Pathologies of MMP-2 underactivity: A perspective on a neglected condition

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TITLE: Pathologies of MMP-2 underactivity: A perspective on a neglected condition

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ABSTRACT

A member of the matrix metalloproteinase family, matrix metalloproteinase-2 (MMP-2, gelatinase A), has been extensively studied for its role in both normal physiology and pathological processes. Whereas most research efforts in recent years have investigated the pathologies associated with MMP-2 overactivity, the pathological mechanisms elicited by MMP-2 underactivity are less well understood. Here, we distinguish between two states and describe their causes: (i) MMP-2 deficiency (complete loss of MMP-2 activity) and (ii) MMP-2 insufficiency (defined as MMP-2 activity below baseline levels). Further, we review the biology of MMP-2, summarizing the current literature on MMP-2 underactivity in both mice and humans, and describe research being conducted by our lab towards improving our understanding of the pathological mechanisms elicited by MMP-2 deficiency / insufficiency. We think that this research could stimulate the discovery of new therapeutic approaches for managing pathologies associated with MMP-2 underactivity. Moreover, similar concepts could apply to other members of the matrix metalloproteinase family.

KEYWORDS: MMP, inflammation, metabolism, MMP-2 deficiency
INTRODUCTION

A member of the matrix metalloproteinase (MMP) family, matrix metalloproteinase-2 (MMP-2, gelatinase A), has been extensively studied for its role in disease processes. One of our lab’s current research interests lies in determining the pathological mechanisms triggered when MMP-2 activity is reduced below baseline (physiological) levels. As reduction in MMP-2 activity can either be complete (genetic MMP-2 deficiency) or partial (upregulation of endogenous inhibitors, pharmacological blockade of MMP-2 activity, partial inactivating mutations of the MMP2 gene), we have introduced the term “MMP-2 insufficiency” to characterize the latter case (Table 1). What pathological mechanisms are elicited by MMP-2 insufficiency? How does MMP-2 insufficiency differ from MMP-2 deficiency? Here we review the biology of MMP-2 and describe ongoing efforts to address these questions. We think similar concepts could apply to other members of the matrix metalloproteinase family, as well.

MATRIX METALLOPROTEINASE-2 (MMP-2)

The discovery of the MMP family dates back to studies on collagen breakdown during tadpole morphogenesis (Gross and Lapiere 1962), which led to the purification of MMP-1 as an important enzyme modulating collagen metabolism (Nagai et al. 1966). Some six decades later, MMPs are now recognized as a family of 24 homologous Zn\(^{2+}\)-dependent proteinases necessary for normal extracellular matrix (ECM) breakdown, growth factor release and cytokine activation during wound healing, tissue remodelling, embryogenesis, neovascularization, cell migration, and regulation of immune responses (Nagase et al. 2006; Visse and Nagase 2003).

Matrix metalloproteinase 2 (MMP-2; gelatinase A, 72 kDa type IV collagenase) is a 660 amino acid secreted endopeptidase (Fernandez-Patron et al. 2016) in the matrix metalloproteinase family. Characteristic structures of MMPs include a catalytic Zn\(^{2+}\) ion,
coordinated in the active site by three histidine residues in a zinc-binding motif (HExxHxxGxxH) (Bode et al. 1993), and their distinct pro-peptide, catalytic and hemopexin-like domains (with the exception of stromelysins which lack a hemopexin-like domain) (Nagase et al. 2006). MMP-2 is secreted as the inactive zymogen proMMP-2, in which a cysteine residue of the pro-peptide domain’s cysteine switch motif (PRCGXPD) binds and inactivates the catalytic Zn$^{2+}$. Its hemopexin-like domain, a 4-bladed β-propeller structure, plays an important role in activation by recruiting the non-inhibitory C-terminus of tissue inhibitor of metalloproteinases (TIMP)-2 (Strongin et al. 1995). This proMMP-2-TIMP-2 complex associates with the membrane bound MT1-MMP (MMP-14) via the N-terminus of TIMP-2, and a nearby TIMP-2 free MT1-MMP then completes proteolytic cleavage of the pro-peptide domain (Nagase 1998). Extracellular activation of proMMP-2 forms a 64 kDa active enzyme, while an alternative intracellular mechanism has been discovered to occur independent of pro-domain proteolysis, thus yielding a 76 kDa active enzyme (Okamoto et al. 2001). This activation mechanism involves S-glutathiolation and occurs mainly in cardiac tissue following oxidative stress.

Active MMP-2 most likely performs proteolysis via polarization of the peptide bond carbonyl by the active Zn$^{2+}$, as well as deprotonation of a water molecule associated with the zinc by a conserved glutamate residue (Glu-404) (Nagase et al. 2006). Deprotonation converts the water molecule into a nucleophile, which attacks the carbonyl carbon of the substrate’s peptide bond to complete hydrolysis. In vivo, MMP-2 activity is modulated by endogenous inhibitors (Visse and Nagase 2003), gene transcription (Chernov et al. 2009; Nagase and Woessner 1999), zymogen activation (Strongin et al. 1995) and substrate accessibility (Klein and Bischoff 2011). MMP-2 (gelatinase A) is distinguishable from other MMPs by three fibronectin-like repeats in its catalytic domain, which assist in the binding and cleavage of gelatin, laminin
and type IV collagen, important components of the ECM (Visse and Nagase 2003). Unlike MMP-9 (gelatinase B), MMP-2 is also capable of degrading fibrillar collagens I, II and III. Though its collagenolytic activity is weaker than that of MMP-1 (interstitial collagenase-1) (Nagase et al. 2006), MMP-2 directly activates proMMP-1 to further increase interstitial collagen breakdown (Crabbe et al. 1994). MMP-2 also influences tissue remodelling via the proteolytic release of latent transforming growth factor (TGF)-β sequestered in the ECM, a cytokine that once active induces the synthesis of ECM molecules including fibronectin, collagen and proteoglycan (Yu and Stamenkovic 2000). MMP-2-mediated tissue remodelling is involved in numerous physiological functions including angiogenesis and neovascularization (Nguyen et al. 2001), embryonic cardiac development (Tuysuz et al. 2009), osteogenesis (Martignetti et al. 2001) and wound healing (Caley et al. 2015; Moses et al. 1996). In addition to ECM substrates, MMP-2 cleaves pro-inflammatory cytokines including monocyte chemoattractant protein (MCP)-3 (CCL7), tumor necrosis factor (TNF)-α and interleukin (IL)-1β, modifying their pro-inflammatory potential (Van Lint and Libert 2007).

**MMP-2 DEFICIENCY**

Human MMP-2 deficiency, a chronic and debilitating pediatric condition involving severe osteolysis and arthritis, arises from autosomal recessive inactivating mutations in the *MMP2* gene (16q12.2) (Martignetti et al. 2001). Most mutations described in the literature are family specific and localized to the Middle East, West Asia and North Africa (Martignetti et al. 2001) – areas of the world where consanguineous marriages are common practice (Hamamy 2012). While parents of affected children (heterozygous carriers) exhibit a 50% reduction in MMP-2 activity (Tuysuz et al. 2009), they are typically asymptomatic (Bhavani et al. 2016). Around 20 unique *MMP2* inactivating mutations have been identified thus far (Bhavani et al. 2016).
While catalytic domain mutations disrupt substrate binding and Zn$^{2+}$-mediated catalysis, inactivating mutations are not just limited to the active site. Nonsense mutations in any region of the polypeptide result in the formation of a truncated, inactive protein. Pro-peptide mutations involving the cysteine motif disrupt zymogen activation via destabilization of the cysteine switch. An altered hemopexin-like domain can inhibit substrate binding as well as disrupt the formation of the MMP-2-TIMP-2-MT1-MMP activation complex (Klein and Bischoff 2011). Promoter mutations can also significantly impact gene expression and disease development by altering transcription factor binding (Vinagre et al. 2013), however they are not typically analyzed as part of genetic diagnostic tests (de Vooght and van Solinge 2009). Expression assays (both mRNA and protein) of the MMP2 gene provide methods for diagnosing promoter-induced MMP-2 deficiency when no exonic mutations are present in suspected patients (Martignetti 2003). Currently, there is no effective treatment for patients with MMP-2 deficiency as little is known about the underlying pathological mechanisms.

MMP-2 INSUFFICIENCY

In contrast to MMP-2 deficiency caused by MMP2 gene inactivating mutations, MMP-2 activity insufficiency is a term that we use to describe the condition characterized by a reduction in MMP-2 activity below baseline levels (Table 1). Possible causes of MMP-2 insufficiency include partially inactivating MMP2 gene or promoter mutations that reduce MMP-2 expression or enzymatic activity, along with aberrant inhibition of MMP-2 by either endogenous or non-endogenous inhibitors. Endogenous MMP-2 inhibitors include: (i) TIMPs (Klein and Bischoff 2011), (ii) α-2-macroglobulin (Nagase et al. 2006), (iii) the membrane-bound angiogenic suppressor RECK (Oh et al. 2001), (iv) a 10 amino acid segment of the secreted form of β-amyloid precursor protein (Higashi and Miyazaki 2003), and (v) a proteolytic fragment of
procollagen C-proteinase enhancer protein (Mott et al. 2000). Upregulation of endogenous MMP-2 inhibitors is seen in diverse pathologies including liver cirrhosis and pancreatitis (Kurzepa et al. 2014), chronic renal failure (de Sain-van der Velden et al. 1998), Chagas disease (Medrano et al. 1996) and tuberculosis (Bapat et al. 2015). Among the non-endogenous MMP-2 inhibitors are several highly prescribed medications including statins, used to lower LDL-cholesterol levels in people at risk of ischemic heart disease (Izidoro-Toledo et al. 2011; Luan et al. 2003), and doxycycline, a commonly prescribed tetracycline-class antibiotic (Golub et al. 1995; Liu et al. 2003).

The prevalence of the above-mentioned conditions exhibiting upregulated endogenous MMP-2 inhibitors, and the high number of prescriptions for statins, doxycycline (and possibly other medications with undesired MMP-2 inhibitory action) suggest that MMP-2 insufficiency is likely much more prevalent, albeit also less noticed, than genetic MMP-2 deficiency in the general population. Thus, an appropriate question to ask, and a concept currently being explored in our laboratory is whether MMP-2 insufficiency and MMP-2 deficiency trigger the same pathological mechanisms? We hypothesize that MMP-2 insufficiency triggers both common and distinct pathological mechanisms to those initiated by MMP-2 deficiency. A greater understanding of the pathological mechanisms elicited by MMP-2 underactivity will be derived from studies of animal models of (or patients with) MMP-2 deficiency and MMP-2 insufficiency.

**TISSUE INHIBITORS OF METALLOPROTEINASES**

TIMPs are a set of four inhibitory proteins (TIMP-1, TIMP-2, TIMP-3 and TIMP-4) consisting of a single subunit (21-29 kDa, ~190 amino acids) with discrete N- and C-terminal domains (Williamson et al. 1990) While all TIMPs exhibit activity towards the full spectrum of
MMPs (apart from TIMP-1 which poorly inhibits the membrane-type MMPs MT1, MT3 and MT5 as well as MMP-19) (Nagase et al. 2006), the interaction between TIMP-2 and MMP-2 has been particularly well characterized. TIMP-2’s smaller C-terminal domain (~65 amino acids) is non-inhibitory and involved in activation, mediating the interaction between MMP-2 and MT1-MMP (Williamson et al. 1994). Its N-terminal domain (~125 amino acids) is shaped like a wedge and slots into the active site of MMP-2 in a 1:1 stoichiometry, much like its natural substrate would (Murphy et al. 1991). Two strictly conserved N-terminal cysteines (positions 1 and 3) are involved in inhibition, with Cys-1 chelating the Zn^{2+} ion and expelling the water molecule, preventing hydrolysis (Fernandez-Catalan et al. 1998). TIMP overexpression-related diseases include liver cirrhosis and pancreatitis, characterized by abnormally high TIMP-2 levels (Kurzepa et al. 2014).

**ACUTE PHASE REACTANTS**

Unlike TIMPs, which are mainly active within tissues, a more effective regulator of MMP-2 within the plasma is the acute phase reactant α-2-macroglobulin (Nagase et al. 2006). α-2-macroglobulin is a 720 kDa homotetrameric protease inhibitor with a broad specificity (Barrett 1981) that facilitates the clearing of proteases from the blood via receptor-mediated endocytosis, rather than direct inhibition of the active site (Strickland et al. 1990). Its polypeptide sequence includes a bait region (amino acids 666-706) that serves as a target for MMP-2-mediated proteolysis (Sottrup-Jensen et al. 1989). Cleavage results in entrapment of MMP-2 within the macroglobulin and subsequent interaction with phagocytic receptors (Barrett 1981). α-2-macroglobulin’s upregulation in inflammation (Jain et al. 2011) could conceivably play a role in MMP-2-mediated inflammatory disease. Vascular smooth muscle cells overexpressing the ligand-activated transcription factor nuclear receptor 4A (NR4A) exhibit decreased MMP-2
mRNA production and enzymatic activity, coinciding with an upregulation in α-2-macroglobulin but not other MMP-2 inhibitors (TIMP-1/TIMP-2) (Rodriguez-Calvo et al. 2015). This suggests that an α-2-macroglobulin dependent mechanism of inhibition could also have implications in vascular physiology and cardiovascular disease (Rodriguez-Calvo et al. 2015). Furthermore, pathological elevation of a-2-macroglobulin is present in chronic hepatic fibrosis (Naveau et al. 1994), kidney disease (de Sain-van der Velden et al. 1998), and infectious diseases such as Chagas disease (Medrano et al. 1996) and tuberculosis (Bapat et al. 2015), and thus MMP-2 activity insufficiency could play a role in development of disease symptoms.

Fibrinogen (FBG), a 340 kDa dimeric glycoprotein consisting of three unique polypeptide chains in the symmetry (AαBβγ)2 (Mosesson et al. 2001), is another positive acute-phase reactant reaching up to ten-fold elevated expression during inflammation (Ebersole and Cappelli 2000). It is the precursor of fibrin in the clotting pathway (Hiller et al. 2000), and its Aα/Bβ chains have been reported to be cleaved by MMP-2 (Monaco et al. 2007). In trying to ascertain the role of MMP-2 in the metabolism of FBG, our lab has investigated the reaction of MMP-2-mediated FBG cleavage. Surprisingly, we have found that FBG is a very poor substrate of MMP-2 relative to its native substrate gelatin (unpublished observations). If that is the case, are the interactions between MMP-2 and FBG important in normal physiology? How about in inflammatory conditions? Or in the settings of thrombosis? We are currently addressing these questions and testing the hypothesis that rather than being a substrate, FBG acts as an inhibitor of MMP-2. If this is the case, our on-going studies could help explain the significance of FBG / MMP-2 interactions in the settings of heart & kidney failure, infections, thrombosis and arthritis - processes in which FBG levels are highly elevated (Davalos and Akassoglou 2012; Kannel et
al. 1992; Prinsen et al. 2003) and could lead to the development of a non-genetic MMP-2 insufficiency.

GENERAL MMP INSUFFICIENCY

In the context of MMP-2 insufficiency induced by elevated expression of the aforementioned endogenous inhibitors, it is important to note that most (if not all) endogenous inhibitors of MMP-2 also inhibit other MMPs to varying degrees (Nagase et al. 2006). As detailed earlier in this article, TIMPs and the broad-spectrum anti-protease α-2-macroglobulin inhibit multiple MMPs in circulation and in the ECM. Besides the inhibition of MMP-2, the side effects of statins and doxycycline also include inhibition of MMP-1, -3, -7 and -9 (Golub et al. 1995; Izidoro-Toledo et al. 2011; Liu et al. 2003; Luan et al. 2003). Therefore, it is likely that inhibitor-induced MMP-2 underactivity overlaps with a general reduction in MMP-mediated proteolysis. Accumulation of a substrate exclusively cleaved by MMP-2 and not other MMPs would distinguish MMP-2 insufficiency from a general MMP insufficiency; however, no such substrate is currently reported in the literature.

PATHOLOGICAL MECHANISMS ELICITED BY MMP-2 UNDERACTIVITY

Inflammation: Studies on MMP-2 deficient (Mmp2−/−) mice report exacerbated allergen-induced inflammation in the lungs and a predisposition to asphyxiation, marked by an accumulation of T_{H}2 cytokines (e.g. IL-13) and inflammatory cells (Corry et al. 2002). During an asthmatic reaction, expression of MMP-2 increases 5-fold as part of an IL-13-mediated regulatory loop that attenuates the inflammatory response. MMP-2 deficiency prevents this upregulation, inhibiting the formation of an MMP-2-mediated cytokine gradient that triggers the migration of eosinophils out of the lung parenchyma. A predisposition to cardiac inflammation following viral infection is also observed in Mmp2−/− mice, suggesting MMP-2 plays a protective role in myocarditis.
Myocardial infection with coxsackievirus B3 (CVB3) in Mmp2-/- leads to increased invasion of CD4+ -activated T-cells and elevated cytokine expression, resulting in cardiac injury and subsequent mortality (Westermann et al. 2011). Using Mmp2-/- mice, our lab discovered that MMP-2 deficiency results in a systemic pro-inflammatory phenotype (Figure 11), characterized by elevated eicosanoids and an exacerbated febrile response to lipopolysaccharide (Berry et al. 2015). These effects are mediated in part by an accumulation of cardiac MCP-3, a pro-inflammatory cytokine normally degraded by MMP-2 to attenuate the inflammatory response (McQuibban et al. 2000). Our research suggests that one of the pathways by which MCP-3 stimulates inflammation is through induction of cardiomyocyte secretion of phospholipase-A2 (cardiac sPLA2), an enzyme whose activity is increased 10 to 1000-fold in mice lacking MMP-2 (Berry et al. 2015). Like other secretory phospholipase-A2 enzymes (Lambeau and Gelb 2008), cardiac sPLA2 is a highly pro-inflammatory phospholipid hydrolase that mobilizes fatty acids including eicosanoid precursors, promoting an upregulation of inflammatory modulators including monocyte-chemoattractant proteins and members of the interferon and TNF superfamilies in both the heart and the liver of Mmp2-/- mice (Berry et al. 2015; Hernandez-Anzaldo et al. 2015). Thus, cardiac secretion of PLA2 activity mediates a cardio-centric mechanism that modulates the inflammatory status of target organs (Berry et al. 2015; Hernandez-Anzaldo et al. 2015). TNF-α is highly pro-inflammatory in atherosclerosis, heart failure, arthritis, autoimmune disease, metabolic syndrome, diabetes, and obesity (Aggarwal et al. 2012; Parameswaran and Patial 2010). It is likely that TNF-α production downstream of MCP-3 contributes to the pathologies elicited by MMP-2 deficiency.

Cardiovascular pathologies: In cardiac pathologies MMP-2 plays a complex role, exhibiting both beneficial and harmful effects (Hardy et al. 2018). Humans with autosomal recessive
inactivating mutations in the hemopexin-like domain of MMP-2 develop congenital heart defects including transposition of the great arteries, a bicuspid aortic valve and both atrial and ventricular septal defects (Tuysuz et al. 2009). This likely arises from the loss of intermittent extracellular matrix breakdown provided by MMP-2, required for the migration of cells during tissue remodelling in embryogenesis. Mouse studies have indicated that MMP-2 activity is deleterious following myocardial infarction, increasing left-ventricular remodelling by stimulating the degradation of type IV collagen, laminin, fibronectin and elastin (Matsumura et al. 2005). MMP-2 deficiency decreases the risk of cardiac rupture by reducing this left-ventricular remodelling and limiting macrophage-mediated removal of infarcted cardiomyocytes. However, MMP-2 activity protects against the development of thoracic aortic aneurysms induced by angiotensin II (Shen et al. 2015) with Mmp2/-/- mice exhibiting more pronounced thoracic aortic dilation upon angiotensin II administration than Mmp2+/+ mice (Shen et al. 2015). Our studies also indicate a protective role of MMP-2 in hypertensive heart disease (Wang et al. 2015) as Mmp2/-/- mice experience more rapid and severe development of cardiac hypertrophy and perivascular fibrosis than their wild-type counterparts. Purportedly, MMP-2 protects from hypertensive heart disease, at least in part, by suppressing the transcriptional activity of SREBP-2 (sterol regulatory element-binding protein-2) (Wang et al. 2015) - a master regulator of isoprenoids and cholesterol biosynthesis (Brown and Goldstein 2009).

Metabolic dysregulation: Mmp2/-/- mice exhibit a rather unique metabolic phenotype (Figure 1), displaying elevated triglyceride levels within the liver and plasma VLDL (very-low density lipoprotein) and insensitivity to dietary cholesterol (Hernandez-Anzaldo et al. 2015). Metabolic dysregulation is likely a consequence of inflammation in Mmp2/-/- mice. One of the inflammatory pathways in MMP-2 deficiency involves the accumulation of cytokines normally
cleaved by MMP-2 such as MCP-3, which stimulates inflammation, at least in part, through induction of cardiac secretion of PLA2 activity (as discussed earlier). Purportedly, the MCP-3/sPLA2 secretion mechanism in Mmp2-/- mice may primarily originate from the heart (Berry et al. 2015), but it enables bidirectional pathways of communication between the heart and other organs (Hernandez-Anzaldo et al. 2015). For instance, the heart utilizes significant amounts of VLDL for energy, particularly in pathological states (Niu and Evans 2011), with the MCP-3/sPLA2 secretion pathway serving as a mechanism for the heart to signal the liver of its energy needs (Hernandez-Anzaldo et al. 2015). Unrelated lines of research suggest that MMP-2 activity is also critical for triglyceride deposition in adipose tissue as Mmp2-/- mice resist diet-induced obesity and are predisposed to adipose tissue fibrosis and subsequent hypotrophy (Van Hul and Lijnen 2008).

Skeletal pathologies: One of the most conclusive and well-studied pathologies of MMP-2 deficiency is the development of osteolytic disorders, otherwise known as ‘vanishing bone’ syndromes. Specific MMP2 inactivating mutations have been discovered in patients suffering from MONA (multicentric osteolysis, nodulosis, and arthritis) (MIM# 259600) (Martignetti et al. 2001), a progressive osteolytic disorder characterized by severe bone demineralization, abnormal long bone and craniofacial development and destruction of joint cartilage (Tuysuz et al. 2009). Additional symptoms include facial anomalies (dysmorphic facies, strabismus, gingival hypertrophy) and skin abnormalities (subcutaneous fibrocollagenous nodules, hyperpigmentation thickening) (Bhavani et al. 2016). Disease symptoms were initially reported to be absent in murine models, with Mmp2-/- mice purportedly displaying normal skeletal development with only a slight growth delay (Itoh et al. 1997). More recent evidence, however, has confirmed that Mmp2-/- mice indeed display attenuated features of the human disease including impaired bone
mineralization, joint erosion and defects in osteoblast and osteoclast growth (Mosig et al. 2007). Winchester syndrome, a symptomatically related osteolytic condition is caused by mutations in the MT1-MMP gene, resulting in reduced proMMP-2 activation (Evans et al. 2012). As MMP-2 cleaves ECM proteins, deficient MMP-2-mediated proteolysis should result in an accumulation of bone mass, not progressive bone loss. How and why MMP2-inactivating mutations result in osteolysis remains unclear. A possible explanation for this paradox is that MMP-2 acts at the level of osteoclast and osteoblast growth and proliferation, rather than through direct degradation of the bone matrix (Mosig et al. 2007). The elevated transcription of SREBP-2 related genes, and subsequent increase in cholesterol seen in MMP-2 deficiency as previously discussed (Berry et al. 2015; Hernandez-Anzaldo et al. 2015), provides one conceivable mechanism for such a role. Cholesterol elevation has been shown to inhibit osteoblast proliferation and differentiation (You et al. 2011) while stimulating the osteoclastic activity of macrophages (Sjögren et al. 2002). In addition to perturbed cholesterol metabolism, the elevation of TNF-α observed in MMP-2-deficient mice (Berry et al. 2015; Hernandez-Anzaldo et al. 2015) could play a role in osteolysis. Studies have demonstrated the ability of TNF-α to stimulate osteoclastic activity and bone resorption both in vitro (Azuma et al. 2000) and in vivo (Kitazawa et al. 1994). Polyarticular idiopathic juvenile arthritis, a condition whose phenotype mimics MONA (Castberg et al. 2013), has been shown to benefit from a treatment protocol involving TNF-α blockade (Gartlehner et al. 2008). MMP-2 activity within the synovial membrane positively correlates with rheumatoid arthritis (RA) progression (Goldbach-Mansky et al. 2000); however, recent research suggests that the elevation of MMP-2 is protective. Loss of MMP-2 in mice exacerbates arthritis and increases cellular infiltration in the hind paws (Itoh et al. 2002; Xue et al. 2014) - a response attenuated by the injection of wild-type embryonic fibroblasts secreting MMP-2 (Itoh et al.
As MMP-2 cleaves and inactivates IL-17, an important cytokine stimulating progression of RA (Hot and Miossec 2011), MMP-2 underactivity could increase joint degeneration via the accumulation of IL-17. A possible mechanism leading to MMP-2 underactivity (amid elevated protein expression) in RA patients could be the binding of MMP-2 to endogenous inhibitors including $\alpha$-2-macroglobulin - as reported (Tchetverikov et al. 2003).

OUTLOOK

While well-studied, an effective treatment option for the debilitating skeletal and inflammatory pathologies of MMP-2 deficiency does not currently exist. Disease progression is typically noticeable by the time a child reaches the age of six and affected individuals are crippled for life – most are wheelchair-bound by the onset of adulthood (Bhavani et al. 2016). The current standard of care is symptomatic and supportive, involving management of joint pain and rehabilitative medicine (Ekbote et al. 2014). Experimental treatments include bisphosphonates (e.g. pamidronate) to improve bone mineralization and bone formation (Al-Mayouf et al. 2006), as well as anti-inflammatory immunosuppressive treatments such as denosumab, methotrexate, etanercept and prednisolone (Castberg et al. 2013). Treatment with bisphosphonates has inconsistently demonstrated a relief of bone pain (Al-Mayouf et al. 2006; Pichler et al. 2016), however no trials have reported success with slowing disease progression or inducing the regression of pathologies.

FUTURE THERAPEUTIC DIRECTIONS

MMP-2 underactivity (whether caused by MMP-2 deficiency or insufficiency) is potentially highly pro-inflammatory because of the accumulation of cytokines normally cleaved and inactivated by MMP-2 (Figure 1). Theoretically, human $MMP2$ gene deficiency could be counteracted by administering MMP-2 protein, mRNA or cDNA in a pharmacologically
acceptable carrier to supplement MMP-2 activity in vivo (Martignetti 2003). Similarly, MMP-2 insufficiency could conceivably be treated by: (i) antagonists of the endogenous MMP-2 inhibitor TIMP-2, such as anti-TIMP-2 antibodies (Martignetti 2003) or phorbol esters (Mackay et al. 1992), (ii) endogenous activators of proMMP-2 like the membrane-bound MT1-MMP, or (iii) agonists of proMMP-2 activators such as thrombin (Lafleur et al. 2001), or the HIV-1 Tat protein in combination with basic fibroblast growth factor (bFGF) (Toschi et al. 2001). However, the feasibility of these approaches has yet to be demonstrated. Alternative personalized treatment approaches could target MCP-3 or downstream mediators such as cardiac sPLA2 and cytokines (or a combination therapy) – whichever are upregulated by MMP-2 underactivity in the specific patient. Further research into the above could lead to the discovery of novel life-changing treatments for patients with genetic MMP-2 deficiency, a currently unsolvable and disabling pathology, and for those affected by MMP-2 insufficiency, a condition that could transiently affect a fairly large cohort of the general population (Table 1).

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REFERENCES


Bode, W., Gomis-Ruth, F.X., and Stockler, W. 1993. Astacins, serralysins, snake venom and matrix metalloproteinases exhibit identical zinc-binding environments (HEXXHXXGXXH and
Met-turn) and topologies and should be grouped into a common family, the 'metzincins'. FEBS Lett. 331(1-2): 134-140.


virus-1 Tat protein and basic fibroblast growth factor. Mol Biol Cell, 12(10): 2934-2946. doi: 10.1091/mbc.12.10.2934.


Figure 1

*Pathological mechanisms triggered by MMP-2 underactivity.* MMP-2 activity can be reduced below baseline via either MMP-2 deficiency or MMP-2 insufficiency, both of which result in accumulation of pro-inflammatory cytokines normally cleaved by MMP-2. The ensuing pro-inflammatory state disrupts the metabolism of target organs thus catalyzing the development of pathologies, including the cardiovascular and skeletal disorders exhibited by MMP-2 deficient patients.

Table 1

*MMP-2 deficiency vs. insufficiency.* Comparison of MMP-2 activity levels, causes, prevalence, and phenotypes of MMP-2 deficiency and MMP-2 insufficiency.
Metabolic dysregulation

Inflammation

Accumulation of pro-inflammatory cytokines normally cleaved and inactivated by MMP-2

MMP-2 deficiency or insufficiency
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<th>MMP-2 Insufficiency</th>
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<tr>
<td><strong>Activity</strong></td>
<td>Complete loss of MMP-2 activity</td>
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<td></td>
<td>MMP-2 activity below baseline levels</td>
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<td><strong>Causes</strong></td>
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<td></td>
<td>Upregulation of endogenous inhibitors, medications w/ MMP-2 inhibitory properties, partially inactivating MMP2 gene or promoter mutations that reduce MMP expression or enzymatic activity</td>
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<td><strong>Prevalence</strong></td>
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<td>Affects a fairly large cohort of the general population?</td>
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<td><strong>Phenotype</strong></td>
<td>Severe, debilitating phenotype</td>
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<tr>
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<td>• Progressive bone and joint degeneration</td>
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<td>• Cardiovascular pathologies</td>
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<td>• Metabolic disruption</td>
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<td>Less severe, transient phenotype?</td>
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<td>• Side effects of statins? (hepatic/renal toxicity, myositis, pancreatitis)</td>
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<tr>
<td></td>
<td>• Doxycycline side effects? (joint inflammation, fever, tachycardia, myalgia, hepatic toxicity)</td>
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