Matrix Metalloproteinase 2 and Type Ⅱ D.M in The Elderly

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Abstract

Background: Matrix metalloproteinases (MMPs) are a family of zinc-binding proteolytic enzymes that normally remodel the extracellular matrix such as collagen, gelatin, fibronectin, laminin, elastin, and proteoglycans and pathologically attack substrates as part of an inflammatory response. Increased activity of MMPs has been reported in numerous disease processes including tumor growth, arthritis and cardiovascular disease. Increased matrix degradation by MMPs within the atherosclerotic plaque has been implicated as one of the key factors that leads to plaque instability, and consequently to cardiovascular events. Deterioration of MMP regulation contributes to the development of arterial lesions, in part, by facilitating monocyte invasion. Gelatin zymography studies have shown that MMPs, especially MMP-2 (72-kDa gelatinase A) and MMP-9 (92-kDa gelatinase B), are involved in remodeling processes associated with atherogenesis. MMPs are synthesized in atheromatous plaques and are present at elevated levels in rupture-prone shoulder regions of arterial blood vessels. Increased MMP activity has also been correlated with cardiovascular pathologies. Since vascular complications such as acute coronary artery syndrome and peripheral arterial disease are significantly more common among diabetics. Chronic low-grade inflammation is a characteristic feature of T2DM. MMPs are involved in modulating the inflammatory response by regulating the migration and activation of immune cells, as well as the release of pro-inflammatory cytokines. Elevated levels of MMPs have been observed in the circulation and tissues of individuals with T2DM, suggesting their potential role in promoting inflammation and contributing to the pathogenesis of the disease

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Introduction:

Matrix metalloproteinases (MMPs) are a family of zinc-binding proteolytic enzymes that normally remodel the extracellular matrix such as collagen, gelatin, fibronectin, laminin, elastin, and proteoglycans and pathologically attack substrates as part of an inflammatory response. Increased

activity of MMPs has been reported in numerous disease processes including tumor growth, arthritis and cardiovascular disease. Increased matrix degradation by MMPs within the atherosclerotic plaque has been implicated as one of the key factors that leads to plaque instability, and consequently to cardiovascular events **(1).**

The major MMP species in the myocardium and vasculature are the gelatinases (MMPs 2 and 9), MMP-1 (interstitial collagenase) and Mt1-MMP. Matrix metalloproteinases in type 2 and MMP-9 specifically attack type IV collagen, laminin, and fibronectin, major components of the basal lamina around blood vessels. Matrix metalloproteinases in type 2 diabetes, found in human arteries, have been implicated in acute myocardial ischemia and reperfusion injury, and along with MMP-9 play a major role in myocardial and vascular matrix remodeling. Furthermore, increased expression of MMP-9 has been demonstrated in the vulnerable regions of human atherosclerotic plaques **(2).**

Metalloproteinases also participate in the formation and destabilization of the atherosclerotic plaque. A key role in this phenomenon is linked with activation of MMP-1, MMP-2, MMP-3, MMP-7 and MMP-9 within atherosclerotic arteries. Circulatory levels of MMP-2 and MMP-9 are reported to be raised in patients with acute coronary syndromes. Increased expression and activation of MMP-2 and MMP-9 were found in cerebral ischemia, whilst increased MMP-9 expression has been related to hemorrhagic transformation of cardio-embolic stroke **(3).**

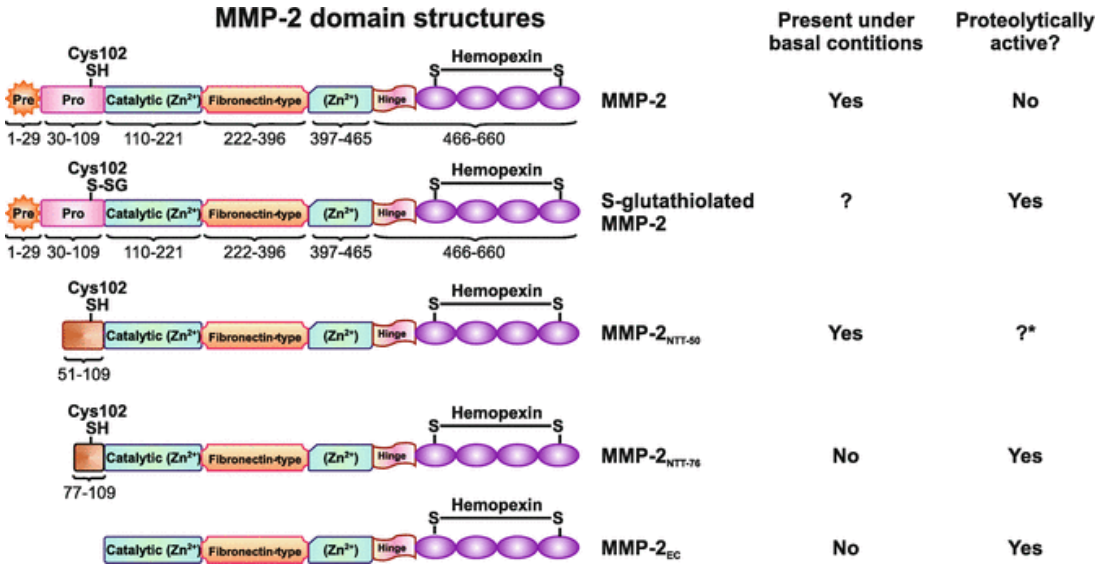

Figure (1): MMP-2 domain structure and its changes under basal condition and when proteolytically active (4).

In comparison with the general population, atherosclerosis in diabetic patients manifests earlier, is more severe and has a more disseminated character. Moreover, diabetic patients die more frequently of myocardial infarction, suffer from recurrent coronary events and are more prone to the development of heart insufficiency. A reason for this adverse prognosis could also be related to disturbed synthesis and activity of MMPs. However, the relationship between MMPs and their inhibitors (tissue inhibitors of matrix metalloproteinases – TIMPs) and diabetic angiopathy is less well defined **(5).**

Hyperglycemia directly or indirectly (e.g., via oxidative stress or advanced glycation products) might increase MMP expression and activity in large vessels. On the other hand, low proteolytic activity of MMPs contributes to diabetic nephropathy, probably via increased glycation-related activity of TIMP-2, the principal inhibitor of MMP-2. Within atherosclerotic plaques an imbalance between MMPs and TIMPs may induce matrix degradation, resulting in an increased risk of plaque rupture. Furthermore, because MMPs enhance blood coagulability they may play a role in acute thrombotic occlusion of vessels and consequent cardiovascular events **(6).**

MMPs have been implicated in the degradation of myelin basic protein, and interleukin as well as proteolytic processing of tumor necrosis factor. MMPs are secreted as proenzymes by many cell types, including leukocytes, macrophages, astrocytes, neurons, and microglia, and are widely distributed in tissues and biological fluids such as blood and urine. They are involved in many physiological processes, including tissue remodeling during development and platelet aggregation. MMPs also have roles in pathophysiological processes such as inflammation, tissue repair, myocardial injury, vascular diseases, tumor invasion, and metastasis **(7).**

Mechanisms have been identified for regulation of both expression and activity of MMPs. Transcription of MMP genes is modulated by growth factors, cytokines, and free radicals. However, MMP-2 differs from other MMPs in that it is constitutively expressed. Activity of MMPs is negatively regulated by tissue inhibitors of matrix metalloproteinases (TIMPs). The in vivo balance between MMPs and TIMPs dictates the level of MMP activity **(2).**

Deterioration of MMP regulation contributes to the development of arterial lesions, in part, by facilitating monocyte invasion. Gelatin zymography studies have shown that MMPs, especially MMP-2 (72-kDa gelatinase A) and MMP-9 (92-kDa gelatinase B), are involved in remodeling processes associated with atherogenesis. MMPs are synthesized in atheromatous plaques and are present at elevated levels in rupture-prone shoulder regions of arterial blood vessels. Increased MMP activity has also been correlated with cardiovascular pathologies. Since vascular complications such as acute coronary artery syndrome and peripheral arterial disease are significantly more common among diabetics **(1).**

Chronic low-grade inflammation is a characteristic feature of T2DM. MMPs are involved in modulating the inflammatory response by regulating the migration and activation of immune cells, as well as the release of pro-inflammatory cytokines. Elevated levels of MMPs have been observed in the circulation and tissues of individuals with T2DM, suggesting their potential role in promoting inflammation and contributing to the pathogenesis of the disease **(6).**

Insulin resistance, a hallmark of T2DM, involves impaired insulin signaling and glucose uptake in target tissues. MMPs may contribute to insulin resistance by affecting insulin signaling pathways. MMPs can cleave insulin receptor substrates (IRS) and insulin-like growth factor-binding proteins (IGFBPs), which are important mediators of insulin signaling and glucose metabolism. Dysfunction and reduced mass of pancreatic beta cells, which are responsible for insulin production, contribute to the development and progression of T2DM. MMPs have been implicated in beta-cell dysfunction and apoptosis. Increased MMP activity can lead to the degradation of the ECM surrounding beta cells, impairing their survival and function **(3).**

Matrix Metalloproteinase 2

Discovery

In the early 1970's, Harris and Krane identified in the rheumatoid synovial tissue culture medium an endopeptidase degrading the denatured form of type I collagen or gelatin, but not its native form . By analogy with a previously described endopeptidase called collagenase because of its catalytic activity against native type I collagen, the gelatin- degrading enzyme was termed gelatinase. The gelatinase was then purified in a latent 72-kDa form from culture media of various tissues and cells, including rabbit bone and transformed human bronchial epithelial cells. The latent gelatinase could be activated to an active form degrading type IV collagen and was therefore also called type IV collagenase **(8).**

This gelatinase/type IV collagenase exhibited the principal characteristics that conventionally define a member of the MMP family, namely secretion as an inactive proMMP, activation, and metallo-dependent degradation of extracellular macromolecules. It was named MMP-2 since it was the second enzyme to enter the family after interstitial collagenase/MMP-1 **(8).**

Biochemical and biological features

MMP-2 is a Zn^{2+}-dependent enzyme encoded by a gene located on the long arm of chromosome 16 at position q12.2. The 27 kb-long *MMP2* gene has 13 exons and is classically transcribed in a 3.1 kb-mRNA. The cDNA for MMP-2 codes for a 660 residues preproenzyme containing a 29 residues signal peptide responsible for translocation to the endoplasmic reticulum and followed by the 72-kDa-proenzyme. ProMMP-2 is composed of a propeptide followed by a catalytic domain that is connected to a hemopexin (Hpx)-like domain through a linker sequence **(9).**

A free zinc-ligating thiol group present in the propeptide maintains the enzyme latency until activation (cysteine switch). The Zn^{2+}-binding motif of the catalytic domain is preceded by three cysteine-rich repeats comparable to the collagen-binding fibronectin type II (FN-II) repeats, which are required for recognizing collagen and elastin **(9).**

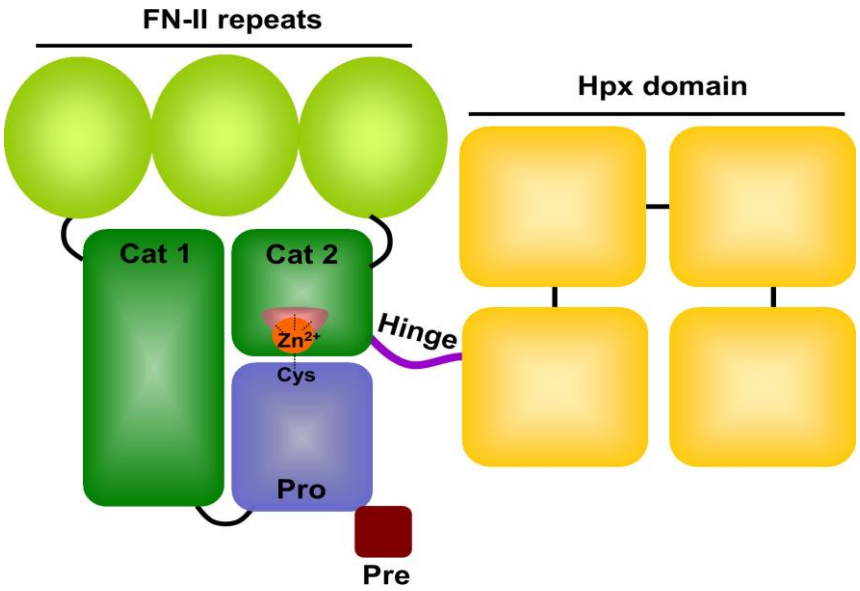

Figure (2): molecular structure of MMP-2 and its domains (9).

Secretion or intracellular activity

The N-terminus of preproMMP-2 is a signal peptide responsible for co-translational translocation into the endoplasmic reticulum (ER). Although MMP-2, like all other MMPs, was initially regarded only as a secreted MMP with extracellular activity, this paradigm was progressively modified to account for the accumulating evidence that MMPs are also intracellularly active. Two major mechanisms are proposed to explain intracellular MMP localization. The first one relies on inefficient signal sequence and results in ER translocation of a variable proportion of the proteins being synthesized, while the other ones remain in the cytosol **(10).**

The other mechanism depends on endocytosis of secreted proteins. MMP-2 can be endocytosed through direct or indirect binding to several membrane proteins. However, its translocation across the endosome membrane towards the cytosol remains unclear. Moreover, besides its transient journey in the secretory pathway (ER, Golgi apparatus and related vesicles), intracellular MMP-2 is not restricted to the cytosol but is also found in organelles including nucleus and mitochondria **(11).**

ProMMP-2 activation

The latent proform of most MMPs is generally processed in the extracellular compartment by proteolytic removal of the ~10-kDa propeptide, resulting in an intermediate form which is further activated by another proteinase into a fully active form. However, proMMP-2 cannot be fully activated by most extracellular proteinases. For example, thrombin alone is unable to fully activate proMMP-2 and activation of proMMP-2 by neutrophil elastase, cathepsin G or proteinase-3 depends on the expression of membrane-type 1 MMP (MT1-MMP) **(12).**

A prominent mechanism for proMMP-2 activation occurs at the cell surface and depends on the MT-MMPs, mainly MT1-MMP. The six MT-MMPs are associated to cell membranes by either a type I transmembrane domain (MT1-, MT2-, MT3- and MT5-MMPs) or a glycosylphosphatidylinositol (GPI) anchor (MT4- and MT6-MMPs). Their pericellular activities are diverse and include ECM degradation and shedding and activation processes **(13).**

Among the six MT-MMPs tested *in vitro*, only MT4-MMP does not activate proMMP-2 while MT6-MMP appears not to be very efficient. The recombinant catalytic domain of MT5-MMP generates the active form of MMP-2. Activation of proMMP-2 by MT3-MMP requires a preliminary interaction between chondroitin sulfate glycosaminoglycans expressed at the cell surface and the Hpx-like domain of proMMP-2. MT2-MMP activates proMMP-2 by directly interacting with the Hpx-like domain of the proenzyme **(14).**

Mechanisms of proMMP-2 activation by MT1-MMP have been extensively studied and involve tissue inhibitor of metalloproteinases-2 (TIMP-2), a physiological inhibitor of MMPs. Briefly, proMMP-2 can be secreted either in a free form or already complexed with TIMP-2 through its C-terminal domain. In the first case, a TIMP-2 that inhibits a cell membrane-anchored MT1-MMP by interaction between its N-terminal inhibitory domain and the catalytic domain of MT1-MMP, acts as a binding site for free proMMP-2 by interaction between the proMMP-2 Hpx-like domain and the TIMP-2 C-terminal domain **(14).**

Interestingly, TIMP-2-deficient mice revealed that TIMP-2 is required for efficient *in vivo* activation of proMMP-2 by MT1-MMP **(15).**

When proMMP-2 is not secreted, it can also be activated by proteolytic cleavage or alternatively by oxidation (by H2O2), S-nitrosylation and S-glutathiolation. For instance, it was shown that intracellular activation of proMMP-2 by oxidative stress contributes to cardiovascular pathologies **(15).**

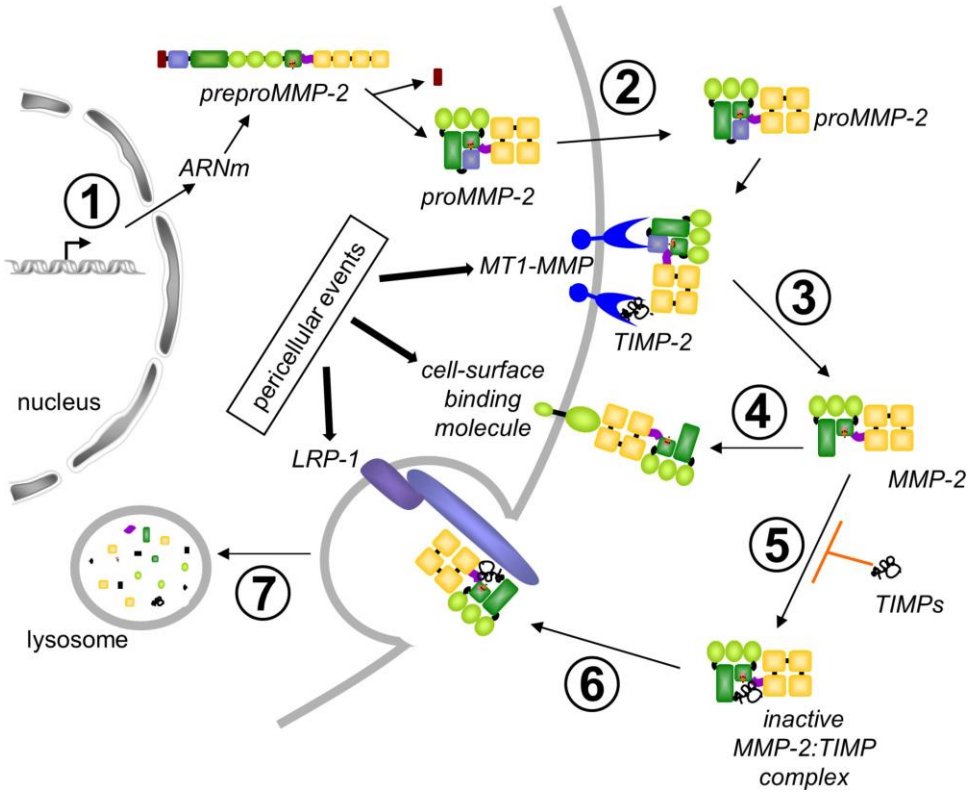

Figure (3): mechanism for proMMP-2 activation (13).

Extra- and peri-cellular substrates

First characterized as a proteinase present in the extracellular compartment for degrading denatured type I collagen and intact type IV collagen, cell-membrane- associated MMP-2 was also reported since the late 1980's. MMP-2 has more than 1,200 extra-, peri- and intra-cellular substrates based on the determination of their cleavage site(s) **(16).**

Cell-surface molecules binding (pro)MMP-2

Besides MT-MMPs, which efficiently bind proMMP-2 to induce its activation, a limited number of cell-surface molecules have been shown to contribute to enhance proteolytic activity at the migrating front of invasive cells by clustering active MMP-2 at the cell membrane. Among them the heat shock protein HSP90α expressed at the surface of tumor cells promotes MMP-2 activity and tumor invasion by binding to the Hpx-like domain of MMP-2. The αvβ3 integrin was first identified as a binding site for the C- terminal Hpx-like domain of MMP-2 in studies investigating *in vivo* and *in vitro* interactions between angiogenic blood vessels and melanoma cells **(17)**

This αvβ3/MMP-2 interaction was further identified in many other invasive cells such as endothelial cells, mesenchymal cells, lung cancer cells and glioblastoma cells. However, the direct interaction between MMP-2 and αvβ3 integrin was challenged. Regardless of these proposed molecules, MT-MMPs represent the major proMMP-2 binding partners at the cell surface **(17)**

MMP-2 binds to members of the low-density lipoprotein receptor-related protein (LRP) family to induce endocytic clearance activity of MMP-2 **(18).**

Cell-surface targets of MMP-2

An extensive review published a decade ago listed the main cell-surface molecules degraded or shed by MMP-2**.** It notably included cytokines and growth factors that can be activated or degraded by MMP-2. Since then, other targets have been identified. As an example, recruitment of MMP-2 by the β1 integrin, concomitant with an altered processing of the β1 integrin, was first reported in apoptotic HUVEC cells **(18).**

They concluded that sheddase activity promoted cell motility by reducing their adhesion to collagen or fibronectin substrates. MMP-2 also binds and cleaves the transmembrane proteoglycans syndecans 1 and 4, members of the syndecan family that exerts important roles in cell proliferation, differentiation, adhesion and migration. The MMP- 2-mediated shedding of human leukocyte antigen-G proteins affects control of pregnancy immune regulation and inflammation. The α-dystroglycan, a subunit of the dystroglycan receptor, was recently identified as a target of MMP-2. Interestingly, α-dystroglycan degradation contributes to progression of cancer and muscular dystrophies **(19).**

From maturation and activation to shedding or degradation, activity of MMP-2 on its various targets on the cell-surface has profound consequences on cell behavior in term of proliferation, differentiation, adhesion and migration, notably in cancer pathologies **(20).**

Non enzymatic membrane-associated activity

Cell membrane-bound MMPs can also act as transducers and trigger outside-in signaling. For instance, binding of proMMP-2 to αvβ3 integrin on the surface of lung cancer cells stimulates tumor angiogenesis by activating PI3K/AKT pathway and HIF-1α, leading to VEGF-A expression **(21).**

Inhibition of MMP-2 activity

A) TIMPs

Like all active endoproteinases, MMPs are inhibited by the "universal" plasma proteinase inhibitor α2-macroglobulin (α2M). MMP activity can also be moderately inhibited by other proteins including domains of netrins, the procollagen C-terminal proteinase enhancer (PCPE), the reversion-inducing cysteine-rich protein with Kazal motifs (RECK), and tissue factor pathway inhibitor (TFPI-2), but their physiological significance remains to be clarified **(21).**

However, the most important inhibitors of MMPs and their close relatives "a disintegrin and metalloproteinases" (ADAMs) and ADAMs with thrombospondin motifs (ADAMTSs) are the tissue inhibitors of metalloproteinases (TIMPs) (reviewed in four members, TIMP-1 to TIMP-4,

make up the TIMP family). All members inhibit active MMP-2 with a binding constant in the low picomolar range. This binding also involves interaction between TIMP and the MMP-2 Hpx-like domain **(22).**

While TIMP-2, TIMP-3 and TIMP-4 are able to strongly interact with proMMP-2, TIMP-2 is the only one contributing to cell-surface activation of the proenzyme by MT1-MMP. Such antagonistic properties of TIMP-2 (inhibition *vs* activation) highlight its seminal role in the control of pericellular MMP-2 activity **(23).**

B) Synthetic MMP inhibitors

The relationship between MMP activity and tumor aggressiveness and metastatic potential prompted laboratories and pharmaceutical companies to undergo massive research activity aimed at designing and developing synthetic MMP inhibitors (MMPIs). Most of these molecules were characterized by their capacity to bind the MMP active site. Unfortunately, these broad spectrum MMPIs presented major side effects and thus failed to pass successfully the phase III clinical trials **(24).**

Different reasons can be pinpointed for explaining such a failure. Among them, the design of these MMPIs was essentially based on *in vitro* data that did not completely reflect the *in vivo* complexity. Moreover, besides contributing to cancer progression (drug targets), some MMPs, such as MMP-8, can also act as antitumor agents and must be considered as anti-targets in cancer therapy. Despite these issues MMP-1, MMP-2 and MMP-7 are still the subjects of research aimed at anti-tumor therapies. Studies are in progress to improve specificity towards MMP-2 target versus MMP anti-targets in cancer **(25).**

C) "Exotic" natural inhibitors

Natural compounds represent a promising source of MMPIs. While the crucial problem to be solved is their selectivity, some of them already present moderate natural specificity. For instance, unsaturated long-chain fatty acids inhibit preferentially MMP-2 and -9 with micromolar Ki values but their inhibition of MMP-1 activity is weak **(26).**

MMP-2 endocytic clearance

Endocytosis allows the transport of material from extracellular environment into intracellular space for its elimination. This mechanism is well known for the clearance of active proteinases complexed to α2M via the α2M receptor. The α2M receptor has been identified as the low-density lipoprotein receptor-related protein (LRP) **(3).**

LRP, now noted LRP-1, is the first member of an endocytic receptor family related to the LDL receptor. Three members of this scavenger receptor family, LRP-1, LRP-1b and LRP-2, are able to endocytose MMP-2 and thus participate in the regulation of extracellular MMP-2 levels **(27).**

A- LRP-1

Binding of (pro)MMP-2 to thrombospondin-2 (TSP-2), which is a ligand of LRP-1 allows efficient clearance of MMP-2. When complexed with TIMP-2, proMMP-2 can also be endocytosed by LRP-1. The involvement of TIMP-2 in both activation of proMMP-2 by MT1-MMP and uptake

of proMMP-2 by LRP-1 strongly suggests the existence of a competition between these two processes. In contrast, the proMMP-2 activator MT1-MMP is able to shed LRP-1. Consequently, it would impede MMP-2 endocytosis by LRP-1 and thus would favor the cell-surface bioavailability of MMP-2 **(28).**

Endocytosis of TIMP-1 and TIMP-3, both able to bind and inhibit active MMP-2, also contributes to the clearance of MMP-2. Moreover, a recent study demonstrates that different glycosaminoglycans including hyaluronan and sulfated hyaluronan impact negatively or positively on MMP-2/TIMP-3 complex formation **(8).**

B- LRP-1b

LRP-1b inactivation in cultured thyroid cancer cells modifies their growth and invasive capacities. Interestingly, restoration of LRP-1b is accompanied by a reduction of MMP-2 in the conditioned medium without concomitant difference in MMP-2 mRNA levels. These data suggest that LRP-1b has the ability to mediate endocytic clearance of MMP-2 **(29).**

C- LRP-2/megalin

Using an in vitro model of BN16 cell cultures, we recently showed that LRP-2 was able to bind and endocytose proMMP-2 complexed to TIMP-2 . Moreover, conditional renal invalidation of LRP-2 in mice resulted in accumulation of proMMP-2 and TIMP-2 in their urine, highlighting the physiological relevance of the binding of the complex to LRP-2. As hypothesized for LRP-1, LRP-2 could also represent an additional mechanism to regulate active MMP-2 at the cell surface depending on the cell type, the extracellular environment and the physio-pathological context **(26).**

Pathologies

From the first correlation established between metastatic potential and degradation of basement membrane type IV collagen by a 65-kDa endopeptidase up to the discovery of MT1-MMP, MMP-2 overexpression was considered as a hallmark of cancer aggressiveness. Meta-analyses found that MMP-2 expression could be correlated (and often proposed to be used as prognostic marker) with pituitary adenomas, breast cancer ovarian cancer, endometrial cancer, gastric cancer, and non-small cell lung cancer **(3).**

However extra/pericellular MMP-2 is also involved in other degenerative pathologies, often in association with other MMPs. MMP-2 is thus involved in neurological disorders including Parkinson's and Alzheimer's diseases and glaucoma . MMP-2 is associated with inflammation of various origins, such as parasitic diseases and osteoarthritis. MMP-2 also contributes to the rupture of atherosclerotic plaques. Meta-analyses also found that MMP-2 is associated with renal fibrosis and thoracic aortic aneurysm **(21).**

Most of the MMP-2 deleterious activities are associated with up-regulation of its expression/activity. In contrast, a recent review presents important data that demonstrate impact of MMP-2 underactivity, either MMP-2 deficiency or insufficiency, on inflammation, metabolic dysregulation and cardiovascular and skeletal pathologies **(6).**

Several mutations in the MMP2 gene can cause Multicentric Osteolysis, Nodulosis, and Arthropathy (MONA), a rare skeletal dysplasia characterized by progressive osteolysis (particularly of the carpal and tarsal bones), osteoporosis, subcutaneous nodules on the palms and soles, and progressive arthropathy (OMIM gene entry 120360) **(19).**

Another genetic disease, the Winchester syndrome (OMIM entry 277650) is also linked to deficient MMP-2 activity since it is caused by mutations in the *MT1-MMP* gene, thereby affecting proMMP-2 activation. Without surprise, the Winchester syndrome shares common symptoms with MONA, including osteolysis and arthropathy. Moreover, recent meta-analyses have concluded that the -1306 C/T and -735 C/T polymorphisms are potentially linked to increased cancer risk **(22).**

Increased expression levels of MMP2 and its inhibitors TIMP1 and TIMP2 were reported in the plasma of T2D patients, compared to healthy individuals **(30).**

Interestingly, a recent study highlighted the impairment of early steps of adipogenesis in type 2 diabetes, independently of obesity, by demonstrating an inverse correlation between the expression of *MMP2* and *AP2* in the stromal vascular fraction isolated from the abdominal subcutaneous adipose tissue of T2D obese patients vs. non-diabetic obese individuals **(31).**In line with these results, it was shown that MMP2 (and MMP7) activities found in the sera change depending on the diabetes stage. Hence, it was observed that during decompensated T2D, the serum activity of MMP and the serum concentration of C-peptide—a marker of insulin secretion—decrease, while pro-insulin concentration robustly increases. On the other hand, the ratio of [proinsulin]/MMP activity was ~1:50 during the compensation phase, whereas in decompensated T2D this ratio was 1:12, suggesting that this ratio may become a diagnostic biomarker to assess diabetic decompensation **(32).**

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