processes is also noted in the long bones of *Mt1-Mmp*$^{-/-}$ mice starting as early as 20 days of age, however osteocyte viability is not affected in these mice [69]. The importance of collagen cleavage for osteocyte viability is not well understood. MT1-MMP is an important component of the triad necessary for MMP2 activation [70–72] and MMP2 activation is severely compromised in *Mt1-Mmp*$^{-/-}$ mice [5]. It is possible that the minimal residual MMP2 activity in *Mt1-Mmp*$^{-/-}$ mice is sufficient to protect osteocytes against the apoptosis observed in *Mmp2*$^{-/-}$ bones. The lack of osteocyte apoptosis in *Mt1-Mmp*$^{-/-}$ mice also suggests that the absence of a well-developed canalicular network is, in itself, insufficient to induce osteocyte death.

The requirement of MMP activity for carving out canalicular spaces is not surprising. Wild type osteocytes express *Mmp2*, *Mmp13* and *Mt1-Mmp* [33,69,73] and MMP-mediated collagen cleavage products are localized around osteocyte bodies and processes [35,69]. As expected, these cleaved collagen fragments are not observed in *Col1a1*$^{r/r}$ mice [35]. Strikingly, however, they are also undetectable in *Mt1-Mmp*$^{-/-}$ mice, suggesting that the MMP13 which is produced cannot sufficiently compensate for the lack of MT1-MMP activity during the invasion of osteocyte process into the bone matrix [69]. It remains possible that MMP13 is not efficiently activated in this case, as both MT1-MMP and MMP2 are known to activate MMP13 *in vitro* [74,75]. To date, the canalicular networks of *Mmp13*$^{-/-}$ mice have not been examined. The link between osteocyte network integrity and increased bone formation may be related to changes in the distribution of osteocyte-produced proteins. Sclerostin is secreted by osteocytes and delivered to osteoblasts at the bone surface where it acts as a negative regulator of osteoblast activity [76,77] (Fig. 3D). Mice overexpressing sclerostin have decreased bone mass and strength, while deletion of this protein causes the opposite effect [78,79]. Osteocyte lacunae in *Mmp2*$^{-/-}$ calvaria and long bones are characterized by a build-up of sclerostin staining [33], suggesting that the inability of sclerostin to reach surface osteoblasts contributes to calvarial thickening in these mice (Fig. 3E). Another osteocyte-produced protein, dentin matrix protein-1 (DMP-1), acts as a nucleation site for calcification, and its absence causes decreased bone mineralization in knockout mice (reviewed in [80]). *Mmp2*$^{-/-}$ osteocytes produce less DMP-1 protein which may contribute to the poor mineralization of *Mmp2* null long bones, but the partially intact canalicular network may still deliver sufficient sclerostin to osteoblasts to prevent bone thickening [33,34]. With respect to the thickened calvaria in *Mmp2*$^{-/-}$ mice, it is suggested that excessive osteoblast activity due to the absence of sclerostin signaling dominates over the impact of reduced DMP-1 on bone mineralization [33].

4. Metalloproteinase activity and joint pathologies

Having examined the roles of metalloproteinases in bone development and remodeling, we will now describe the contributions of metalloproteinase activity to articular cartilage integrity and the progression of arthritic disease. Articular cartilage is organized into four layers, namely the superficial zone, intermediate zone, radial zone and zone of calcified cartilage (reviewed in [18]). Maintenance of articular cartilage is essential for cushioning joint surfaces and protecting the underlying bone. Also important to the function of joints between long bones is the synovial membrane which secretes a lubricating fluid to facilitate joint motion. Loss of articular cartilage integrity and inflammation of the synovial membrane are key features of arthritic disease, which can be grouped into two conditions differentiated by their cause and clinical features. Osteoarthritis, a condition associated with age, gender, obesity and genetic factors, is the most common form of arthritis and involves the loss of articular cartilage integrity leading to degradation of the underlying bone and localized synovial inflammation (reviewed in [81]). On the other hand, joint inflammation is an early and sustained event in the progression of rheumatoid arthritis, in which an infiltration of inflammatory cells leads to the expansion of the synovium and eventually to cartilage and bone destruction (reviewed in [82]). Here we discuss separately the contributions of MMP, ADAMTS and ADAM enzymes to the development of joint pathologies, again focusing on genetic mouse models that either spontaneously develop joint phenotypes or exhibit changes in response to the experimental induction of arthritis.

4.1. The debated contributions of the MMP family in joint pathology

Mmp2$^{-/-}$, *Mt1-Mmp*$^{-/-}$ and transgenic mice expressing activated *Mmp13* develop joint disease. As young as 12 weeks of age, *Mmp2*$^{-/-}$ mice show early onset arthritis and joint contractures characterized by articular cartilage destruction and erosion of the underlying bone surface, although a normal gait is maintained in 2-year-old mice [34]. During routine handling of *Mmp2*$^{-/-}$*Col1a1*$^{r/r}$ mice, investigators noted a decreased range of motion at the knee, hip, shoulder, and elbow joints but no histological examinations of the joints were performed [36]. Joints in *Mt1-Mmp*$^{-/-}$ mice also exhibit articular cartilage destruction, along with ankylosis, a stiffening of the joints [6]. Other contributing factors to the ankylosis in *Mt1-Mmp*$^{-/-}$ mice include an overgrowth of hypercellular and abnormally vascularized synovial tissue, increased vascularity of the tendons and ligaments, and the presence of TRAP-positive cells in articular and periarticular soft tissues [6]. Once again, the phenotypic similarities between the *Mt1-Mmp*$^{-/-}$ and *Mmp2*$^{-/-}$ mice are notable. While joint aberrations are not reported in *Mmp13*$^{-/-}$ mice, the induction of chondrocyte-specific, constitutively active MMP13 in transgenic mice at weaning age leads to the development of osteoarthritic changes [83], although this model turns out to be complex. Here, Neuhold et al. introduce a tetracycline-regulatable transgene into mice such that active MMP13 is constitutively expressed under the control of the *Col2a1* promoter in the absence of doxycycline, which is administered until weaning. Embryonic lethality was observed even in the presence of doxycycline, suggesting that the promoter was leaky. Interestingly, it has since been shown that a mutant proMMP13, which folds improperly, autoactivates in the rough endothelial reticulum, autodegrades, and additionally activates wild type MMP13 and possibly other metalloproteinases [84].

With respect to arthritis models in MMP knockout mice, early work focused on *Mmp3*$^{-/-}$ mice, which have no reported developmental bone abnormalities. Following antigen-induced arthritis, the absence of MMP3 activity appears sufficient to prevent the detection of neoepitopes resulting from MMP-specific aggrecan (VDIPEN) or type II collagen cleavage (COL2–3/4C_{short} fragment) [85,86]. However, proteoglycan loss was found to be equivalent between knockout and wild type mice, suggesting that MMP cleavage of aggrecan is not essential for proteoglycan loss [85,86]. Equivalent disease progression between *Mmp3*$^{-/-}$ and wild type mice is similarly observed when using collagen to induce arthritis, but no change in VDIPEN levels was detected using the same antibody as in the antigen-induced model [87]. Interestingly, *Mmp3*$^{-/-}$ mice have also been subjected to osteoarthritis via surgical induction, and here they display an accelerated progression [88]. In another model of antigen-induced arthritis, *Mmp2*$^{-/-}$ mice develop joint pathology characterized by increased cellular infiltration and proteoglycan depletion compared with wild type controls as well as *Mmp2*$^{-/-}$*Mmp9*$^{-/-}$ mice, while *Mmp9*$^{-/-}$ mice display lower levels of

both infiltration and proteoglycan depletion [89]. The role of MMP12 has been tested in another species; inducing antibody-mediated arthritis in rabbits overexpressing human MMP12 results in increased inflammatory cell infiltration, proteoglycan loss and cartilage erosion [90]. In knock-in mice named 'Chloe' where the MMP-cleavage site of aggrecan is mutated, the progression of surgically-induced osteoarthritis and antibody-mediated arthritis is comparable to that in wild type mice, indicating that MMPs are not the primary mediators of joint destruction [28]. Currently, the extent and nature of the contribution of MMP-mediated aggrecan cleavage to the pathology of human arthritis is under debate. As reviewed by Sandy [91], the majority of recent evidence points to such cleavage as not being detrimental in human disease, although it has been suggested that MMP cleavage contributes as much as 22% of the total destructive activity in human osteoarthritis [92].

4.2. The ADAMTS family is an important mediator of arthritis

When aggrecanase 1 was cloned and identified as ADAMTS4 in 1999, it was already known that, despite aggrecan cleavage between Asn^{341} and Phe^{342} by MMP activity, the primary aggrecan fragments found in synovial fluid from arthritic patients were cut by 'aggrecanases' between Glu^{373} and Ala^{374} [93]. That same year, aggrecanase 2 was also cloned (now referred to as ADAMTS5, aggrecanase 2 was originally identified as ADAMTS11) [94]. Since then, models of induced arthritis in mice deficient in *Adamts1*, *Adamts4* and *Adamts5* have served well towards unraveling the *in vivo* importance of individual aggrecanase activity in joint disease. Using surgical transection of the medial meniscotibial ligament to destabilize the joint and cause osteoarthritic changes, Glasson et al. show that *Adamts4*$^{-/-}$ mice are not protected from aggrecan loss [25]. Similarly, *Adamts1*$^{-/-}$ mice remain susceptible to aggrecan loss in a model of antigen-induced arthritis [95]. It is now clearly established that ADAMTS5 is the primary mediator of arthritic cartilage destruction, as shown by independently generated *Adamts5* knockouts subjected to two different models of arthritis [24,26]. Notably, the protective effect of the lack of ADAMTS5 is specific to proteoglycan destruction and cartilage erosion, since equivalent development of synovitis, joint space exudate, pannus-mediated erosion and bone destruction is observed between *Adamts5*$^{-/-}$ and wild type controls [24]. The importance of ADAMTS5 is further enforced by a recent study showing a comparable lack of proteoglycan loss between *Adamts5*$^{-/-}$ and *Adamts4*$^{-/-}$*Adamts5*$^{-/-}$mice in a model of osteoarthritis [29]. Studies using explanted human tissue however, have shown that both ADAMTS4 and ADAMTS5 are important during the progression of arthritis, and this may be attributable to the differential regulation of these ADAMTS proteases in mice and humans [96].

The normal preferred cleavage sites of both ADAMTS4 and ADAMTS5 are located in the chondroitin sulfate-2 (CS-2) domain, not at the Glu^{373}–Ala^{374} bond in the interglobular domain (IGD) of aggrecan [97]. However, cleavage in the CS-2 region is less detrimental to cartilage integrity and function, as only a fraction of aggrecan-bound glycosaminoglycan chains are lost, compared to full loss following cleavage within the IGD which is predominant in arthritic joints [28]. This damaging cleavage is detectable in joints with osteoarthritis and inflammatory arthritis (reviewed in [98]). Little et al. highlighted the importance of the Glu^{373}–Ala^{374} cleavage site using knock-in mice called 'Jaffa' which carry a cleavage-preventing mutation at this site [28]. Induction of either osteoarthritis or inflammatory arthritis in these mutants results in decreased structural damage and aggrecan loss compared to Chloe mice harbouring aggrecan with a mutated MMP-cleavage site as well as wild type controls [28].

Currently, ADAMTS4 and ADAMTS5 are of major interest as possible drug targets for arthritic patients since the current therapeutic regimens of non-steroidal anti-inflammatory drugs and steroids alleviate symptoms but do not prevent disease progression [99]. Also of interest, therefore, are endogenous modulators of aggrecanase activity. Like most metalloproteinases, the aggrecanases are secreted as inactive zymogens. Recently, the proprotein convertase PACE-4 was shown to be secreted by human osteoarthritic chondrocytes, activating ADAMTS4 and ADAMTS5, but PACE-4 is not produced by healthy articular cartilage [100]. Of the TIMPs, only TIMP3 is an efficient inhibitor of ADAMTS4 and ADAMTS5 [101–103]. Decreased proteoglycan staining is detected in the articular cartilage of aged *Timp*3$^{-/-}$ mice 8 months and older, where staining for both the MMP and ADAMTS cleavage-generated aggrecan neo-epitopes is increased, with a greater increase in the MMP neo-epitope [104]. Aged *Timp*3$^{-/-}$ joints are characterized by increased collagen cleavage products, especially in superficial zone chondrocytes [104]. In a model of antigen-induced arthritis, *Timp*3$^{-/-}$ mice display an initial increase in the severity of arthritic symptoms, specifically synovial inflammation, but this was equivalent to the wild type arthritic score by 2 weeks after injection; no differences in neo-epitope staining were observed at any timepoint [105]. This immediate arthritic response is associated with increased serum tumor necrosis factor (TNF) levels in *Timp*3$^{-/-}$ mice 1 h following antigen administration [105].

4.3. ADAMs and joint pathologies

ADAMs primarily act as sheddases, cleaving substrates from cell surfaces. Given that this metalloproteinase family plays a less prominent role in modifying the ECM, the specific contribution of different ADAMs to the development of arthritis is less well studied than those of MMPs and ADAMTSs. Overall, the expectation is that ADAMs, particularly TACE, contribute to the progression of rheumatoid arthritis by modulating the immune response [106,107]. TACE sheds TNF from the cell surface making it locally and systemically available [108,109] and TNF plays an important role in the progression of rheumatoid arthritis; anti-TNF treatments used clinically are effective at reducing disease symptoms (reviewed in [110]). However, the absence of viable adult TACE deficient mice has made it difficult to experimentally examine the contribution of TACE to arthritis progression. In addition to TNF shedding, TACE and ADAM10 activity also affect the bioavailability of a number of immune-related cytokines, chemokines and receptors (reviewed in [111]), potentially altering the overall balance of pro-inflammatory and anti-inflammatory signals in rheumatoid arthritis. Additionally, as reviewed by Murphy and Nagase, many cytokines and interleukins, including TNF and IL-1, induce the expression of a variety of MMPs in joint tissues [107].

It has been suggested recently that ADAM8 may play a role in the development of rheumatoid arthritis by positively affecting osteoclastogenesis [112], and further study is required to determine if ADAM8 significantly affects the differentiation of osteoclasts in arthritic joints *in vivo*. Notably, unchallenged *Adam8*$^{-/-}$ mice do not have detectable defects in bone development or remodeling up to 10 weeks of age [113]. The effect of aging on *Adam8*$^{-/-}$ joints has not been reported, although it has been examined in *Adam15*$^{-/-}$ mice. In 12–14-month-old *Adam15* null mice, osteoarthritic changes range from mild synovial membrane hyperplasia to cartilage proteoglycan loss to severe erosion of the articular cartilage [114]. Notably, these changes are only observed in aged male mice, the joints of female *Adam15*$^{-/-}$ knockouts are comparable to wild type, indicating a sex-specific protective effect of this protease against osteoarthritis in males [114]. *In vitro* experiments suggest that this protection occurs through an improved ability of chondrocytes to bind collagen types II and VI through integrins and establish survival signals [114]. Consistent with this idea, α1 integrin deficient mice display a similar, age-dependent increase in osteoarthritic changes [115]. Overall, much work remains to be carried out before we fully understand the function of the ADAM family of metalloproteinases in the development of arthritic disease.

5. Conclusions and perspectives

Over the last decade, the identification of skeletal developmental defects, imbalances in bone remodeling and joint disease in metalloproteinase-deficient mice have cemented a role for these proteases in bone biology and pathology. Moving forward, the major challenge will be to tease out molecular mechanisms that explain the observed phenotypes. Given that each metalloproteinase cleaves a range of targets, this is not a trivial undertaking, as it is expected that multiple biological processes may be perturbed in parallel. As also discussed, metalloproteinases mediate communication between specific bone cell types, and focusing on these paired interactions may facilitate mechanistic determination.

Just as the importance of metalloproteinases in bone biology has been increasingly appreciated in recent years, so too has the role of these proteases in immunology and inflammation; genetic mouse models deficient in metalloproteinases are similarly helping to elucidate their role in the immune system. Concurrently, the interplay between the skeletal and immune systems has been recognized in the relatively new field of osteoimmunology. Metalloproteinases can shed molecules common to skeletal and immune cells such as RANKL, are transcriptionally regulated by inflammatory cytokines such as TNF, and in turn control cytokine bioavailability. Thus, metalloproteinases have the potential to act as key molecules in osteoimmunology. Understanding these links will help unravel the complex biological functions of metalloproteinases in skeletal biology and disease.

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