Destroy to Rebuild: The Connection Between Bone Tissue Remodeling and Matrix Metalloproteinases

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Bone is a dynamic organ that undergoes constant remodeling, an energetically costly process by which old bone is replaced and localized bone defects are repaired to renew the skeleton over time, thereby maintaining skeletal health. This review provides a general overview of bone’s main players (bone lining cells, osteocytes, osteoclasts, reversal cells, and osteoblasts) that participate in bone remodeling. Placing emphasis on the family of extracellular matrix metalloproteinases (MMPs), we describe how: (i) Convergence of multiple protease families (including MMPs and cysteine proteinases) ensures complexity and robustness of the bone remodeling process, (ii) Enzymatic activity of MMPs affects bone physiology at the molecular and cellular levels and (iii) Either overexpression or deficiency/insufficiency of individual MMPs impairs healthy bone remodeling and systemic metabolism. Today, it is generally accepted that proteolytic activity is required for the degradation of bone tissue in osteoarthritis and osteoporosis. However, it is increasingly evident that inactivating mutations in MMP genes can also lead to bone pathology including osteolysis and metabolic abnormalities such as delayed growth. We argue that there remains a need to rethink the role played by proteases in bone physiology and pathology.

Keywords: bone, remodeling, metabolism, matrix metalloproteinase, deficiency, underactivity

INTRODUCTION

Bone is a hard, dense, rigid form of highly specialized connective tissue making up the skeleton of vertebrates. Bone protects internal organs, supports body structures, and aids in locomotion (Maffioli and Derosa, 2015). In addition, bone provides an environment for hematopoiesis (i.e., formation and development of blood cells) in the bone marrow, and acts as a homeostatic reservoir of calcium, phosphorus, insulin-like growth factors, transforming growth factor-β, and cytokines. Bone buffers the blood against drastic pH changes, thus detoxifying the circulation from heavy metals (Rauner et al., 2012). Bone develops by intramembranous ossification (e.g., bone of the clavicle, some skull bones), endochondral ossification (e.g., the appendicular and axial skeleton) or pseudo-metamorphic ossification (Rauner et al., 2012).

Bone remodeling is a complex process involving the sequential resorption of bone tissue and deposition of new bone at the same site (Kerschan-Schindl and Ebenbichler, 2012). Together with bone structure, geometry, size, and density, remodeling determines bone’s overall mechanical properties (e.g., the strength) (Mosekilde et al., 1993; Jiang et al., 1997; Ikeda et al., 2003;
The bone remodeling process consists of four distinct consecutive phases spanning over 3–6 months (Datta et al., 2008). The first phase of bone remodeling is known as the ‘activation phase’ and can be triggered by mechanical and nutritional stress on the bone as well as by hormones (e.g., parathyroid hormone, estrogen) (Parra-Torres et al., 2013). As described in Table 1, terminally differentiated osteocyte is a key player in the activation phase (Rauner et al., 2012; Parra-Torres et al., 2013).

The second phase lasts 8–10 days (Teitelbaum, 2007) and is called the ‘bone resorption phase’ – a process by which large multinucleated osteoclast cells break down old bone organic matrix impregnated with minerals (e.g., calcium phosphate nanocrystals), as described in Table 2.

The third ‘reversal phase’ connects osteoclastic bone tissue resorption and osteoblastic bone tissue formation (Delaisse, 2014) and lasts 7–14 days (Pettit et al., 2008; Hienz et al., 2015). After departure of the osteoclast from a cavity in bones undergoing resorption, which is a resorptive lacuna known as the Howship’s lacuna, bone lining cells occupy the Howship’s lacuna and clean it (Everts et al., 2002). The cleaning process occurs by enwrapping and digesting non-mineralized collagenous proteins protruding from the bone surface left by osteoclasts. This cleaning process is a requirement for the subsequent deposition of a first layer of collagen along the Howship’s lacuna (Everts et al., 2002). Four types of osteoclast-derived coupling factors stimulate bone formation during the reversal phase: (i) Matrix-derived factors including transforming growth factor-β, bone morphogenetic protein-2, platelet-derived growth factor, and insulin-like growth factors, which are released during bone tissue resorption, (ii) Osteoclast-secreted factors, including cathepsin-1, sphenomucin-1-phosphate, collagen triple helix repeat containing 1, and complement factor 3a, (iii) Osteoclast membrane-bound factors such as EphrinB2 and Semaphorin D, and (iv) Structural changes brought about by the osteoclast on the bone tissue surface (Sims and Martin, 2014). Reversal cells originating from pre-osteoblast cells (Andersen et al., 2013) colonize the osteoclast-eroded surface and respond to osteoclast-derived messages and coupling factors along with fibroblast-like cells covering the surface of bone (known as bone lining cells), osteoblast precursors, and canopy cells (Delaisse, 2014; Sims and Martin, 2014; Lassen et al., 2017; Pirapaharan et al., 2019).

Shahnazari et al., 2009) as well as enables the repair of damaged bone and the adaption of bone to changing biomechanical forces (Kerschan-Schindl and Ebenbichler, 2012).

We review here the prevailing view of the bone remodeling process with an emphasis on well-accepted and newly emerging roles played by matrix metalloproteinases (MMPs) and cysteine proteinases in this process. Finally, we review the increasing number of instances in which inactivating mutations in MMP genes are found to lead to bone pathology including osteolysis and metabolic abnormalities such as delayed growth.

**GENERAL OVERVIEW ON THE CYCLE OF BONE REMODELING**

The fourth phase of the bone remodeling cycle is ‘formation,’ when mononucleate osteoblast cells synthesize new bone organic matrix formed by collagen fibers and non-collagenous proteins (e.g., bone sialoprotein, osteopontin, osteocalcin, proteoglycans) that later becomes surrounded and impregnated with mineral deposit mainly in the form of calcium hydroxypatite. A summary of osteoblastogenesis, the roles played by osteoblasts during this last phase, and the fate of osteoblasts is described in Table 3.

While bone formation surpasses resorption during childhood, bone formation and resorption are in balance during young adulthood. However, an unbalanced bone loss occurs with aging (Datta et al., 2008; Rauner et al., 2012; Brandi and Piscitelli, 2013) and could predispose an individual to skeletal disorders including: (i) inflammatory bone loss in periodontal disease, (ii) arthritis (stimulation of bone resorption and inhibition of bone formation by prostaglandins and cytokines), (iii) osteoporosis (bone resorption outpaces bone formation), (iv) hyperparathyroidism and hyperthyroidism (greatly increased rate of bone resorption and formation), (v) Paget’s disease (increased and abnormal [shape, weakness, and brittleness] bone formation), (vi) osteomalacia (delayed/deficient bone mineralization), and (vi) osteoporosis (failure of osteoclasts to resorb bone) (Roodman et al., 1992; Delmas, 1995; Gallagher, 1997; Mills and Frausto, 1997; Raizs, 1997; Charles and Key, 1998; Schneider et al., 1998; Siris, 1998; Kini and Nandeesh, 2012).

**MATRIX METALLOPROTEINASES: MODULATORS OF BONE REMODELING**

Matrix metalloproteinases are a family of at least 24 highly homologous, multi-domain enzymes (Figure 1) with the capacity to degrade virtually all extracellular matrix components including collagen, aggrecan, elastin, and fibronectin (Lu et al., 2011; Fernandez-Patron et al., 2016).

All MMP family members are synthesized as catalytically inactive (latent) pro-enzymes (pro-MMPs) that contain a signal N-terminal peptide sequence (~20 amino acids), pro-peptide domain (~80 amino acids), catalytic domain (approximately 160 amino acids), hinge (linker peptide) region of variable length (10–30 amino acids), and a hemopexin-like C-terminal domain (Hpx) (~210 amino acids). The smallest MMPs (MMP-7 and MMP-26) lack the hinge and hemopexin domains, and therefore exhibit a reduced affinity for gelatin. MMP-23 has unique domains (such as the cysteine array, IgG-like domain, interleukin-1 type II receptor-like domains) instead of the hemopexin domain (Massova et al., 1998; Pei et al., 2000; Bode and Maskos, 2003; Visse and Nagase, 2003; Nagase et al., 2006; Piccard et al., 2007; Lopez-Otin et al., 2009; Bonnans et al., 2014; Vandooren et al., 2014; Vandenbroucke and Libert, 2014; Cui et al., 2017). The amino-terminal signal peptide targets the pro-MMPs to the rough endoplasmic reticulum, whereas the C-terminus harbors a cysteine residue and a furin cleavage site (PRCGXPD), both of which are important for conversion into the mature, active enzyme (Bonnans et al., 2014). Presence
TABLE 1 | Osteocytes and the activation phase of bone remodeling.

| Origin of osteocytes | Differentiation stages: (i) From mature osteoblasts to preosteocyte type I with dendritic projections formation; (ii) from preosteocyte type I to preosteocyte type II with cytoskeleton rearrangement; (iii) from preosteocyte type II to preosteocyte type III (mature osteocyte trapped within the mineralized bone matrix) with canaliculce formation (Hirao et al., 2007; Paiva and Granjeiro, 2017).
| Important factors involved in osteocytogenesis | (i) Pre-osteoblasts (Stro1, CD29, CD105, CD166); (ii) Osteoblast (Cbfal and ostein for differentiation, alkaline phosphase and collagen for the production of osteoid, osteocalcin, casein kinase II); (iii) Osteoid osteocyte (Phex and MEPE for regulation of biomineralization and mineral metabolism, E11/gp38 and MMP-14 for dentine/canalicular formation, destrin for cytoskeleton rearrangement); (iv) Mineralizing osteocyte (DMP1 for regulation of biomineralization and mineral metabolism, CapG for cytoskeleton regulation); (v) Mature osteocyte (sclerostin, FGF23 for regulation of renal phosphate excretion, OPG150 for preserving viability in a hypoxic environment) (Bonenal, 2011). Other factors include TGF-β (D’Angelo et al., 2001; Karsdal et al., 2002), MMP-2/MMP-13/MMP-14 proteolytic axis (Barthelmi et al., 2012), Cx43, Dkk-1, Fetal A, RANKL, MCSF, and osteoprotegerin (Chen et al., 2018).
| Key signaling events involved in osteocytogenesis | Osteocalcin, ALP, and other genes specific for osteoblast differentiation gradually downregulate (Paiva and Granjeiro, 2017). At the same time, different genes specific for osteocyte differentiation upregulate (such as CD44 [Hughes et al., 1994], E11/gp38 [Zhang et al., 2006], Phex [Fuch et al., 2002; Westbroek et al., 2002], Fimbrin [Tanaka-Kamioka et al., 1998], MEPE [Fowe et al., 2004], DMP1 [Feng et al., 2006; Toyosawa et al., 2012], sclerostin [Poole et al., 2005; Balemans et al., 2008], ORP150 [Poole et al., 2010], and FGF23 [Liu et al., 2008]). Transcription factors involved in the process of osteoblast/osteocyte transition are ATF-4, whose expression is regulated by JNK, and members of the AP-1 group (Matsuguchi et al., 2009).
| Role of osteocytes | (i) Maintain physical connections with each other, and also other players (osteocytes, osteoblasts) of the bone remodeling cycle through a widespread network of tiny channels called canaliculi (Civitelli, 2008); (ii) May remodel the periacellular matrix (e.g., during lactation) by expressing cathepsin K and acid phosphatase. (ii) Regulate bone remodeling by expressing M-CSF and RANKL (stimulate osteoclast formation and activity) as well as NO and OPG (inhibit osteoclast formation and activity). Also, osteocytes control bone formation by secreting activators (e.g., NO, ATP, PE23) and inhibitors (e.g., sFRP1, Dkk1, sclerostin) of the Wnt signaling pathway. (iv) Source of factors (e.g., sclerostin) and regulators (e.g., FGF-23, DMP-1, Phex, MEPE) of phosphate metabolism. (v) Manage the bone’s reservoir of calcium. (v) Function as mechanosensory cells (Bonenal, 2011; Dallas et al., 2013; Bellido, 2014).
| Molecular mechanism that underlies the function of osteocytes as mechanosensory cells | Osteocytes are good mechanosensors (i.e., they detect changes of mechanical stimuli) in bone tissue which serve to sense and respond to alterations produced when a bone is mechanically loaded. Such alterations may be physical deformation of the bone matrix, fluid flow shear stress generated by variations in canalicular fluid flow and electrical streaming potentials (Bonenal and Mundy, 1990; Mundy, 1993; Manolagas, 2000; Miyauchi et al., 2000; Bonenal and Bowland, 2000; Datta et al., 2008; Parra-Torres et al., 2013; Takemura et al., 2019). Mechanical strain signal is converted into a cellular response (i.e., biochemical signals) with the participation of membrane proteins (such as CD44, connexins, integrins, and ion channels) and downstream mediators of intracellular signaling (such as guanine regulatory proteins, mitogen activated protein kinase, cyclic adenosine monophosphate, inositol triphosphate, and intracellular calcium) (Flawinson et al., 1996; Burger and Klein-Nulend, 1996; Mikuni-Takagaki, 1999; Miyauchi et al., 2000; Gu et al., 2001; Alford et al., 2003; Kapur et al., 2003; Plotkin et al., 2005; Ruben et al., 2006; Miyauchi et al., 2006). On the other hand, bone remodeling is also controlled by upregulation of RANKL and sclerostin in response to a decrease in mechanical signals (Parra-Torres et al., 2013). The precise signaling biochemical pathways (e.g., Wnt/β-catenin) and regulatory mechanisms may be mediate adaptive responses activated by mechanical loading and unloading in bone remain to be completely delineated (Dallas et al., 2013; Parra-Torres et al., 2013).
| Other consequences of osteocyte activities on bone remodeling | Retraction of the bone lining cells (elongated mature osteoblasts) on the endosteal surface (which is a thin layer of cell-rich connective tissue), and also digestion of the underlying collagenous membrane by collagenases (Murray et al., 1995; Dallas et al., 2001; Datta et al., 2008; Kerschan-Schindl and Ebenbichler, 2012).

MM P, matrix metalloproteinase; TGF, transforming growth factor; RANKL, receptor activator of nuclear factor kappa B ligand; MAPK, mitogen-activated protein kinase; TIMP, tissue inhibitor of metalloproteinase; ALP, alkaline phosphatase; Phex, phosphate-regulating endopeptidase homolog X-linked; MEPE, matrix extracellular phosphoglycoprotein; Cx, connexin; Dkk, Dickkopf WNT signaling pathway inhibitor; Phex, phosphate-regulating endopeptidase homolog X-linked; DMP, dentin matrix acidic phosphoprotein precursor; M-CSF, Macrophage colony-stimulating factor; ORP, oxygen regulated protein; PPI, provitamin K; Zn, zinc; AP, alkaline phosphatase; OPG, osteoprotegerin; ATP, adenosine triphosphate; NO, nitric oxide; sFRP1, secreted frizzled-related protein 1; DKK1, Dickkopf WNT signaling pathway inhibitor 1; PEG2, prostataglandin E2; Wnt, Wingless-type MMTV integration site family.

of an intact pro-peptide accounts for the latency of pro-MMPs, which can be overridden through the activation of a “cysteine-switch” mechanism (Van Walt and Birkedal-Hansen, 1990). The pro-peptide contains a cysteine residue that prevents catalytic activity when it is coordinated with a Zn(II)-ion in the catalytic domain (Springman et al., 1990; Van Walt and Birkedal-Hansen, 1990). The cysteine-Zn(II) interaction can be disrupted by alkylating compounds such as the organomercurial 4-aminophenylmercuric acetate as well as by serine proteases and other MMPs such as membrane-type MMPs, which act at the cell surface to which they anchor through their transmembrane domain/short cytoplasmic tail or by glycosylphosphatidylinositol linkage (Bonnans et al., 2014). MMP autolysis is another mechanism of activation mediated by allosteric perturbation of the inactive proenzyme (Springman et al., 1990; Van Walt and Birkedal-Hansen, 1990;
TABLE 2 | Osteoclasts and the bone resorption phase.

| Origin of osteoclasts | Differentiation stages: Hematopoietic stem cell precursors differentiate into monocyte and macrophage, and then they fuse into end-differentiated multinucleated (bone resorbing) cells (Tanaka et al., 1993; Quinn et al., 1998; Rodman, 1999; Udagawa et al., 1999; Holmbeck and Szabo, 2006; Bar-Shavit, 2007; Brazzanti and Baron, 2006). Osteocyte apoptosis is thought to contribute to the recruitment of osteoclast precursors by diminishing the secretion of osteocyte-derived factors (e.g., TGF-β) that have inhibitory effect on osteoclast formation (Heino et al., 2002; Aguirre et al., 2006). |
| Main factors involved in osteoclastogenesis | Osteoblasts, osteocytes, RANKL, M-CSF, OPG, TNF, ILs, mineralized bone particles containing osteocalcin, DC-STAMP, OC-STAMP (Tanaka et al., 1993; Roach, 1994; Wiebe et al., 1996; Kotake et al., 1999; Udagawa et al., 1999; Mare, 2003; Miyamoto, 2006; Kim et al., 2011; Hienz et al., 2015; Plotkin and Brazzanti, 2019), |
| Key signaling events involved in osteoclastogenesis | After the induction of PU.1, the stem cell precursor is determined to the osteoclast lineage (Tondravi et al., 1997). Then, cell proliferation is induced following expression and activation of c-fms by the precursor. RANK is subsequently expressed and activated by RANKL, after which RANK interacts with the TRAF family members (e.g., TRAF2, TRAF6) and lead to downstream activation of MAP kinases and NF-κB. This process is aided by co-signaling from other receptors (such as TREM2, OSCAR, DAP 12, and FcγR) (Koga et al., 2004; Mocsai et al., 2004). The interaction between immunoreceptors (e.g., TREM2, OSCAR) and FcγR/FcγR adapters, activates Syk kinases, leading to PLCε activation. Callli, which is mobilized from the intracellular stores, activates calcineurin, resulting in dephosphorylation of NFATc1. Moreover, the activation of calcineurin involves the activation of phospholipase-Cy and Tec kinases (Mocsai et al., 2004; Facchio et al., 2005; Wada et al., 2005). In general, most signaling pathways (MAPKs, NF-κB, AP-1, Callli, Src/Pi3K/Akt) which are activated in the osteoclast convege to induce the activity of NFATc1 (Ceri et al., 2000; Ishida et al., 2002; Takayanagi et al., 2002; Matsuo et al., 2004; Paiva and Granjeiro, 2017; Plotkin and Brazzanti, 2019; Zheng et al., 2019). Upon translocation to the nucleus, NFATc1 acts together with c-fos to promote the expression of key osteoclast genes. Some of the osteoclast differentiation genes to which NFATc1 binds directly are OSCAR (Kim Y. et al., 2005), cathepsin K (Matsumoto et al., 2004), calcinonin receptor (Matsurom et al., 2004), integrin β3 (Crotti et al., 2006, 2008), MMP-9 (Sundaram et al., 2007), and TRAP (Matsurom et al., 2004; Paiva and Granjeiro, 2017). Of note, another factor which controls NFATc1 is OPG, which functions as a decoy receptor for RANKL, thus inhibiting the differentiation of osteoclasts (Lacey et al., 1998). Osteoclastogenesis is regulated by the RANKL/OPG balance. Opposing effects on RANK during osteoclast differentiation is exerted by LGR4 which signals through G-protein or Wnt signaling pathways (Luo et al., 2016). Cytokines which inhibit RANK signaling on osteoclasts are IL-10, IFNs (α, β, and γ), and GM-CSF. |
| Mechanisms that underlie the action of osteoclasts | During initiation of the resorption phase, the mature osteoclasts (1-2% of bone cells) attach to the bone surface via αvβ3, αvβ5, αvβ1, and αvβ1 integrins (Vaanenan and Horton, 1995; Datta et al., 2008; Rauner et al., 2012; Plotkin and Brazzanti, 2019). At the bone/osteoclast surface, a ruffled border which is entirely surrounded by a sealing zone is formed, thereby creating an isolated resorption (Howship's) lacuna (i.e., scalloped erosion) (Miyaura et al., 1991; Miruna et al., 1994; Teitelbaum, 2000; Teitelbaum and Ross, 2003). Osteoclasts dissolve mineral (hydroxyapatite) and organic components (e.g., type I collagen) of the bone matrix in the resorption lacuna (Teitelbaum et al., 1995; Rauner et al., 2012). This resorption process is mediated by the secretion of hydrogen ions, to acidify the resorption compartment beneath osteoclasts and dissolve hydroxyapatite crystals (Blair et al., 1989; Teit et al., 1989). Hydrogen ions, supplied by the reaction of water and carbon dioxide and catalyzed by carbonic anhydrase II, are transported into the resorption lacuna by ATPases located in the ruffled border of osteoclasts (Baron, 1989; Mattsson et al., 1994; Li et al., 1999; Brazzanti and Baron, 2006; Hienz et al., 2015). Hydrochloric acid formed with chloride ions pumped into the resorption lacuna dissolves the mineralized bone matrix (Silver et al., 1988; Plotkin and Brazzanti, 2019). In addition, lysosomal enzymes (e.g., cathepsin K), bone-derived collagenases, and other proteinases (e.g., trypsin-like acid phosphatase) act in concert to mediate the resorption process (Sord et al., 1996; Gelb et al., 1996; Saliht et al., 1998; Boyle et al., 2003; Teitelbaum, 2007; Hienz et al., 2015). Osteoclast-mediated bone resorption, which takes a few (2-4) weeks during each remodeling cycle, results in Howship's lacuna on the surface of trabecular bone and cylindrical Haversian canals in cortical bone (Brazzanti and Baron, 2006; Teitelbaum, 2007; Hienz et al., 2015). After one resorption lacuna is completed, the osteocell cells die by apoptosis (Plotkin and Brazzanti, 2019) or move along the bone surface to resume resorption. This phase lasts approximately 8-10 days (Teitelbaum, 2007). |
| Systemic and local factors that stimulate bone resorption | Osteocytes as the major source of RANKL; thyroid hormones; PTH/PTHrP; calcitriol; glucocorticoids; growth factors (FGF, PDGF, EGF); TNF-α, colony-stimulating factors (M-CSF, GM-CSF); IL-1, -6, -7, -8, -11, -15, -17; PGE1, 2, 12; PGH2 (MacDonald, 1986; Dempster et al., 1993; Raisz, 1993; Kawaguchi et al., 1994, 1995; Nash et al., 1994; Holt et al., 1996; Lanske et al., 1999; Rodman, 1999; Lam et al., 2000; Compston, 2001; Ragabi et al., 2002; Sher et al., 2004; Eikens et al., 2005; Dai et al., 2006; Zhang et al., 2008; Kini and Nandeesh, 2012; Rauner et al., 2012; Parra-Torres et al., 2013; Paiva and Granjeiro, 2017; Hachemi et al., 2018; Belloco and Gallant, 2019). |

RANK, receptor activator of nuclear factor kappa B; RANKL, receptor activator of nuclear factor kappa B ligand; GM-CSF, granulocyte-macrophage colony-stimulating factor; OPG, osteoprotegrin; TNF, tumor necrosis factor; IL, interleukin; DC-STAMP, dendritic-cell specific transmembrane protein; DC-STAMP osteoclast stimulatory transmembrane protein; NFκB, nuclear-factor kappa B; TRAF6, TNF receptor-associated factor 6; TREM2, triggering receptor expressed on myeloid cells-2; OSCAR, osteoclast-associated receptor; DAP, DNA-activating protein; FcγR, Fc receptor y chain; FcγR, soluble Fc receptor from a group C streptococcus; Syk, spleen tyrosine kinase; PLC, phospholipase C; NFATc1, nuclear factor of activated T cell cytoplasmic 1; Tec, tyrosine protein kinase; AP, activator protein; Src, receptor tyrosine kinase activator; P38K, phosphatidylinositol 3-phosphate kinase; TRAP, tartrate-resistant acid phosphatase; LGR, leucine-rich repeat-containing G protein-coupled receptor; IFN, interferon; PTH, parathyroid hormone; PTHrP, PTH-related protein; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; M-CSF, macrophage colony-stimulating factor; PGE, prostaglandin E; PGH, prostaglandin H.
TABLE 3 | Osteoblasts and the bone formation phase.

| Origin of osteoblasts | Differentiation stages: (i) From stem cell to mesenchymal (adult) stem cell; (ii) from mesenchymal stem cell to preosteoblast (immature); (iii) from preosteoblast to mature osteoblast (Datta et al., 2008). |
| Key factors involved in osteoblastogenesis | Hormones (such as PTH, glucocorticoids, estrogen, leptin, 1,25-dihydroxyvitamin D3) (Datta et al., 2008; Mohanakrishnan et al., 2018; Arumugam et al., 2019; Plotkin and Bruzzaniti, 2019), growth factors (such as EGF, TGF-β, IGF) (Datta et al., 2008; Canalis, 2009; Plotkin and Bruzzaniti, 2019), local factors (such as the family of intracellular glycoproteins known as BMPs – 2, – 4, – 6, – 7) (Shore et al., 2006; Wutzl et al., 2010), members of the Wnt family in a paracrine/autocrine fashion (Bodine and Komm, 2006), Sonic and Indian hedgehogs (Maecla et al., 2007; Guan et al., 2009), cell-to-cell communication through receptors (such as Notch, Ephrin-Ephrin) and connexins (e.g., Cx43) (Plotkin and Bruzzaniti, 2019). |
| Key signaling events involved in the canonical Wnt/β-catenin pathway | Wnt proteins bind to Fzd receptor and its co-receptor (e.g., LR4, LR5, LR6). CK1α then phosphorylates Dvl and in turn the complex Dvl-Frat1-axin-LRP5/6-Fzd is formed. These events result in GSK3β inhibition, thereby avoiding modification (degradation, phosphorylation) of β-catenin. The stable β-catenin is then translocated to the nucleus to activate transcription factors (e.g., TCF, LEF), thus inducing the transcription of Wnt target genes (e.g., osteoprotegerin) (Datta et al., 2008; Plotkin and Bruzzaniti, 2019). Wnt signaling is regulated by a variety of molecules at the levels of extracellular inhibition of Wnt ligands or LRPs/Lrp6, co-receptors, intracellular signaling, and transcription (Gong et al., 2001; Boyden et al., 2002; Tian et al., 2003; Logan and Nusse, 2004; Semenov et al., 2005; Datta et al., 2008; Chen et al., 2010). Besides the canonical Wnt/β-catenin pathway, Wnt ligands can also activate other different signaling cascades (such as the Wnt-Ca++), planar cell polarity, and protein kinase A pathways. |
| Roles played by osteoblasts | Osteoblasts have a created a resorption cavity and detached from the bone surface, osteoblasts move into the cavity to initiate bone formation (Datta et al., 2008). Osteoblasts synthesize and lay down new unmineralized bone matrix (osteoid), which is subsequently mineralized (e.g., forming hydroxyapatite) over a period of about 20 days. Osteoblasts also synthesize and secrete the bone matrix proteins osteopontin, osteocalcin, bone sialoprotein, proteoglycans, and alkaline phosphatase (Baron, 1989; Roach, 1994; Ducy et al., 2000; Datta et al., 2003; Hienz et al., 2015). The synthesis of non-collagenous bone matrix proteins help to coordinate matrix mineralization and are essential for cellular adhesion (such as chemoattractant activity by osteocalcin), and regulation of cell activity (such as the osteopontin- and osteoectein-displayed cell activities) during coupling of bone resorption and formation (Robey, 1989; Raynal et al., 1996; Hienz et al., 2015). There is another function of osteoblasts that is worth highlighting. Osteoblasts also inhibit the ability of osteoclasts to degrade osseous tissue (Datta et al., 2008). |
| Stimulators of osteoblast functions | The increased formation of osteoid to build bone is stimulated by hormones (such as the pituitary-secreted growth hormone, sex hormones [estrogens and androgens], and thyroid hormone) (Kini and Nandeesh, 2012). Other factors that have stimulating effect on bone formation are insulin, vitamin D metabolites, IGF-I, IGF-II, TGF-β, BMP-2, BMP-4, BMP-7, IL-13, IFN, and OPG (Baylink et al., 1993; Cohick and Clemmons, 1993; Fraher, 1993; Rosen and Donahue, 1998; Yamaguchi et al., 2000; Canalis et al., 2003; Lovibond et al., 2003; Datta et al., 2008; Tang et al., 2009; Ruan et al., 2010; Kini and Nandeesh, 2012; Xian et al., 2012; Hienz et al., 2015). |
| Osteoblast fate | Bone-forming osteoblasts become encased in the mineralized matrix surrounding them, turning into osteocytes that gradually stop synthesizing osteoid (i.e., the newly formed unmineralized organic bone matrix) (Datta et al., 2008; Rauner et al., 2012). Osteocytes are evenly distributed throughout the bone matrix which enables contact with osteoblasts and vasculature (Kamioka et al., 2001; Plotkin et al., 2002; Zhao et al., 2002; Plotkin et al., 2008). Osteocytes not only facilitate mechanosensation as described in Table 1, but also control bone structure (amount and quality) through mineralization inhibitors such as dentin matrix protein-1, fetuin-A, and Wnt inhibitor (Poole et al., 2005; Feng et al., 2006; Coen et al., 2009; Liu et al., 2009; Rauner et al., 2012). Although it was thought that osteocytes remain inactive until the next bone remodeling cycle (Mikuni-Takagaki, 1999; Kamioka et al., 2001; Zhao et al., 2002; Knothe-Tate et al., 2004; Datta et al., 2008), it is now accepted that osteocytes constantly remodel the surrounding extracellular matrix (Yee et al., 2019). Another fate of osteoblasts is to become bone lining cells, which cover the freshly formed bone surface thus forming a physical barrier to avoid the process of osteoclast adhesion and bone resorption. |

Pei and Weiss, 1995; Pei et al., 2000; Meng et al., 2016). The cat lidatic domain harbors the Zn(II)-binding motif HEXXHXXGXXH, a catalytic Zn(II), a structural Zn(II), specific pockets related to specificity (S1, S2,...Sn and S1', S2'...Sn') and coordinated Ca(II) ions which confer stabilization. The catalytic Zn(II) is coordinated by three histidine residues (Bode and Maskos, 2003; Bonnans et al., 2014; Vandenbroucke and Libert, 2014). The hinge domain is flexible and mediates interactions with substrates, cell-surface proteins, and tissue inhibitors (Cui et al., 2017; Liu and Khalil, 2017). The hemopexin domain modulates substrate recognition and specificity, binding to cell-surface receptors and inhibitors,
activation of MMPs, and cellular MMP internalization for degradation (Visse and Nagase, 2003; Nagase et al., 2006; Piccard et al., 2007).

Matrix metalloproteinases expression and activity are tightly regulated at various levels: gene transcription, translation and secretion of the inactive enzyme precursor, proteolytic activation of the zymogen, spatial localization, interaction with specific extracellular matrix proteins, and inhibition by endogenous inhibitors (such as tissue inhibitors of MMPs [TIMPs 1-4], α2-macroglobulin, and human fibrinogen) (Sottrup-Jensen, 1989; Overall et al., 1991; Kusano et al., 1998; Zeng et al., 1998; Sternlicht and Werb, 2001; Han et al., 2003; Greenlee et al., 2007; Clark et al., 2008; Fanjul-Fernandez et al., 2010; Hadler-Olsen et al., 2011; Arpino et al., 2015; Sarker et al., 2019). Despite their similar names, TIMPs 1-4 exhibit large differences in their primary sequence, tissue expression, transcriptional regulation and in their inhibitory spectrum (Brew et al., 2000). In bone, TIMP-2 and TIMP-3, unlike TIMP-1, are effective inhibitors of the membrane-type MMPs (e.g., MMP-14), while TIMP-3 displays the broadest inhibitory actions of all TIMPs against metalloproteinases. Unlike TIMP-1, -2, and -4, which are soluble, TIMP-3 has basic amino acid residues in its C- and N-termini through which TIMP-3 attaches to heparan and chondroitin sulfate in the extracellular matrix and inhibits both MMPs and members of a disintegrin and metalloproteinase (ADAM) and a disintegrin and metalloproteinase with thrombospondin domains (ADAMTS) family including ADAM-17 and ADAMTS-4/5 (Porter et al., 2005; Javaheri et al., 2016). Deficiency of tissue inhibitors (TIMP-1, -2, or -4) has minor impact on bone phenotype. However, both Timp3 deficiency and transgenic overexpression alters craniofacial bones of endochondral and intramembranous origins in mice, while the growth plates appear normal in these mice (Javaheri et al., 2016). Paradoxically, mice deficient in RECK (an MMP inhibitor anchored on the cell membrane with inhibitory actions against MMP-2, -9, and -14 and ADAM-10) die in utero displaying a perturbed extracellular matrix organization (Javaheri et al., 2016).

These observations suggest that bone remodeling may not be solely defined by the balance/imbalance between MMPs and TIMPs. Rather, other molecules expressed and released in the settings of bone physiology and pathology such as RECK (Paiva and Granjeiro, 2014) and some acute phase reactants (alpha 2-macroglobulin, fibrinogen) may regulate/dysregulate MMP activity in inflammatory conditions thus perturbing the normal bone remodeling process (Cook et al., 2018; Sarker et al., 2019). A consequence implied by the latter notion is that MMPs, ADAMs and ADAMTS molecules may be released from bone or non-bone tissues to influence bone remodeling through autocrine and paracrine actions. In other words, MMPs likely circulate bound to non-classical inhibitors (such as acute phase reactants) being recruited to sites of active bone remodeling, where local substrates act as chemoattractants and local activators (other proteases, reactive oxygen species) activate them.

The aforementioned levels of regulation effectively dissociate MMP expression from MMP activity (e.g., since overexpression of endogenous MMP inhibitors would effectively reduce MMP activity). Current biochemical techniques for assessing MMP activity are non-reliable. However, as research requires a proxy, MMP expression is often used as a surrogate (albeit incorrectly) for MMP activity. There remains an urgent need for highly sensitive, specific, and robust methods for assessing the activity potential of individual MMPs such that therapeutic strategies can be designed to specifically reduce the activity of overactive MMPs (i.e., those whose activity levels are above baseline) or to increase the activity of underactive MMPs (i.e., those whose activity levels are below baseline).

### Roles of MMPs Associated to Bone Development and Remodeling

The biochemical actions of MMPs are intimately linked to their cells of origin. Table 4 describes cell-specific roles of MMPs in physiological bone remodeling. Osteoclast-mediated bone resorption in calvaria and long bones requires normal enzymatic activity of MMPs and cysteine proteinases such as...
### TABLE 4 | Specific roles of MMPs under physiological conditions in bone remodeling.

| Entity                  | MMP                        | Role                                                                 | References                                                                 |
|-------------------------|----------------------------|---------------------------------------------------------------------|---------------------------------------------------------------------------|
| Cartilage and bone cells| Network of multiple MMPs (mainly widely expressed MMP-2, -7, -9, -12, -13, -14, -16) | Maintain bone and cartilage health by their normal proteolytic activity. | Everts et al., 1992; Meikle et al., 1992; Mattot et al., 1995; Apte et al., 1997; Johansson et al., 1997; Bord et al., 1998; Jimenez et al., 1999; Filani et al., 2000 |
|                         |                            | Control bone tissue remodeling at the levels of osteocyte viability and activities, osteoclast recruitment and function, bone matrix solubilization, coupling of bone resorption and formation, osteoblast recruitment and survival, cell-extracellular matrix interaction, and cell–cell interaction. | Blavier and Delaisse, 1995; Bord et al., 1998; Engsig et al., 2000; Hou et al., 2004; Inada et al., 2004; Karsdal et al., 2004; Holmbeck et al., 2005; Kasper et al., 2007; Manduca et al., 2009; Lu et al., 2010; Ortega et al., 2010; Tang et al., 2012; Madsen et al., 2013; Lozito et al., 2014; Almalki and Agrawal, 2016 |
|                         |                            | Regulate the bioavailability of soluble RANKL, thereby promoting the formation of multinucleated osteoclast cells, acquisition of osteoclast-specific differentiation markers, binding of osteoclasts to bone surfaces, promotion of osteoclast survival, and stimulation of bone resorption. | Bellido et al., 2019 |
| Mesenchymal stem cells   | Network of multiple MMPs, tissue inhibitors of MMPs and RECK | (i) Modulates the commitment and differentiation of mesenchymal stem cells. (ii) Impacts osteoblastic migration, spreading, and differentiation. | Kasper et al., 2007; Lu et al., 2010; Lozito and Tuan, 2011; Egea et al., 2012; Almalki and Agrawal, 2016; Mahl et al., 2016 |
|                         | MMP-16                     | Controls mesenchymal stem cells viability.                          | Paiva and Granjeiro, 2017 |
|                         | MMP-2 and MMP-9            | Promote the directional migration of bone marrow mesenchymal stem cells. | Lv et al., 2017 |
| Osteocytes              | MMP-2, MMP-13 and MMP-14   | Modulate the formation of the osteocyte canalicular network.         | Barthelemi et al., 2012 |
|                         | MMP-13                     | Regulates the remodeling of the osteocyte lacunar-canicular network in mid-cortical bone matrix, which is critical for the active maintenance of bone quality (matrix composition, organization, fracture resistance). | Tang et al., 2012; Alliston, 2014 |
|                         | MMP-14                     | Essential for cell adhesion, invasion, and cell–cell communication events. | Hughes et al., 1994; Paiva and Granjeiro, 2017 |
| Osteoclasts             | MMP-9                      | Participates in cell recruitment (by generating collagen-derived endostatin which prevents osteoclast chemotaxis), survival (e.g., by activating pro-TNF-α), adhesion (e.g., by cleaving intercellular adhesion molecule-1), as well as in degradation of cytokines important to osteoclastogenesis such as IL-1β. | Gearing et al., 1995; Ito et al., 1996; Ferreras et al., 2000; Fiore et al., 2002 |
|                         | MMP-12                     | Modulates the interaction between osteoclasts and bone matrix through multiple mechanisms including: (i) cleavage of osteopontin, vitronectin, bone sialoprotein and osteonectin, (ii) activation of TNF-α, (iii) generation of endostatin from collagen, and (iv) digestion of urokinase-type plasminogen activator receptor/uPAR. | Koolwijk et al., 2001; Hou et al., 2004; Paiva and Granjeiro, 2017 |
|                         | MMP-14                     | Sheds CD14 receptor to impinge on osteoclast adhesion and migration as well as being involved in monocyte/macrophage fusion (e.g., by modulating the Rac1 pathway). | Kajita et al., 2001; Vivinus-Nebot et al., 2004; Gonzalo et al., 2010 |
|                         | The CD44/MMP-9/MMP-14 axis | Mediates pro-MMP-9 activation on the osteoclast membrane thereby modulating osteoclast migration in bone tissue resorption. | Chellaiah and Ma, 2013 |
|                         | MMP-14 and MMP-7           | Promote RANKL availability, which implicates the RANKL/RANKL/osteoprotegerin axis in osteoclast maturation and activation. | Lynch et al., 2005; Hikita et al., 2006; Aiken and Khokha, 2010 |

(Continued)
cathpsin K whose deficiency impairs bone remodeling (Everts et al., 1999; Delaisse et al., 2003). This is evidenced in osteoclasts from patients with pycnodysostosis (an osteopetrosis-like bone disease related to loss-of-function mutations in the cathepsin K gene) and osteoclasts from cathepsin K-deficient mice which are unable to efficiently digest organic bone matrix, resulting in large, mineral-free areas of bone matrix (Everts et al., 1998, 2009). Cysteine proteinases synthesized and used by the different osteoclasts for bone matrix digestion (Everts et al., 2006) can degrade intramembranous bones as well as osteoclast-derived MMPs (Everts et al., 2009). Cysteine proteinases are secreted to act in the low pH environments formed by osteoclasts in the resorption sites, with MMPs degrading the rest of the bone matrix when the pH increases (Everts et al., 1998) as well as contributing to the digestion of fibrillar, non-mineralized collagen in Howship’s lacunae abandoned by osteoclast cells (Everts et al., 2002). These complementary and overlapping contributions of the MMP and cysteine proteinase families make the process of bone tissue remodeling both complex and robust.

The involvement of MMPs in bone remodeling has become clear with the aid of animal models such as MMP-deficient mice, which show a variety of bone abnormalities (Table 5). Impaired bone tissue remodeling in Mmp2−/− mice (Table 5, row 2) is characterized by a reduced number of osteoblasts and osteoclasts, disruption of the canicular network exacerbating osteocyte death, disruption of the Mmp-2, osteopontin-bone sialoprotein axis, and promotion of osteolysis (Martignetti et al., 2001; Inoue et al., 2006; Mosig et al., 2007; Malaponte et al., 2016). MMP-9-deficient mice show alterations in cartilage-bone replacement during endochondral ossification (Vu et al., 1998) (Table 5, row 3). This phenotype may be explained by an inefficient degradation of the cartilage matrix, which leads to a diminished bioavailability of extracellular matrix-derived vascular endothelial growth factor and consequently effects osteoclasts and endothelial cells movement into the cartilage (Ortega et al., 2010). Bone tissue modeling and remodeling processes are altered in MMP-13 deficient mice (Table 5, row 4) (Inada et al., 2004; Stickens et al., 2004; Ortega et al., 2005). MMP-14 deficiency (Table 5, row 5), which is associated with high lethality, results in the most drastic skeletal phenotype among MMP-deficient mice (Holmbeck et al., 1999; Zhou et al., 2000). Double gene-deficient mice lacking at least one MMP gene have been engineered and their bone phenotype have been studied. For instance, double-knockout mice lacking MMP-2 and uPARAP/Endo180 (endocytic collagen receptor of collagen and collagen fragments for degradation in the lysosomes) show reduced bone mineral density, short long bones, and poor trabecular bone quality (Madsen et al., 2013). MMP-8 and MMP-13 double-deficient mice have abnormal growth plate as well as augmented metaphyseal trabecular bone mineral density (Inada et al., 2001, 2002; Stickens et al., 2004). Double knockout mice lacking MMP-9 and MMP-13 exhibit expanded growth plates, disorganized hypertrophic chondrocyte zone, increased number of end-differentiated hypertrophic cells, and

| Entity | MMP | Role | References |
|--------|-----|------|------------|
| Bone matrix | MMP-1, -2, -8, -9, -13, -14, and -15 | Necessary for extracellular matrix turnover. | Paiva and Granjeiro, 2017 |
| MMPs -2, -3, -7, -9, -12, -14 | Cleave and regulate bone matrix-associated non-collagenous proteins (such as osteonectin, vitronectin, osteopontin, bone sialoprotein) as well as cell membrane- and matrix-anchored latent growth factors. | Sasaki et al., 1997; Agnihotri et al., 2001; Sage et al., 2003; Lindsey et al., 2015 |
| MMP-14 | The collagen fragments produced by MMP-14 are endocytosed via uPARAP/Endo180 for total lysosomal degradation. | Lafleur et al., 2006; Lee et al., 2006; Messaritou et al., 2009 |
| Osteoblasts | MMP-2 | Critical for osteoblast differentiation and survival. | Paiva and Granjeiro, 2017 |
| MMP-14 | Serves to preserve osteoblast survival once osteoblasts have stopped the synthesis of new bone matrix, thus aiding in the transition from osteoblasts to osteocytes. | Karsdal et al., 2004 |
| Bone remodeling | MMPs from osteoblasts and bone lining cells | Preceding osteoclast adhesion and resorption, MMPs participate in the cleavage of organic matrix (such as cathepsin-cleaved collagen and non-collagenous proteins). | Holliday et al., 1997; Stahle-Backdahl et al., 1997; Yamagiwa et al., 1999; Paiva and Granjeiro, 2017 |
| MMP-13 | Active in regulating bone mass through osteoblasts, and forming osteocyte canalicular network. | Page-McCaw et al., 2007; Bartheleimi et al., 2012 |
| MMP-14/CD44 | Activates Pro-MMP-9 on osteoclast membrane surface during osteoclast recruitment, adhesion, resorption and migration. | Paiva and Granjeiro, 2017 |

MMPs, matrix metalloproteinases; RECK, reversion-inducing cysteine-rich protein with Kazal motifs; TNF, tumor necrosis factor; IL, interleukin; Rac1, Ras-related C3 botulinum toxin substrate 1 pathway; RANK, receptor activator of nuclear factor kappa B; RANKL, RANK ligand; uPARAP/Endo180, endocytic collagen receptor of collagen and collagen fragments for degradation in the lysosomes.
delayed formation of the bone marrow cavity (Kennedy et al., 2005; Paiva and Granjeiro, 2014). The bone phenotype of mice with a double knockout for MMP-14 and MMP-2 reassembles that of MMP-14-deficient mice (Oh et al., 2004). MMP-14 and MMP-16 double-knockout mice develop a bone phenotype that affects ossification (intramembranous and endochondral) and is characterized by severe irregularities, including (i) high mortality associated to developmental defects, (ii) noticeable craniofacial malformations such as cleft palate, thinner cranial vault bones, deformed parietal bone vault, and more frontal and nasal bones, (iii) altered growth plate, and (iv) cortical bone shortening (Paiva and Granjeiro, 2014). MMP-14 and uPARAP/Endo180 double-knockout mice die soon after birth (Wagenaar-Miller et al., 2007). As listed in Table 6, MMP activity contributes to numerous bone phenotypes including arthritis, osteoporosis, osteonecrosis, periodontalitis, sinusonal osteitis, degenerated lumbar disk tissues, and bone cancer metastasis (Aiken and Khoka, 2010; Koskinen et al., 2011; Mittal et al., 2016; Rose and Kooymen, 2016; Lazarus et al., 2017; Paiva and Granjeiro, 2017; Tauro and Lynch, 2018; Zhang et al., 2018). The roles played by MMPs in these pathways are influenced by non-matrix proteins such as TIMPs, transforming growth factor, vascular endothelial growth factor, bone morphogenic proteins, activated protein C, and the Wnt [Wingless-type MMTV integration site family]-β-catenin (Table 7).

**MMPs as Sheddases**

Beyond the direct degradation of extracellular matrix substrates (e.g., collagen), MMP-mediated cleavage of substrates can lead to the release (sheding) into the extracellular matrix of soluble fragments of cell membrane-anchored receptor ligands. This extracellular event enables ligand-mediated activation of cognate receptors and elicits downstream intracellular signal transduction cascades which modify gene transcription and, ultimately, cell behavior. A prominent example pertinent to osteoblasts is the release of RANKL, which is the ligand of receptor activator of nuclear factor kappa B (RANK), by MMP-14. This MMP-14/RANKL/RANK/signal transduction axis regulates osteoblastogenesis and osteoclastogenesis, making MMP-14 crucial for normal bone formation (Bonfil et al., 2007; Thiolloy et al., 2009; Sabbota et al., 2010; Bonfil and Cher, 2011). The ligand shedding activity of

**TABLE 5 | Selected skeletal phenotypes associated to MMP deficiency in mice.**

| Genotype | Phenotype | References |
|---------|-----------|------------|
| Mmp2−/− | MMP-2 knockout (vs. wild-type) mice show: (i) craniofacial defects (such as shorter and broader snouts, hypertelorism, smaller jaws, dome-shaped and taller skulls), (ii) severe arthritis and joint contractures (even in young mice) with articular cartilage destruction and erosion of the underlying bone surface, (iii) joint pathology with increased cellular infiltration and proteolytic depletion in antigen-induced arthritis, (iv) diminished bone integrity (such as long bones with osteopenia, fractured tibiae), (v) abnormal bone development (e.g., reduced number of long bones, decreased femur and tibia length in adult mice, calvarial bones with a greater [48%] thickness by 55 weeks of age, trabecular bone with fewer osteocytes), (vi) progressive decrease in bone mineral density and increase in bone porosity (characterized by e.g., low trabecular connectivity density, reduced mineral-collagen relation, thinner diaphyseal cortex, less nanoindentation modulus), (vii) increased number of empty lacunae as the mice aged (e.g., about 3-fold by 55 weeks of age), (viii) loss of the canalicular network architecture in calvariae and slighter in long bones, and (ix) presumably expression of bone sialoprotein (which increases osteoblast differentiation and activity) and osteopontin (which increases osteoclast activity). | Inoue et al., 2006; Mosig et al., 2007; Lieu et al., 2011; Nyman et al., 2011; Madsen et al., 2013 |
| Mmp9−/− | MMP-9 knockout (vs. wild-type) mice show: (i) long bones (e.g., metatarsals) with increased (e.g., 4-8-fold for 3 weeks old mice) hypertrophic (cartilage) zones, (ii) 10% shorter long bones, which is the only remaining phenotype in older MMP-9 deficient mice, (iii) irregularly shaped bone spicules, (iv) delayed endochondral ossification, (v) expanded zone of hypertrophic chondrocytes in the growth plate, (vi) reduced vascular invasion into the hypertrophic cartilage, (vii) slowed apoptosis of hypertrophic chondrocytes, (viii) impaired osteoclast/condroclast recruitment, (ix) abnormal growth in trabecular bone mass, and (x) improved connectivity density of the tibia trabeculae. This phenotype eventually resolve, resulting in correction of bone growth defects after approximately 4 weeks of age. | Vu et al., 1998; Ortega et al., 2003; Nyman et al., 2011; Kojima et al., 2013 |
| Mmp13−/− | MMP-13−/− (vs. Mmp13+/+) mouse embryos show: (i) progressive changes in the embryonic growth plates (e.g., increased length which persisted in adults), (ii) delayed endochondral ossification, (iii) augmented metaphyseal trabecular bone mass as the mice aged (e.g., 3 months old), (iv) diminished resistance to fracture in long bones, (v) delay in fracture repair, (vi) defective vascular penetration and chondroclast attraction to the fracture callus, (vii) noticeable expression of collagen type X, osteopontin, and VEGF by hypertrophic chondrocytes. | Inada et al., 2001; Inada et al., 2002; Inada et al., 2004; Stickens et al., 2004; Kosaki et al., 2007; Tang et al., 2012; Singh et al., 2013 |
| Mmp14−/− | MMP-14 knockout (vs. wild-type) mice show: (i) progressive disturbances (e.g., smaller body size and weight, very high postnatal mortality), possibly caused by deprived feeding and therefore malnutrition, (ii) craniofacial dysmorphism in surviving mice (e.g., short snout, hypertelorism, dome-shaped skull, orbital protrusions, unclosed cranial sutures), (iii) incomplete cartilage remodeling, (iv) impaired formation of secondary ossification centers in the epiphyses, (v) arthrosis resulting from joints with arthritis and other factors (e.g., greater vascularity of the ligaments and tendons, overgrowth of hypercellular and wrongly vascularized synovial tissue), (vi) augmented bone resorption, (vii) osteopenia, (viii) osteoporosis, (ix) dwarfism, (x) mesenchymal stem cells commitment to chondrogenesis and adipogenesis instead of osteogenesis. | Holbeck et al., 1999; Zhou et al., 2000; Holbeck et al., 2003 |
| Mmp16−/− | MMP-16 knockout (vs. wild-type) mice show shorter size associated with reduced viability of mesenchymal cells in bone tissues. | Shi et al., 2008; Loffek et al., 2011 |

MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor.
TABLE 6 | Involvement of MMPs in bone pathologies.

| MMP         | Reported involvement                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | References                                                                                   |
|-------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| MMP-1       | Abundant in the diaphysis and metaphyses of long bones being upregulated in arthritis.                                                                                                                                                                                                                                                                                                                                                                                       | Gack et al., 1995; Wu et al., 2008; Rose and Kooyman, 2016                                    |
| MMP-2       | Required for maintenance of bone mineral density and strength and in bone development (e.g., by affecting intramembranous and endochondral ossification); however, deregulated MMP-2 expression is observed in the settings of metabolic syndrome, osteoporosis, osteonecrosis of the jaws, ligamentum flavum degeneration in lumbar spinal canal stenosis, as well as in bone pre-metastatic niche formation. | Duerr et al., 2004; Suh et al., 2004; Tester et al., 2004; Durie et al., 2005; Lynch, 2011; Fernandez-Patron et al., 2016; Rose and Kooyman, 2016; Sugimoto et al., 2018 |
| MMP-3       | Overexpressed in osteoarthritis (in cartilage and the synovium) and also acts on primary tumor growth.                                                                                                                                                                                                                                                                                                                                                                      | Okada et al., 1992; Tester et al., 2004; Lynch, 2011; Paiva and Granjeiro, 2017               |
| MMP-8       | Modulates human dentin and remodeling, but its deregulation may exacerbate periodontitis although it may be protective against inflammatory arthritis.                                                                                                                                                                                                                                                                                                                                   | Sulkala et al., 2007; Cox et al., 2010; Mauramo et al., 2018                                 |
| MMP-9       | Participates in chondrocyte biology; specific processes in which the enzyme is involved are apoptosis of hypertrophic chondrocytes present in the plate, bone development (e.g., by being highly active to angiogenesis in the growth plate), strength and toughness of bone, as well as the regulation of gene pathways responsible for osteoclastogenesis. In turn, MMP-9 overexpression contributes to sinonasal ostesitis, rheumatoid arthritis, and degenerated lumbar disk tissues. Osteoporotic bone (vs. normal bone) tissues express higher MMP-9 levels. Involved in secondary (metastatic) breast cancer in the bone (e.g., by promoting angiogenesis, regulating VEGF bioavailability, contributing to bone remodeling) or prostate cancer (e.g., by influencing bone osteoblastic and osteoclastic activity). | Vu et al., 1998; Liang et al., 2016; Mittal et al., 2016; Ahrens et al., 1996; Li et al., 2017 |
| MMP-13      | Required for bone development; it participates in the transition from cartilage to bone at the growth plates of long bones and in the remodeling of bone spicules. In turn, MMP-13-mediated degradation of articular cartilage exacerbates osteoarthritis. In linking osteoarthritis to metabolic syndrome, the presence of adiponectin positively correlates with the presence of membrane-expressed PGE synthase and MMP-13. Overexpressed in congenital spondyloepiphyseal dysplasia which results in early development of osteoarthritis. In addition to typical bone collagen matrix degradation, MMP-13 regulates bone resorption in periodontal disease through osteoclast differentiation (by inactivating galectin-3, an inhibitor of osteoclastogenesis) and osteoclast activation (by activating osteoclast-secreted pro-MMP-9 and favoring RANKL and TGF-β signaling). In breast cancer resulting from bone metastasis, MMP-13 deregulation may alter osteoblast morphology and bone resorption through differentiation of pre-osteoclasts, osteoclast activation, and osteolysis. | Inada et al., 2004; Stickens et al., 2004; Page-McCaw et al., 2007; Holmbeck et al., 1999; Mittal et al., 2016; Rose and Kooyman, 2016 |
| MMP-14      | Contributes to bone development (endochondral and intramembranous ossification) and remodeling. Extracellular matrix remodeling by MMP-14 influences cell shape inducing the formation of a complex between MMP-14 and beta1-integrin, which activates the Rho/ROSA(S) cascade leading to nuclear translocation of YAP and TAZ – this series of signaling events is necessary for mesenchymal stem cells commitment during development. Palmitoylation (i.e., addition of 16-carbon palmitate to proteins) enables MMP-14 to anchor to cell membrane. This post-translational modification of MMP-14 has a major impact on bone development and bone tissue metabolism likely through influencing MMP-14 correct membrane localization and also decreasing the expression of osteocalcin and vascular endothelial growth factor in osteoblasts and chondrocytes. In turn, MMP-14 is critical for osteoclast resorption thus contributing to the pathogenesis of osteoporosis. | Holmbeck et al., 1999, 2003; Zhou et al., 2000; Liao et al., 2004; Hienz et al., 2004; Stickens et al., 2004; Page-McCaw et al., 2007; Shah et al., 2012 |
| MMP-3 and MMP-9 | Contribute to cartilage endplate degeneration.                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | McGowan and Duffy, 2008; Rowe and Weiss, 2009; Paiva and Granjeiro, 2017                    |
| MMP-2, MMP-9, and MMP-13 | In experimental glucocorticoid-induced osteoporosis and osteocytic osteolysis, these three enzymes are upregulated in the trabecular bone of the metaphysis whereas MMP-2 and MMP-13 are expressed in the cortical bone diaphysis. | Zhang et al., 2018; Sun et al., 2016                                                        |

VEGF; vascular endothelial growth factor; PGE; prostaglandin E synthase; RANKL, receptor activator of NF-kappa B ligand; TGF transforming growth factor; MMP, matrix metalloproteinase; YAP, Yes-association protein; TAZ, transcriptional coactivator with PDZ-binding motif.

MMPs influences the propensity to cancer metastasis and bone disease. For instance, MMP-14-mediated shedding of RANKL and downstream activation of RANK in the left supraclavicular lymph node cells of the prostate stimulates the non-receptor tyrosine kinase, SRC, to effectively increase the migration of prostate tumor cells which can metastasize.
TABLE 7 | Interactions of MMPs with other proteins in bone development/remodeling.

| Protein | Effect on the partner | Effect on bone development/remodeling |
|---------|-----------------------|---------------------------------------|
| TIMPs   | Inhibit all MMPs       | Control bone resorption and formation  |
|         |                       | (Bord et al., 1999; Sobue et al., 2001; Huang et al., 2002; Geoffroy et al., 2004; Haesler et al., 2005; Sahebjam et al., 2007; Shen et al., 2010; Miller et al., 2017). |
| MMP-2/MMP-9 | Control TGF-β (bioavailability and bioactivity) | Decrease the mechanical properties (modulus, hardness) of mice bones, when TGF-β signaling is augmented (Dajas et al., 1995; Balloch et al., 2005; Nyman et al., 2011) |
| MMP-9   | Regulates VEGF (bioavailability and bioactivity) | Exerts chemotactic action on osteoclasts, which affects osteoclast recruitment during bone resorption (Bergers et al., 2000; Engsig et al., 2000; Ortega et al., 2010). |
| MMP-14  | Activates TGF-β        | Helps to preserve the survival of osteoblasts and their differentiation into osteocytes (Karsdal et al., 2002). |
| TGF-β   | Upregulates MMP-13     | Promotes bone resorption associated to changes in osteoblast morphology (Karsdal et al., 2001). |
| BMPs    | Regulates MMP-2        | Obstructs tissue remodeling and regeneration in Poecilia latipinna (Rajaram et al., 2016). |
|         | Regulates MMP-9        | Impairs bone remodeling (e.g., augmented bone mass during early development) and chondrocyte commitment (e.g., in the mouse C3H10T1/2 stem cell line) (Kamiya et al., 2008; Choi et al., 2009; Rajaram et al., 2016). |
| Wnt/β-catenin | Regulates MMP-2      | Affects bone development (cartilage formation, endochondral ossification, growth plate organization, chondrocyte function) (Tamamura et al., 2005). |
|         | Upregulates MMP-9      | Modulates cartilage degradation and bone resorption (Tamamura et al., 2005). |
|         | Regulates MMP-13       | Modulates cartilage vascularization (Tamamura et al., 2005; Nakashima and Tamura, 2006; Chen et al., 2008; Papathanasiou et al., 2012). |
| aPC     | Upregulates MMP-2 activity | Suppresses cartilage and bone degradation as well as pro-inflammatory signaling in rheumatoid arthritis patients (Nguyen et al., 2000; Buisson-Legendre et al., 2004; Xue et al., 2007). |
|         | Downregulates MMP-9 activity | Suppresses cartilage pro-inflammatory signaling as well as cartilage and bone degradation in rheumatoid arthritis patients (Xue et al., 2007; Xue et al., 2010). |

TIMPs, tissue inhibitor of MMPs; MMP, matrix metalloproteinase; TGF-β, transforming growth factor; VEGF, vascular endothelial growth factor; BMP, bone morphogenic protein; aPC, activated protein C.

to bone (Sabbota et al., 2010). Similarly, osteoclast-derived MMP-7 solubilizes osteoblast-bound RANKL whose release into the tumor-bone microenvironment promotes osteoclast activation in bone metastatic sites contributing to prostate and mammary tumor-induced osteolysis (Lynch et al., 2005; Thiolloy et al., 2009).

MMP-Generated Neoepitopes

The proteolytic action of MMPs on extracellular matrix macromolecules can result in the exposure of neo-epitopes (i.e., unique bioactive MMP-generated fragments). Compared to healthy subject controls, patients with ankylosing spondylitis (which is a form of arthritis that causes inflammation of the vertebrae) show significantly higher levels of different neo-epitopes such as C1M, C2M, C3M, C4M, C5M, C6M, and C7M from collagen type I, II, III, IV, V, VI, and VII (Veidal et al., 2012; Genovese and Karsdal, 2016). Some of these neo-epitopes have been combined (e.g., C2M, C3M, and C6M) for diagnostic purposes (Bay-Jensen et al., 2012). IPEN341-342FGV is an MMP cleavage site which could be useful as diagnostic and prognostic markers for osteoarthritis (Bay-Jensen et al., 2011). Similarly, other MMP-generated neo-epitopes derived from collagen type II (e.g., C2C, C2M, C-terminal telopeptide of type II collagen (CTX-II), and TIINE) hold biomarker potential for osteoarthritis (Karsdal et al., 2010; Qvist et al., 2010; Karsdal et al., 2011).

Over-Overexpression of MMPs

Over-expression of MMPs is frequently reported in arthritis (Burrage et al., 2006; Tokito and Jougasaki, 2016). Collagenolytic MMPs (such as MMP-1, -2, -8, -13, and -14) are expressed in the arthritic joint and likely participate in the degradation of cartilage type II collagen, while MMP-3, -7, and -9 can degrade aggrecan leading to joint destruction (Puliti et al., 2012; Tokito and Jougasaki, 2016). Such a pathological mechanism has been proposed for MMP-3 and MMP-13 in degenerative joint disease in the elderly (Neuhold et al., 2001; Troeberg and Nagase, 2012; Jackson et al., 2014; Pap and Korb-Pap, 2015). Other contributions to osteoarthritis from activities related to MMP-3 include MMP-3-mediated activation of MMP-1 and MMP-13 (Mancini and di Battista, 2006; Tokito and Jougasaki, 2016). In rheumatoid arthritis, MMP-14 is greatly expressed in fibroblast-like synoviocytes and macrophages, and it could be an effector to cartilage destruction (Pap et al., 2000; Sabeh et al., 2010). MMP-1 and MMP-3 likely participate in cartilage destruction in rheumatoid arthritis and osteoarthritis (Burrage et al., 2006; Fiedorczyk et al., 2006; Tokito and Jougasaki, 2016). As a result, MMP overexpression could be therapeutically
targeted in arthritis (Tokito and Jougasaki, 2016). Whether reducing MMP expression (or activity) levels provides a clinical benefit is unclear. In experimental models, many synthetic MMP inhibitors have shown positive effects (Ishikawa et al., 2005). At the clinical level, however, all efforts with MMP inhibitors to block the damaging activity of MMPs in arthritis and other non-neoplastic conditions were regrettably unsuccessful (Burrage et al., 2006; Tokito and Jougasaki, 2016). Reasons for these failures include: (i) deficient clinical trial designs (Burrage et al., 2006), (ii) unwanted characteristics of MMP inhibitors (side effects including musculoskeletal pain, low oral bioavailability, short in vivo half-lives, and lack of selectivity [Iyer et al., 2012; Fields, 2015; Tokito and Jougasaki, 2016]), (iii) inability of MMP inhibitors to infiltrate the cartilage/bone/synovial interface (Burrage et al., 2006), (iv) neglect of the highly complex functions served by MMPs in physiological and disease states (Iyer et al., 2012; Li et al., 2013; Sawicki, 2013) and (v) broad tissue distribution and substrate promiscuity exhibited by MMPs and their substrates (Burrage et al., 2006; Tokito and Jougasaki, 2016). To date, there remains a need for highly selective MMP inhibitors and for better information on the disease-specific substrates, which could be therapeutically targeted as shown by recent studies with MMP-13 in osteoarthritis (Li et al., 2011) as well as for more efficient and reliable techniques to sensitively measure condition-specific MMP activity potential (not just MMP expression levels).

**MMP Gene Polymorphism**

A nucleotide polymorphism, by which an additional guanine creates an ETS transcription factor binding site (5'-GGA-3') at position 1607 in the promoter sequence of the MMP-1 gene, has been related to bone mineral density (BMD) (Rutter et al., 1998). This polymorphism is associated with increased transcription of the MMP-1 gene and elevated MMP-1 activity. Among 819 postmenopausal Japanese women, BMD (e.g., D50, D100) for the distal radius had a lower value in women with the GG/GG genotype (47.9%) than in those with other (e.g., G/G [41.9%], G/G [10.3%], G/G + G/G [52.1%]) genotypes. A -1562C3 thymine polymorphism in the promoter of MMP-9, which shows greater transcriptional activity than T/C alleles, was found to be correlated with more severe stages of disk herniation (Jing et al., 2018); while the T allele of rs17576 appears to correlate with more severe stages of disk degeneration.

**MMP Deficiency and Insufficiency in Humans**

Having discussed the roles of MMPs under physiological and pathological conditions, we will next discuss how their deficiency and insufficiency relates to bone metabolic abnormalities. MMP-2 gene deficiency leads to a rare human skeletal disorder¹, which was first reported in consanguineous Saudi Arabian families, and is characterized by severe bone alterations (Martignetti et al., 2001). Osteolytic and metabolic changes linked to MMP-2 deficiency affect tarsal, carpal, and phalangeal bones, cause severe arthropathy, osteoporosis, fibrous nodules, distinctive craniofacial defects such as exophthalmos, brachycephaly, and flattened nasal bridges and dwarfism (Al-Aqeel et al., 2000; Al-Mayouf et al., 2000; Al-Aqeel, 2005; Mosig et al., 2007; Page-McCaw et al., 2007; Castberg et al., 2013). This complex syndrome is currently categorized as a form of Torg syndrome and results from homoallelic mutations in the gene for MMP-2 located at 16q12-21 (Martignetti et al., 2001; Liang et al., 2016). A Tyr codon in the MMP-2 prom domain is replaced with the Y244X stop codon and an Arg is replaced with a His (R101H) in the cysteine-containing domain (PRCGNPD substituted by PHCGNPD). The R101H mutation is suggested to perturb coordination of Cys102 to the catalytic Zn(II) domain, consequently activating intracellular pro-MMP-2 and leading to its auto-degradation (Kennedy et al., 2005; Krane and Inada, 2008). A homoallelic missense mutation in the catalytic Zn(II) domain (E404K) has been revealed in Winchester syndrome (another variant of multicentric osteolysis) (Zankl et al., 2005). These rare Torg and Winchester arthritic syndromes together with others (such as multicentric osteolysis with nodulosis and arthropathy [known as MONA]) belong to a general family of hereditary autosomal dominant and recessive skeletal disorders with progressive bone loss and joint destruction (Al-Mayouf et al., 2000; Martignetti et al., 2001; Al-Aqeel, 2005; Zankl et al., 2005; Rouzier et al., 2006; Mosig et al., 2007; Tuysuz et al., 2009).

Similar to MMP-2, a homozygous dominant mutation (Ser substituted by Phe [F56S]) in the pro-region domain of MMP-13 also results in a bone development disorder known as spondyloepimetaphyseal dysplasia-Missouri type (Kennedy et al., 2005)². This disorder, which appears to spontaneously resolve by adolescence, is characterized by anomalous modeling of long bones, mild defects in epiphysis, moderate to severe changes in the metaphysis morphology, pear-shaped vertebrae, femoral and tibial bowing, genu varum deformities, and osteoarthritis. While the biochemical mechanisms linking MMP-13 to these bone abnormalities remain unclear, the phenotype of MMP-13 deficiency could be due to a late exit of chondrocyte cells from the growth plate (Kennedy et al., 2005).

MMP-14 is widely considered one of the physiological activators of MMP-2 as it converts pro-MMP-2 into mature MMP-2 at the cell surface (Fernandez-Patron et al., 2016). An MMP-14 homoallelic mutation (T > R replacement in the signal peptide domain) destabilizes the interaction (e.g., recognition and binding) of the MMP-14 signal peptide with the signal recognition particle complex, thus affecting MMP-14 targeting to the plasma membrane (Evans et al., 2012). This MMP-14 homoallelic mutation causes an apparent

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¹ [https://www.omim.org/entry/120360](https://www.omim.org/entry/120360)

² [https://www.omim.org/entry/600108](https://www.omim.org/entry/600108)
deficiency of biochemically active MMP-14 at the cell membrane which impairs pro-MMP-2 activation and causes a condition of MMP-2 activity deficiency with Winchester syndrome (Evans et al., 2012)\(^3\).

A missense homozygous mutation (g.16250T \(>\) A, which replaces His226 of the Zn(II) catalytic domain with Gln [p.H226Q]), in the MMP20 gene disrupts the metal-binding site and prevents MMP-20 proteolytic activity regarding enamel matrix proteins (Ozdemir et al., 2005)\(^4\). This mutation may lead to autosomal-recessive hypomaturation amelogenesis imperfecta, a group of inherited heterogeneous diseases that alter enamel development (amount, composition, structure) in humans (Kim J.W. et al., 2005). Another mutation in the intron 6 splice acceptor (g.30561A \(>\) T) that causes this disease is specifically characterized by pigmented teeth with a mottled and rough surface (Kim J.W. et al., 2005).

Partial loss of MMP activity or impaired MMP secretion can lead to MMP activity insufficiency. A pervasive cause of MMP insufficiency can be medications with such MMP inhibitory actions including: (i) Statins (200 million prescriptions in the United States/year; 14 million prescriptions for lovastatin alone in 2014)\(^5\) which can cause myositis and rhabdomyolysis (Luan et al., 2003; Thompson et al., 2003). (ii) Doxycycline (7 million prescriptions in 2014)\(^5\) with side-effects including joint inflammation in humans and cardiac inflammation in mice (Berry et al., 2015). (iii) Therapeutic antibodies against MMPs and MMP inhibitor drugs for treating patients with rheumatoid arthritis, severely active Crohn's disease, and cystic fibrosis\(^6\). If these antibodies reduce MMP activity below baseline levels, they would cause MMP insufficiency with unpredictable consequences. Pharmacological MMP-inhibitors in Phase 3 clinical trials conducted during 1997 and 1998 in patients with advanced cancers led to an as of yet poorly understood, very severe inflammatory musculoskeletal syndrome (Zucker et al., 2000; Coussens et al., 2002). Another common cause of MMP insufficiency could be the pathological elevation of...

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\(^3\)https://www.omim.org/entry/600754
\(^4\)https://www.omim.org/entry/604629
\(^5\)http://clincalc.com/DrugStats
\(^6\)http://www.gilead.com

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**FIGURE 2** Schematic representation of the bone remodeling cycle with emphasis on the manifold roles played by matrix metalloproteinases. (A) Osteocytes detect mechanical stress or respond to biochemical stimuli. (B) Lining cells of the endosteal bone surface retract and proteases (e.g., MMPs) remove bone underlying membrane. (C) Osteoclasts are attracted and fused to become activated. (D) The underlying bone is digested by active multinucleated osteoclasts. (E) Osteoblasts are recruited to the bone resorption cavity. (F) New osteoid is formed by osteoblasts, and then mineralized (Datta et al., 2008; Fernandez-Patron et al., 2016; Paiva and Granjeiro, 2017; Cook et al., 2018). Other pathologies related to inactive/underactive MMPs are excessive inflammation, cardiovascular disorders, and metabolic dysregulation. MMP underactivity could also result from undesired side effects of common medications with MMP inhibitory actions (e.g., statins) (Cook et al., 2018). MSCs, mesenchymal stem cells; GFs, growth factors; RUNX2, runt-related transcription factor 2; RANKL, receptor activator of NF-kappa B ligand.
endogenous MMP inhibitors (e.g., tissue inhibitors of MMPs, α-2-macroglobulin, RECK) (Mott et al., 2000; Oh et al., 2001; Nagase et al., 2006; Klein and Bischoff, 2011). In addition, there is fibrinogen, an acute phase reactant in arthritis, which our laboratory discovered recently to inhibit MMP-2 in a cohort of rheumatoid arthritis patients (Sarker et al., 2019).

**SUMMARY**

In summary, bone lining cells, osteocytes, osteoclasts, reversal cells, and osteoblasts are responsible for constant bone tissue remodeling (Figure 2). The activation of this multicellular unit and the intense communication between the bone cells is tightly regulated by mechanical stimuli, apoptosis, as well as systemic and local factors such as hormones and cytokines including RANKL, CSF-M, IL-3, and IL-6. Proteases of the MMP and cysteine protease families converge in the modulation of bone remodeling. Whereas proteolytic activity has long been thought to be required for the degradation of bone tissue in osteoarthritis and osteoporosis, inactivating mutations in MMP genes can also lead to bone pathology including osteolysis and metabolic abnormalities such as delayed growth. Thus, there remains a need to rethink the role played by proteases in bone physiology and pathology. More specific information related to bone remodeling and presumed pathways by which proteases, in particular MMPs, contribute to bone tissue remodeling in health and disease is provided in previous excellent reviews (Kini and Nandeesh, 2012; Rauner et al., 2012; Hienz et al., 2015; Liang et al., 2016; Mittal et al., 2016; Franco et al., 2017; Paiva and Granjeiro, 2017; Tauro and Lynch, 2018; Plotkin and Bruzzaniti, 2019).

**REFERENCES**

Agnihotri, R., Crawford, H. C., Haro, H., Matrisian, L. M., Havrda, M. C., and Liaw, I. (2001). Osteopontin, a novel substrate for matrix metalloproteinase-3 (Stromelysin-1) and matrix metalloproteinase-7 (Matrilysin). *J. Biol. Chem.* 276, 28261–28267. doi: 10.1074/jbc.M103608200

Aguirre, J. J., Plotkin, L. I., Stewart, S. A., Weinstein, R. S., Parfitt, A. M., Manolagas, S. C., et al. (2006). Osteocyte apoptosis is induced by weightlessness in mice and precedes osteoclast recruitment and bone loss. *J. Bone Miner. Res.* 21, 605–615. doi: 10.1359/jbmr.060107

Ahrens, D., Koch, A. E., Pope, R. M., Stein-Picarella, M., and Niedbala, M. J. (1996). Expression of matrix metalloproteinase 9 (96-kd gelatinase B) in human rheumatoid arthritis. *Arthritis Rheum.* 39, 1576–1587. doi: 10.1002/art.178039019

Aiken, A., and Khokha, R. (2010). Unraveling metalloproteinase function in skeletal biology and disease using genetically altered mice. *Biochim. Biophys. Acta* 1803, 121–132. doi: 10.1016/j.bbamcr.2009.07.002

Al-Aqeel, A., Al Sewairi, W., Edress, B., Gorlin, R. J., Desnick, R. J., and Martignetti, J. A. (2000). Inherited multicentric osteolysis with arthritis: a variant resembling Al-Mayouf, S. M., Majeed, M., Hugosson, C., and Bahabri, S. (2000). New form of osteolysis. *J. Bone Miner. Res.* 15, 1130–1139. doi: 10.1002/jbmr.102126

Apte, S. S., Fukai, N., Beier, D. R., and Olsen, B. R. (1997). The matrix metalloproteinase-14 (MMP-14) gene is structurally distinct from other MMP genes and is co-expressed with the TIMP-2 gene during mouse embryogenesis. *J. Biol. Chem.* 272, 25511–25517. doi: 10.1074/jbc.272.41.25511

Arpino, V., Brock, M., and Gill, S. E. (2015). The role of TIMPs in regulation of extracellular matrix proteolysis. *Matrix Biol.* 4, 247–254. doi: 10.1016/j.matbio.2015.03.005

Arumugam, B., Vairamani, M., Partridge, N. C., and Selvamurugan, N. (2018). Characterization of Runx2 phosphorylation sites required for TGF-β1-mediated stimulation of matrix metalloproteinase-13 expression in osteoblastic cells. *J. Cell Physiol.* 233, 1082–1094. doi: 10.1002/jcp.25964

Bar-Shavit, Z. (2007). The osteoclast: a multinucleated, hematopoietic origin, bone-resorbing multinucleated cell. *Biochimie* 89, 43–52. doi: 10.1016/j.biochi.2008.05.007

Balemans, W., Pitters, E., Cleiren, E., Ai, M., Van Wesenbeeck, L., Warman, M. L., et al. (2008). The binding between sclerostin and LRP5 is altered by DKK1 and by high-bone mass LRP5 mutations. *Calcif. Tissue Int.* 82, 445–453. doi: 10.1007/s00223-008-9130-9

Balooch, G., Balooch, M., Nalla, R. K., Schilling, S., Vafaroff, E. H., Marshall, G. W., et al. (2005). TGF-beta regulates the mechanical properties and composition of bone matrix. *Proc. Natl. Acad. Sci. U.S.A.* 102, 18813–18818. doi: 10.1073/pnas.0507417102

Baron, R. (1989). Molecular mechanisms of bone resorption by the osteoclast. *Anat. Rec.* 224, 317–324. doi: 10.1002/ar.1092240220

Bar-Shavit, Z. (2007). The osteoclast: a multinucleated, hematopoietic origin, bone-resorbing osteoclast-like cell. *J. Cell Biochem.* 102, 1130–1139. doi: 10.1002/jcb.21533

Barthelemi, S., Robinet, J., Garnotel, R., Antonicelli, F., Schittly, E., Hornebeck, W., et al. (2016). Effects of matrix metalloproteinases on the fate of mesenchymal stem cells. *Stem Cell Res. Ther.* 7, 1–12. doi: 10.1186/s13287-016-0393-1

Bay-Jensen, A. C., Leeming, D. J., Kleyer, A., Veidal, S. S., Schett, G., and Karsdal, M. A. (2012). Ankylosing spondylitis is characterized by an increased turnover

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of several different metalloproteinase-derived collagen species: a cross-sectional study. *Rheumatol. Int.* 32, 3565–3572. doi:10.1136/ard.49.10.727
Bay-Jensen, A. C., Liu, Q., Bryjalsen, I., Li, Y., Wang, J., Pedersen, C., et al. (2011). Enzyme-linked immunosorbent assay (ELISAs) for metalloproteinase derived type II collagen neoepitope, CIIIM—increased serum CIIM in subjects with severe radiographic osteoarthritis. *Clin. Biochem.* 44, 423–429. doi:10.1016/j.clinbiochem.2011.01.001
Baylink, D. J., Finkelman, R. D., and Mohan, S. (1993). "Growth factors to stimulate Bone Remodeling and Matrix Metalloproteinases" in Buisson-Legendre, N., Smith, S., March, L., and Jackson, C. (2004). Elevation of activated protein C in synovial joints in rheumatoid arthritis and its correlation with matrix metalloproteinase 2. *Arthritis Rheum.* 50, 2151–2156. doi:10.1002/art.20313
Burger, E. H., and Klein-Nulend, J. (1999). Mechanotransduction in bone-role of the laculocanalicular network. *FASEB J.* 13, S101–S112.
Burre, P. S., Mix, K. S., and Brinckerhoff, C. E. (2006). Matrix metalloproteinases: role in arthritis. *Front. Biosci.* 11:529–543.
Canalis, E. (2009). Growth factor control of bone mass. *J. Cell Biochem.* 108, 697–777. doi:10.1002/jcb.22322
Canalis, E., Economides, A. N., and Gazzero, E. (2003). Bone morphogenic proteins, their antagonists, and the skeleton. *Endocr. Rev.* 24, 218–235. doi:10.1210/er.2002-0023
Castberg, F. C., Kjaergaard, S., Mosis, R. A., Lobl, M., Martignetti, C., Martignetti, J. A., et al. (2013). Multicentric osteolysis with nodulosis and arthropathy (MONA) with cardiac malformation, mimicking polyarticular juvenile idiopathic arthritis: case report and literature review. *Eur. J. Pediatr.* 172, 1657–1663. doi:10.1007/s00431-013-2102-8
Cavalla, F., Hernandez-Rios, P., Sorsa, T., Biguetti, C., and Hernandez, M. (2017). Matrix metalloproteinases as regulators of periodontal inflammation. *Int. J. Mol. Sci.* 18:440. doi:10.3390/ijms1804440
Charles, J. M., and Key, L. L. (1998). Developmental spectrum of children with congenital osteoporosis. *J. Pediatr.* 132, 371–374. doi:10.1016/s0022-3476(98)70467-6
Chelliaia, M. A., and Ma, T. (2013). Membrane localization of membrane type 1 matrix metalloproteinase by CD44 regulates the activation of pro-matrix metalloproteinase 9 in osteoclasts. *Biomed. Res. Int.* 2013:30292. doi:10.1155/2013/30292
Chen, D., Xie, R., Shu, B., Landay, A. L., Wei, C., Reiser, J., et al. (2019). Wnt signaling in bone, kidney, intestine, and adipose tissue and interorgans interaction in aging. *Ann. N. Y. Acad. Sci.* 1442, 48–60. doi:10.1111/nyas.13945
Chen, M., Zhu, M., Awad, H., Li, T. F., Sheu, T. J., Boyce, B. F., et al. (2008). Inhibition of beta-catenin signaling causes defects in postnatal cartilage development. *J. Cell Sci.* 121, 1455–1465. doi:10.1242/jcb.200712.224
Chen, X., Wang, L., Zhao, K., and Wang, H. (2018). Osteocystogenesis: roles of physiocochemical factors, collagen cleavage, and exogenic molecules. *Tissue Eng. Part B Rev.* 24, 215–225. doi:10.1089/ten.teh.2017.0378
Choi, Y. A., Kang, S. S., and Jin, E. J. (2009). BMP-2 treatment of C3H10T1/2 mesenchymal cells blocks MMP-9 activity during chondrocyte commitment. *Cell Biol. Int.* 33, 887–902. doi:10.1016/j.cellbi.2008.04.020
Civitelli, R. (2008). Cell-cell communication in the osteoblast/osteocyte lineage. *Arch. Biochem. Biophys.* 473, 188–192. doi:10.1016/j.abb.2008.04.005
Clark, I. M., Swinger, T. E., Sampieri, C. L., and Edwards, D. R. (2008). The regulation of matrix metalloproteinases and their inhibitors. *Int. J. Biochem. Cell Biol.* 40, 1362–1378. doi:10.1016/j.biocel.2007.12.006
Coen, G., Ballanti, P., Silvestrini, G., Mantella, D., Manni, M., Di Giulio, S., et al. (2009). Immunohistochemical localization and mRNA expression of matrix Gliprotein and fetuin-A in bone biopsies of hemodialysis patients. *Vrchnos Avd Arch.* 454, 263–271. doi:10.1007/s00428-008-0072-4
Cohick, W. S., and Clemons, D. R. (1993). The insulin-like growth factors. *Annu. Rev. Physiol.* 55, 131–153.
Compston, J. E. (2001). Sex steroids and bone. *Physiol. Rev.* 81, 419–447.
Cook, R., Sarker, H., and Fernandez-Patron, C. (2018). Pathologies of matrix metalloproteinase-2 underactivity: A perspective on a neglected condition. *Can. J. Physiol. Pharmacol.* 97, 1–7. doi:10.1139/cjpp-2018-0525
Cousins, L. M., Fingleton, B., and Matrisian, L. M. (2002). Matrix metalloproteinase inhibitors and cancer: trials and tribulations. *Science* 295, 2387–2392. doi:10.1126/science.1067100
Cox, J. H., Starr, A. E., Kappelhoff, R., Yan, R., Roberts, C. R., and Overall, C. M. (2010). Matrix metalloproteinase 8 deficiency in mice exacerbates inflammatory arthritis through delayed neutrophil apoptosis and reduced caspase 11 expression. *Arthritis Rheum.* 62, 3645–3655. doi:10.1002/art.27757
Crottì, T. N., Flannery, M., Walsh, N. C., Fleming, J. D., Goldring, S. R., and McHugh, K. P. (2006). NFAc1l regulation of the human beta integrin
Hirao, M., Hashimoto, J., Yamasaki, N., Ando, W., Tsuboi, H., Myoui, A., et al. (2016). Protein degradation fragments as diagnostic and prognostic biomarkers of connective tissue diseases: understanding the extracellular matrix message and implication for current and future serological biomarkers. Expert Rev. Proteomics 13, 213–225. doi: 10.1586/14789450.2016.1134327

Geoffroy, V., Marty-Morieux, C., Le Goupil, N., Clement-Lacroix, P., Terraz, C., Frain, M., et al. (2004). In vivo inhibition of osteoblastic metalloproteinases leads to increased trabecular bone mass. J. Bone Miner. Res. 19, 811–822. doi: 10.1359/bmr.040119

Gong, Y., Skee, R. B., Fukai, N., Rawadi, G., Roman-Roman, S., Reginato, A. M., et al. (2001). LDL receptor-related protein 5 (LRPS) affects bone accrual and eye development. Cell 107, 513–523.

Gonzalo, P., Guadamillas, M. C., Hernandez-Riquer, M. V., Pollan, A., Grande-Garcia, A., Bartolome, R. A., et al. (2010). MT1-MMP is required for myeloid cell fusion via regulation of Rac1 signaling. Dev. Cell 18, 77–89. doi: 10.1016/j.devcel.2009.11.012

Gori, F., Hofbauer, L. C., Dunstan, C. R., Splegberg, T. C., Khosla, S., and Riggs, B. L. (2000). The expression of osteoprotegerin and RANK ligand and the support of osteoclast formation by stromal-osteoblast lineage cells is developmentally regulated. Endocrinology 141, 4768–4776. doi: 10.1210/endo.141.12.7840

Greenlee, K. J., Werb, Z., and Kershmadand, F. (2007). Matrix metalloproteinases in lung: multiple, multifarious, and multifaceted. Physiol. Rev. 87, 69–98. doi: 10.1152/physrev.00022.2006

Gu, Y., Preston, M. R., El Haj, A. J., Howl, J. D., and Publicover, S. J. (2001). Three types of K+ currents in murine osteocyte-like cells (MLO-Y4). Bone 28, 29–37. doi: 10.1016/s8756-3822(00)00439-7

Guan, C. C., Yan, M., Jiang, X. Q., Zhang, P., Zhang, X. L., Li, J., et al. (2009). Sonic hedgehog alleviates the inhibitory effects of high glucose on the osteoblastic differentiation of bone marrow stromal cells. Bone 45, 1146–1152. doi: 10.1016/j.bone.2009.08.009

Hachemi, Y., Rapp, A. E., Picke, A. K., Weidinger, G., Ignatius, A., and Ivanovski, S. (2015). Mechanisms of bone: a novel marker of osteocytic differentiation. J. Bone Miner. Metab. 33, 779–787. doi: 10.1007/s00777-015-0548-6

Ikeda, S., Morishita, Y., Tsutsushi, H., Ito, M., Shiraiishi, A., Arita, S., et al. (2003). Reductions in bone turnover, mineral, and structure associated with mechanical properties of lumbar vertebra and femur in glucocorticoid-treated growing minipigs. Bone 33, 779–787. doi: 10.1016/s7356-3282(03)00263-1

Inada, M., Wang, Y., Byrne, M. H., Miyaura, C., and Krane, S. M. (2001). Mice with null mutation in collagenase-3 (Matrix Metalloproteinase [MMP]-13) exhibit altered bone remodeling and increased bone mass. J. Bone Miner. Res. 16, S149.

Inada, M., Wang, Y., Byrne, M. H., Miyaura, C., and Krane, S. M. (2002). Loss of function of matrix metalloproteinase-13 (MMP-13) affects collagen accumulation and bone formation. J. Bone Miner. Res. 16, S171.

Ito, A., Mukaiyama, A., Itoh, Y., Nagase, H., Thogersen, I. B., Englund, J. J., et al. (1996). Degradation of interleukin 1 beta by matrix metalloproteinases. J. Biol. Chem. 271, 14657–14660.

Iyer, R. P., Patterson, N. L., Fields, G. B., and Lindsey, M. L. (2012). The history of matrix metalloproteinases: milestones, myths, and misperceptions. Am. J. Physiol. Heart Circ. Physiol. 303, H919–H930. doi: 10.1152/ajpcell.00577.2012

Holliday, L. S., Welgus, H. G., Fliszar, C. J., Veith, G. M., Jeffrey, J. I., and Gluck, S. L. (1997). Initiation of osteoclast bone resorption by interstitial collagenase. J. Biol. Chem. 272, 20253–20258. doi: 10.1074/jbc.272.35.20253

Holmbek, K., Bianco, P., Ciceri, J., Yamada, S., Kromer, M., Kuznetsov, S. A., et al. (1999). MT1-MMP-deficient mice develop dwarfishism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. Cell 99, 81–92. doi: 10.1016/s0092-8674(00)00641-4

Holmbek, K., Bianco, P., Chryssovergis, K., Yamada, S., and Birkedal-Hansen, H. J. (2003). MT1-MMP-dependent, apoptotic remodeling of unmineralized cartilage: a critical process in skeletal growth. Cell Biol. 163, 661–671. doi: 10.1083/jcb.200307061

Holmgren, K., Bianco, P., Pidoux, L., Inoue, S., Billinghurst, R. C., Wu, W., et al. (2005). The metalloproteinase MT1-MMP is required for normal development and maintenance of osteocyte processes in bone. J. Cell Sci. 118, 147–156. doi: 10.1242/jcs.01581
Jackson, M. T., Moradi, B., Smith, M. M., Jackson, C. J., and Little, C. B. (2014). Activation of matrix metalloproteinases 2, 9, and 13 by activated protein C in human osteoarthritic cartilage chondrocytes. *Arthritis Rheumatol.* 66, 1525–1536. doi: 10.1002/art.38401

Javaheri, B., Hopkins, M., Poudet, B., Pollard, A. S., Shelef-Belina, S. J., Chang, Y. M., et al. (2016). Deficiency and also transgenic overexpression of TIMP-3 both lead to compromised bone mass and architecture in vivo. *PLoS One* 11:e0159657. doi: 10.1371/journal.pone.0159657

Jiang, Y. B., Zhao, J., Genant, H. K., Dequequer, J., and Geusens, P. (1997). Long-term changes in bone mineral and biomechanical properties of vertebral and femur in aging, dietary calcium restricted and/or estrogen-deprived/-replaced rats. *Bone Miner. Res.* 12, 820–831. doi: 10.1035/jbmr.1997.12.5.820

Jimenéz, M. J., Balbin, M., Lopez, J. M., Alvarez, J., Komori, T., and Lopez-Otin, C. (1999). Collagenase 3 is a target of Cbfα1, a transcription factor of the runt gene family involved in bone formation. *Mol. Cell. Biol.* 19, 4431–4442. doi: 10.1128/mcb.19.6.4431

Jing, R., Liu, Y., Guo, P., Ni, T., Gao, X., Mei, R., et al. (2018). Evaluation of common variants in matrix metalloproteinase-9 gene with lumbar disc herniation in Han Chinese population. *Genet. Test Mol. Biomarkers* 22, 622–629. doi: 10.1089/gtmb.2018.0080

Johannson, N., Saarialho-Kere, U., Airola, K., Herva, R., Nissinen, L., Westermark, J., et al. (1997). Collagenase-3 (MMP-13) is expressed by hypertrophic chondrocytes, perioseal cells, and osteoblasts during human fetal bone development. *Dev. Dyn.* 208, 387–397. doi: 10.1002/(sici)1097-0777(199703)

Kajita, M., Itoh, Y., Chiba, T., Mori, H., Okada, A., Kinoh, H., et al. (2001). Membrane-type 1 matrix metalloproteinase cleaves CD44 and promotes cell migration. *J. Cell Biol.* 153, 893–904. doi: 10.1083/jcb.153.5.893

Kamioka, H., Honjo, T., and Takano-Yamamoto, T. (2001). A three-dimensional distribution of osteocyte processes revealed by the combination of confocal laser scanning microscopy and differential interference contrast microscopy. *Bone* 28, 145–149. doi: 10.1016/s8756-3282(00)00421-x

Kim, H. J., Zhao, H., Kitaura, H., Bhattacharyya, S., Brewer, J. A., Muglia, L. J., et al. (2006). Glucocorticoids suppress bone formation via the osteoclast. *J. Clin. Invest.* 116, 2152–2160. doi: 10.1172/jci28804

Kim, J. W., Simmer, J. P., Hart, T. C., Hart, P. S., Ramaswami, M. D., Barlett, J. D., et al. (2005). MP-20 mutation in autosomal recessive pigmented dyspatogenesis amelogenesis imperfecta. *J. Med. Genet.* 42, 271–275. doi: 10.1136/jmg.2004.024505

Kim, M. H., Park, M., Baek, S. H., Kim, H. J., and Kim, S. H. (2011). Molecules and signaling pathways involved in the expression of OC-STAMP during osteoclastogenesis. *Amino Acids* 40, 1447–1459. doi: 10.1007/s00726-010-0754-9

Kim, Y., Sato, K., Asagiri, M., Morita, I., Soma, K., and Takayanagi, H. (2005). Contribution of nuclear factor of activated T cells cI to the transcriptional control of immunoreceptor osteoclast-associated receptor but not triggering receptor expressed by myeloid cells-2 during osteoclastogenesis. *J. Biol. Chem.* 280, 32905–32913. doi: 10.1074/jbc.m505820200

Kini, U., and Nandeesh, B. N. (2012). "Physiology of bone formation, remodeling, and metabolism," in *Rudimentum and Hybrid Bone Imaging*, eds I. Fogelman, G. Gnanasegaran, and H. Van der Wall, (Berlin: Springer-Verlag), 29–57. doi: 10.1182/blood.v97.10.3123

Kothe-Tate, M. L., Adamson, J. R., Tami, A. E., and Bauer, T. W. (2004). The osteoint. *Int. J. Biochem. Cell Biol.* 36, 1–8.

Koga, T., Inui, M., Inoue, K., Kim, S., Suematsu, A., Kobayashi, E., et al. (2004). Costimulatory signals mediated by the ITAM motif cooperate with RANKL for bone homeostasis. *Nature* 428, 758–763. doi: 10.1038/nature02444

Koga, T., Matsui, Y., Asagiri, M., Kodama, T., de Crombrugghe, B., Nakashima, K., et al. (2005). NEAT and Osterix cooperatively regulate bone formation. *Nat. Med.* 11, 880–885. doi: 10.1038/nm1270

Kojima, T., Hasegawa, T., de Freitas, P. H., Yamamoto, T., Sasaki, M., Horiiuchi, K., et al. (2013). Histochemical aspects of the vascular invasion at the erosion zone of the epiphyseal cartilage in MMP-9-deficient mice. *Biomed. Res. 34, 119–128.*

Kooijman, S. A., Matziolis, G., et al. (2013). Proteolysis of the urokinase-type plasminogen activator receptor by metalloproteinase-12: implication for angiogenesis in fibrin matrices. *Blood* 97, 3123–3131. doi: 10.1182/blood.v97.10.3123

Koskinen, A., Vuotteenaho, K., Nieminen, R., Moilanen, T., and Moilanen, E. (1995). The role of prostaglandins in the regulation of bone metabolism. *Clin. Orthop.* 313, 36–46.

Kotake, S., Udagawa, N., Takahashi, N., Matsuzaki, K., Itoh, K., Ishiyama, S., et al. (2011). Biochemical markers of ongoing joint damage from OA patients. *Clin. Exp. Rheumatol.* 29, 57–64. doi: 10.1182/blood.v97.10.3123

Krause, M. S., and Inada, M. (2008). Matrix metalloproteinases and bone. *Bone* 43, 7–18. doi: 10.1016/j.bone.2008.03.020

Krischan, V., Bryant, H. U., and Macdougald, O. A. (2006). Regulation of bone mass by Wnt signaling. *J. Clin. Invest.* 116, 1202–1209. doi: 10.1172/jci28551
Liu, S., Zhou, J., Tang, W., Jiang, X., Rowe, D. W., and Quarles, D. L. (2006). Pathogenic role of Fgf23 in Hyp mice. Am. J. Physiol. Endocrinol. Metab. 291, E38–E49. doi: 10.1152/ajpendo.00582.2005

Liu, Y. H., Tang, Z., Kundu, R. K., Wu, L., Luo, W., Zhu, D., et al. (1999). Msx2 gene dosage influences the number of proliferative osteogenic cells in growth centers of the developing murine skull: a possible mechanism for MSX2-mediated craniosynostosis in humans. Dev. Biol. 205, 260–274. doi: 10.1006/dbio.1998.9114

Lofek, S., Schilling, O., and Franzke, C. W. (2011). Series “matrix metalloproteinases in lung and health disease”: biological role of matrix metalloproteinases: a critical balance. Eur. Respir. J. 38, 191–208. doi: 10.1183/09031936.00146510

Logan, C. Y., and Nusse, R. (2004). The Wnt signaling pathway in development and disease. Annu. Rev. Cell Dev. Biol. 20, 781–810.

Lopez-Otin, C., Palavalli, L. H., and Samuels, Y. (2009). Protective roles of matrix metalloproteinases: from mouse models to human cancer. Cell Cycle 8, 3657–3662. doi: 10.4161/cc.8.22.9956

LoviBond, A. C., Haque, S. J., Chambers, T. J., and Fox, S. W. (2003). TGF-beta-induced SOCS3 expression augments TNF-alpha-induced osteoclast formation. Biochim. Biophys. Acta. 1647, 233–243. doi: 10.1016/j.bbamcr.2005.03.003

Luo, J., Yang, Z., Ma, Y., Yue, Z., Lin, H., Qu, G., et al. (2016). LGR4 is a receptor for RANKL and negatively regulates osteoclast differentiation and bone resorption. Nat. Med. 22, 539–546. doi: 10.1038/nm.4076

Lynch, C. C. (2011). Matrix metalloproteinases as master regulators of the vicious cycle of bone metastasis. Bone 48, 44–53. doi: 10.1016/j.bone.2010.06.007

Lynch, C. C., Hikosaka, A., Acuff, H. B., Martin, D. M., Kawai, N., Singh, R. K., et al. (2005). MMP-7 promotes prostate cancer-induced osteolysis via the solubilization of RANKL. Cancer Cell 7, 485–496. doi: 10.1016/j.ccr.2005.04.013

MacDonald, B. R. (1986). Parathyroid hormone, prostaglandins and bone resorption. World Rev. Nutr. Diet. 47, 163–201. doi: 10.1159/000412334

Madsen, D. H., Jürgensen, H. J., Ingvarsen, S., Melander, M. C., Albrechtsen, R., Hald, A., et al. (2013). Differential actions of the endocytic collagen receptor uPARAP/Endo180 and the collagenase MMP-2 in bone homeostasis. PLoS One 8:e71261. doi: 10.1371/journal.pone.0071261

Maeda, Y., Nakamura, E., Nguyen, M. T., Suva, L. J., Swain, F. L., Razaque, M. S., et al. (2007). Indian Hedgehog produced by postnatal chondrocytes is essential for maintaining a growth plate and trabecular bone. Proc. Natl. Acad. Sci. U.S.A. 104, 6382–6387. doi: 10.1073/pnas.0608449104

Maffioli, P., and Derosa, G. (2015). “Overview of biochemical markers of bone metabolism,” in Bone Disease, Biomarkers in Disease: Methods, Discoveries and Applications, ed. V. R. Preedy, (Dordrecht: Springer Science+Business Media), 1–19. doi: 10.1007/978-94-007-7745-3_1-1

Mahl, C., Egea, V., Megens, R. T. A., Pitsch, T., Santovito, D., Webster, C., et al. (2014). RECK (reversion-inducing cysteine-rich protein with Kazal motifs) regulates migration, differentiation and Wnt/β-catenin signaling in human
mesenchymal stem cells. *Cell Mol. Life Sci.* 73, 1489–1501. doi: 10.1007/s00018-015-2054-4

Malaponte, G., Hafsi, S., Polese, J., Castellano, G., Spessotto, P., Guarneri, C., et al. (2016). Tumor microenvironment in diffuse large B-cell lymphoma: matrix metalloproteinases activation is mediated by osteopontin overexpression. *Biochem. Biophys. Acta* 1863, 483–489. doi: 10.1016/j.bbamcar.2015.09.018

Mancini, A., and di Battista, J. A. (2006). Transcriptional regulation of matrix metalloprotease gene expression in health and disease. *Front. Biosci.* 11:423–446.

Manduca, P., Castagnino, A., Lombardini, D., Marchisio, S., Soldano, S., Ulivi, V., et al. (2009). Role of MT1-MMP in the osteogenic differentiation. *Bone* 44, 251–265. doi: 10.1016/j.bone.2008.10.046

Mannello, F., Tonti, G., and Papa, S. (2005). Matrix metalloproteinase inhibitors as anticancer therapeutics. *Curr. Drug Targets* 5, 285–298. doi: 10.2174/1389450054046151

Manolagas, S. C. (2000). Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. *Endoc. Rev.* 21, 115–137. doi: 10.1209/edrv.21.2.0395

Marie, P. J. (2003). Fibroblast growth factor signaling controlling osteoblast differentiation. *Skelet. Cell. Mol. Biol. Rev.* 25, 61–75. doi: 10.2174/1389450033826221

Martignetti, J. A., Aqeel, A. A., Sewairi, W. A., Boumah, C. E., Kambouris, M., Mayouf, S. A., et al. (2003). Fibroblast growth factor signaling controlling osteoblast differentiation. *J. Cell Sci.* 116, 3569–3576.

Matsuo, K., Galson, D. L., Zhao, C., Peng, L., Laplace, C., Wang, K. Z., et al. (2009). JNK activity is essential for Atf4 expression and late-stage osteoblast differentiation. *J. Biol. Chem.* 284, 26176–26182. doi: 10.1074/jbc.M108.678285

Matsumoto, M., Kogawa, M., Wada, S., Takayanagi, H., Tsujiimoto, M., Katayama, S., et al. (2004). Essential role of p38 mitogen-activated protein kinase in caspase K gene expression during osteogenesis through association of NFATC1 and PU.1. *J. Biol. Chem.* 279, 45969–45979. doi: 10.1074/jbc.m40879200

Matsuo, K., Galson, D. L., Zhao, C., Peng, L., Laplace, C., Wang, K. Z., et al. (2004). Nuclear factor of activated T-cells (NFAT) regulates osteoclastogenesis in precursors lacking c-Fos. *J. Biol. Chem.* 279, 26475–26480. doi: 10.1074/jbc.m313973200

Mattsuk, T., Chiba, N., Bandow, K., Kakimoto, K., Masuda, A., and Ohnishi, T. (2009). JNK activity is essential for ATF4 expression and late-stage osteoblast differentiation. *J. Bone Miner. Res.* 24, 398–410. doi: 10.1359/jbmr.081107

Mattson, J. P., Schlesinger, P. H., Keeling, D. J., Teitelbaum, S. L., Stone, D. K., and Mosekilde, L., Danielsen, D. D., and Knudsen, U. B. (1993). The effect of aging on bone remodeling in precursors lacking c-Fos. *Bone* 122, 4042–4048. doi: 10.1242/jb.015-2054-4

Mey, S. C., et al. (2004). The immunomodulatory adapter proteins DAP12 and Fc receptor gamma-chain (FcGamma) regulate development of functional osteoclasts through the Syk tyrosine kinase. *Proc. Natl. Acad. Sci. U.S.A.* 101, 6158–6163. doi: 10.1073/pnas.0410620101

Mohanakrishnan, V., Balusabramanian, A., Mahalingam, G., Partridge, N. C., Ramachandran, I., and Selvamurugan, N. (2018). Parathyroid hormone-induced down-regulation of miR-532-5p for matrix metalloproteinase-13 expression in rat osteoblasts. *J. Cell Biochem.* 119, 6181–6193. doi: 10.1002/jcb.26872

Moskilde, L., Danielsen, D. D., and Knudsen, U. B. (2013). The effect of aging and ovarioectomy on the vertebral bone mass and biomechanical properties of mature rats. *Bone* 14, 1–6. doi: 10.1016/j.bone.2007.08.014

Motsch, C., et al. (2007). Loss of MMP-2 disrupts skeletal and craniofacial development and results in decreased bone mineralization, joint erosion and defects in osteoblast and osteoclast growth. *Hum. Mol. Genet.* 16, 1113–1123. doi: 10.1093/hmg/ddm60

Mott, J. D., Thomas, C. L., Rosenbach, M. T., Takahara, K., Greenspan, D. S., and Chen, Y. (2005). Intricate functions of matrix metalloproteinases in physiological and pathological situations. *J. Cell Sci.* 118, 1667–1678.

Munoz, G. (2017). Tumor microenvironment in diffuse large B-cell lymphoma: matrix metalloproteinases activation is mediated by osteopontin overexpression. *Biochem. Biophys. Acta* 1863, 483–489. doi: 10.1016/j.bbamcar.2015.09.018

Mucenic, A., and di Battista, J. A. (2006). Transcriptional regulation of matrix metalloprotease gene expression in health and disease. *Front. Biosci.* 11:423–446.
Overall, C. M., Wrana, J. L., and Sodek, J. (1991). Transcriptional and...
Vu, T. H., Shipley, J. M., Bergers, G., Berger, J. E., Helms, J. A., Hanahan, D., et al. (1998). MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. *Cell* 93, 411–422. doi: 10.1016/s0092-8674(00)81169-1

Wada, T., Nakashima, T., Oliveira-dos-Santos, A. J., Gasser, J., Harla, H., Schett, G., et al. (2005). The molecular scaffold Gab2 is a crucial component of RANK signaling and osteoclastogenesis. *Nat. Med.* 11, 394–399. doi: 10.1038/nmm1203

Wagenaar-Miller, R. A., Engelholm, L. H., Gavard, J., Yamada, S. S., Gutkind, J. S., Behrendt, N., et al. (2007). Complementary roles of intracellular and pericellular collagen degradation pathways in vivo. *Mol. Cell. Biol.* 27, 6309–6322. doi: 10.1128/mcb.00291-07

Westbrook, I., De Rooij, K. E., and Nijweide, P. J. (2002). Osteocyte-specific monoclonal antibody MAb OB7.3 is directed against Phex protein. *J. Bone Miner. Res.* 17, 845–853. doi: 10.1359/jbmr.2002.17.5.845

Wiebe, S. H., Hafezi, M., Sandhu, H. S., Sams, S. M., and Dixon, S. J. (1996). Osteoclast activation in inflammatory periodontal diseases. *Oral Dis.* 2, 167–180. doi: 10.1111/j.1601-0825.1996.tb00218.x

Wu, H., Du, J., and Zheng, Q. (2008). Expression of MMP-1 in cartilage and synovium of experimentally induced rabbit ACLT traumatic osteoarthritis: immunohistochemical study. *Rheumatol. Int.* 29, 31–36. doi: 10.1007/s00296-008-0636-2

Wutzl, A., Rauner, M., Seermann, R., Millei, W., Krepler, P., Pietschmann, P., et al. (2010). Bone morphogenetic proteins 2, 5, and 6 in combination stimulate osteoblasts but not osteoclasts in vitro. *J. Orthop. Res.* 28, 1431–1439. doi: 10.1002/jor.21144

Xian, L., Wu, X., Pang, L., Lou, M., Rosen, C. J., Qiu, T., et al. (2012). Matrix IGF-1 maintains bone mass by activation of mTOR in mesenchymal stem cells. *Nat. Med.* 18, 1095–1101. doi: 10.1038/nm.2793

Xue, M., March, L., Sambrook, P. N., and Jackson, C. J. (2007). Differential regulation of matrix metalloproteinase 2 and matrix metalloproteinase 9 by activated protein C: relevance to inflammation in rheumatoid arthritis. *Arthritis Rheum.* 56, 2864–2874. doi: 10.1002/art.22844

Xue, M., McKelvey, K., Shen, K., Minhas, N., March, L., Park, S. Y., et al. (2014). Endogenous MMP-9 and not MMP-2 promotes rheumatoid synovial fibroblast survival, inflammation and cartilage degradation. *Rheumatology* 53, 2270–2279. doi: 10.1093/rheumatology/keu254

Yamada, Y., Ando, F., Niino, N., and Shimokata, H. (2004). Association of a polymorphism of the matrix metalloproteinase-9 gene with bone mineral density in Japanese men. *Metabolism* 53, 135–137. doi: 10.1016/j.metabol.2003.09.003

Yamagishi, H., Tokunaga, K., Hayami, T., Hatano, H., Uchida, M., Endo, N., et al. (1999). Expression of matrix metalloproteinase-13 (collagenase-3) is induced during fracture healing in mice. *Bone* 25, 197–203. doi: 10.1016/s8756-3282(99)00157-x

Yamaguchi, A., Komori, T., and Suda, T. (2000). Regulation of osteoblast differentiation mediated by bone morphogenetic proteins, hedgehogs, and Chba1. *Endocrinology* 21, 393–411. doi: 10.1210/edrv.21.4.0403

Yee, C. S., Schurman, C. A., White, C. R., and Alliston, T. (2019). Investigating osteocytic pericellular/canalicular remodeling. *Curr. Osteoporos Rep.* 17, 157–168. doi: 10.1007/s11914-019-00514-0

Zankl, A., Bonafe, L., Calcatera, V., Di Rocco, M., and Superti-Furga, A. (2005). Winchester syndrome caused by a homozygous mutation affecting the active site of matrix metalloproteinase 2. *Clin. Genet.* 67, 261–266. doi: 10.1111/j.1399-0004.2004.00402.x

Zeng, Y., Rosborough, R. C., Li, Y., Gupta, A. R., and Bennett, J. (1998). Temporal and spatial regulation of gene expression mediated by the promoter for the human tissue inhibitor of metalloproteinases-3 (TIMP-3)-encoding gene. *Dev. Dyn.* 211, 228–237. doi: 10.1002/(sici)1097-0177(199803)211:3<228::aid-aja4>3.0.co;2-j

Zhang, B., Henney, A., Eriksson, P., Hamsten, A., Watkins, H., and Ye, S. (1999). Genetic variation at the matrix metalloproteinase-9 locus on chromosome 20q12.2-13.1. *Hum. Genet.* 105, 418–423. doi: 10.1007/s004399900167

Zhang, J. F., Wang, G. L., Zhou, Z. J., Fang, X. Q., Chen, S., and Fan, S. W. (2018). Expression of matrix metalloproteinases, tissue inhibitors of metalloproteinases, and interleukins in vertebral cartilage endplate. *Oral Surg.* 10, 306–311. doi: 10.1111/oms.12409

Zhang, K., Barragan-Adjemian, C., Ye, L., Kotha, S., Dallas, M., Lu, Y., et al. (2006). E11/gp38 selective expression in osteocytes: regulation by mechanical strain and role in dendrite elongation. *Mol. Cell. Biol.* 26, 4539–4552. doi: 10.1128/mcb.02120-05

Zhang, W., Yang, N., and Shi, X. M. (2008). Regulation of mesenchymal stem cell osteogenic differentiation by glucocorticoid-induced leucine zipper (GILZ). *J. Biol. Chem.* 283, 4723–4729. doi: 10.1074/jbc.b70417200

Zhao, H., Cai, G., Du, J., Xia, Z., Wang, L., and Zhu, T. (1997). Expression of matrix metalloproteinase-9 mRNA in osteoprototic bone tissues. *J. Tongji. Med. Univ.* 17, 28–31. doi: 10.1002/jbm.22887998

Zhao, S., Zhang, Y. K., Harris, S., Ahuja, S. S., and Bonewald, L. F. (2002). MLO-Y4 osteocyte-like cells support osteoclast formation and activation. *J. Bone Miner. Res.* 17, 2068–2079. doi: 10.1359/jbmr.2002.17.11.2068

Zheng, H., Liu, J., Tycksen, E., Nunley, R., and McAlinden, A. (2019). MicroRNA-181a/b-1 over-expression enhances osteogenesis by modulating PTEN/P13K/AKT signaling and mitochondrial metabolism. *Bone* 123, 92–102. doi: 10.1016/j.bone.2019.03.020

Zhou, Z., Apte, S. S., Soininen, R., Cao, R., Baaklini, G. Y., Rauser, R. W., et al. (2000). Impaired endochondral ossification and angiogenesis in mice deficient in membrane type matrix metalloproteinase I. *Proc. Natl. Acad. Sci. U.S.A.* 97, 4052–4057. doi: 10.1073/pnas.060037197

Zucker, S., Cao, J., and Chen, W. T. (2000). Critical appraisal of the use of matrix metalloproteinase inhibitors in cancer treatment. *Oncogene* 19, 6642–6650. doi: 10.1038/sj.onc.1204097

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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