Modulatory effects of proteoglycans on proteinase activities

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Abstract

Proteoglycans (PGs), composed of a core protein and one or more covalently attached sulfated glycosaminoglycan (GAG) chains, interact with a wide range of bioactive molecules, such as growth factors and chemokines, to regulate cell behaviors in normal and pathological processes. Additionally, PGs, through their compositional diversity, play a broad variety of roles as modulators of proteinase activities. Interactions of proteinases with other molecules on the plasma membrane anchor and activate them at a specific location on the cell surface. These interactions with macromolecules other than their own protein substrates or inhibitors result in changes in their activity and/or may have important biological effects. Thus, GAG chains induce conformational changes upon their binding to peptides or proteins. This behavior may be related to the ability of GAGs to act as modulators for some proteins 1) by acting as crucial structural elements by the control of proteinase activities, 2) by increasing the protein stability, 3) by permitting some binding to occur, exposing binding regions on the target protein, or 4) by acting as co-receptors for some inhibitors, playing important roles for the acceleration of proteinase inhibition. Understanding the modulatory effects exerted by PGs on proteinase activities is expected to lead to new insights in the understanding of some molecular systems present in pathological states, providing new targets for drug therapy.

Key words :

Proteinase activities, Proteoglycans, Glycosaminoglycans

Introduction

Proteoglycans (PGs) are a family of biomolecules that are composed of a core protein and one or more covalently attached sulfated glycosaminoglycan (GAG) chains. Synthesis and sulfation of GAGs occur on genetically distinct acceptor core proteins within the Golgi, followed by rapid translocation to the cell surface *(1).* GAGs are linear polymers of repeated disaccharidic units of hexosamine and hexuronic acid, except for keratan sulfate in which hexuronic acid is replaced by galactose. The presence of either two hexosamine isomers, D-glucosamine or D-galactosamine, divides the GAGs into two groups: glucosaminoglycans [heparin (HP)/heparan sulfate (HS) and keratan sulfate (KS)] and galactosaminoglycans [chondroitin (CS)/ dermatan sulfate (DS)] *(2).* Hexuronic acid is also present as two epimers: D-glucuronic acid and L-iduronic acid. Hyaluronic acid, which is not attached to a protein core, is a non-sulfated GAG composed of D-glucuronic acid and D-glucosamine. The degree and position of the sulfate group/moiety as well as the degree and position of 50 epimerisation are extremely variable in sulfated GAGs depending on the tissular/cellular/metabolic context, ensuring structural variability of these polysaccharides *(3).*

PGs are ubiquitous, being present as cell surface molecules anchored in the plasma membrane, as components of the extracellular matrix or as soluble molecules present in extracellular matrix and serum. Soluble PGs as well as those bound within the extracellular matrix are derived from cell secretions or by shedding from the cell surface. PGs play a role in both cell–cell and cell–extracellular matrix adhesion and can also act to promote assembly of extracellular matrix molecules *(4).* Additionally, PGs interact with a wide range of bioactive molecules such as growth factors and chemokines via their GAG chains to regulate cell behaviors in normal and pathological processes. Thus, these molecules are increasingly thought to participate in regulating a wide variety of biological processes, including the inflammatory response and tumor cell metastasis. Their ability to control proteinase activities

is established. Thus, GAGs induce conformational changes upon their binding to peptides or proteins. This behavior may be related to the ability of GAGs to act as modulators for some proteins by changing their catalytic activity, by increasing protein stability or by permitting some binding to occur, by exposing binding regions on the target protein.

Proteinases

Proteinases comprise a group of enzymes that catalyze the cleavage of a peptide bond in a protein or a peptide by nucleophilic attack on the carbonyl carbon. This is mediated by an amino acid (cysteine, serine, threonine) or by water molecules either fitted between two aspartate amino acids and/or complexed by an ionized metal on cysteine, serine, aspartate and matrix metalloproteinases (MMPs), respectively, or by some other mechanism *(5).*

Proteinases are also classified according to their substrate specificity, which implies the recognition of peptide bonds or residues at the amino or carboxyl terminus of the molecule as well as side chains of the surrounding amino acids at the bond to be cleaved. These endoproteinases play an important role in cell migration and invasion, the remodeling of the extracellular matrix, and the liberation and modification of growth factors. These proteinase activities are ultimately regulated by the balance between activation of inactive proforms and pre-proforms, and the levels of their endogenous inhibitors in cellular or tissue compartments, of which tissue inhibitors of metalloproteinases (TIMPs), serine proteinase inhibitors (Serpin) and cystatins represent major classes *(6).* Many of these proteinases act on the cell surface either because they contain a membrane spanning or binding domain or as the result of their interaction with specific receptors on the cell surface (Table 1).

MMP Docking

Proteinases can be either free in the cytosol or bound to the surface of a cell. The cell surface-bound forms are thought to enhance inflammatory cell functions. It remains unclear how these enzymes make it to the correct location at the cell surface and how the proteolytic activity is controlled at the pericellular space. However, it is becoming increasingly clear that extracellular proteolysis is a cell-regulated process. After all, cells do not release proteinases indiscriminately. Rather they rely on precise interactions to accurately degrade, cleave, or process specific substrates in the pericellular space. Indeed, an emerging concept is that MMPs, as for some serine and cysteine proteinases *(7)*, are anchored to the cell membrane, thereby targeting their catalytic activity to specific substrates within the pericellular space. However, it has been suggested that proteinase binding to cell-surface proteins can affect intracellular signaling, facilitate proenzyme localization and activation, mediate cell motility by disruption of cell contacts with the extracellular matrix (ECM), and promote internalization of the enzyme. For example, integrins are shown to act as receptors for several proteinases (8*)*. Such interactions have been detected in caveolae, invadopodia, and at the leading edge of migrating cells, where directed proteolytic activity is needed.

Metalloproteinases are secreted proteins belonging to a family of zinc metalloendopeptidases that have the capacity to cleave ECM and a variety of extracellular protein substrates. Using oriented peptide libraries to identify potential metalloproteinase cleavage sites, several integrins, PGs and chemokines or their receptors have been identified as substrates. Thus, metalloproteinases can release growth factors from the ECM or the cell surface. They can modify both cell–cell and cell–ECM interactions by the proteolysis of cell surface growth factors and adhesion receptors, and they are key regulators of inflammatory responses, which can be pro- or anti-inflammatory *(9).* This activity makes available active growth factors and cytokines. Because their substrates are diverse, metalloproteinases are involved in a variety of homeostatic functions, such as bone remodeling, wound healing, and

several aspects of immunity. However, metalloproteinases are also involved in a number of pathological processes, such as tumor progression, fibrosis, chronic inflammation and tissue destruction. These metalloproteinases can be subdivided into three sub-families: MMPs (matrix metalloproteinases), ADAM (a disintegrin and metalloproteinase domain) and ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) *(10).*

Specific cell-MMP interactions have been reported, such as the binding of MMP-2 to integrin αvβ3 *(11),* MMP-9 to integrin α4β1 *(12),* and MMP-1 to integrin α2β1 *(13).*The membrane-type MMPs (MMP-14, -15, -16, -17, -24, and -25) are single-pass transmembrane proteins that are fixed and active at the cell surface and, in addition to acting as proteinases, may provide docking sites for other MMPs. Thus, MMP-14 activates proMMP-2 at the cell surface *(14).* Tissue Inhibitor of MetalloProteinase-2 (TIMP-2) has a specialized role in the activation of proMMP-2 by MMP-14. The N terminal domain of TIMP-2 forms an inhibitory complex with the active site of MMP-14, while the C terminal domain interacts with the hemopexin domain of MMP-2. This trimeric complex is essential for activation of this gelatinase *(15).* It is likely that other MMPs are also attached to cells via specific interaction to membrane proteins, and determining these anchors will lead to identifying activation mechanisms and relevant substrates.

Previous studies have shown that cell surface HSPGs function as docking sites for MMPs at the cell surface *(16)* (Figure 1), and the cell surface localization of MMPs is important for their ability to regulate carcinogenesis *(17).* Anchoring MMPs to the cell surface or extracellular matrix would not only prevent them from rapidly diffusing away but would also enable the cell to keep them under close regulatory control. For example, syndecan-2 acts as a docking receptor for pro-MMP-7. At the cell surface, syndecan-2 interacts directly with pro-MMP-7 at the plasma membrane, enhancing its processing into active MMP-7, which in turn regulates tumorigenic activities of colon cancer cells *(18).*

Another PG, the highly polymorphic facultative cell surface PG CD44, is implicated in a variety of cellular functions, including adhesion, migration, activation, invasion, and cell survival. Several of these functions have been attributed to the ability of CD44 to bind hyaluronan *(19),* for which CD44 appears to be a major cell surface receptor. CD44 may also dock MMP-7 *(20)* and MMP-9 *(21)* to the cell surface. Therefore, the formation of MMP-adhesion receptor complexes appears to be a common pathway through which soluble MMPs are localized to the cell surface. Their localization on the cell surface confers resistance to TIMP inhibition *(22)* and may be responsible for the increase in pericellular proteolytic activity. Together with the cell surface activation of MMP-2 and MMP-7, these results/observations suggest that localization of MMPs to the cell surface is a general means of regulating MMP activity.

Zymogen activation

A great majority of proteinases are produced as zymogens, with a signal sequence and a propeptide segment that must be proteolytically processed to be activated. For example, zymogen activation of both MMPs and ADAMTS requires, at least, the removal of the N-terminal prodomain. ADAMTS and a subset of MMPs, including the membrane bound MMPs, contain a furin recognition sequence between their propeptide and catalytic domains, allowing cleavage and activation by furin convertase enzymes in the Golgi apparatus *(10)*. Prodomain removal can also be achieved by the action of other MMPs, such as the MMP-14-mediated activation of pro-MMP-2, or through activation cascades involving co-activators such as plasmin *(23).*

Sulfated GAGs could play important roles in controlling the activation and thereby the activity of MMP (Figure 1). The autolytic activation of proMMP2 is enhanced by HP *(24),* suggesting that sulfated GAGs may have wide roles in controlling MMP proteolysis. HS

regulates ADAM 12 through a molecular switch mechanism. The noncovalently associated prodomain in concert with the catalytic domain of ADAM 12 form a novel molecular switch critical for the regulation of the ADAM 12 proteolytic activity by HSPGs *(25).* Via direct interaction with proMMP-7, sulfated GAGs such as chondroitin-4,6-sulfate act as allosteric modulators promoting the autolytic activation of the proteinase. Once activated, GAGs may facilitate proteolysis of certain substrates by interacting with the substrate, the enzyme, or both. Activation of pro-MMP-2 by MMP-16 is also significantly enhanced in the presence of excess chondroitin 4-sulfate (C4S), whereas chondroitin 6-sulfate or low-molecular-mass hyaluronan was ineffective. C4S, which is expressed on the tumor cell surface, can bind pro-MMP-2 and facilitate its activation by MMP-16-expressing tumor cells to enhance invasion and metastasis

(26).

Among the seven aggrecanases, ADAMTS-4 is mainly expressed in an active form in osteoarthritic cartilage, suggesting that ADAMTS-4 may play an important role in the degradation of aggrecan in human osteoarthritic cartilage *(27).* However, suppression of ADAMTS-4 and ADAMTS-5, individually or in combination, attenuated the degradation of aggrecan in cytokine-stimulated normal cartilage. The active form of ADAMTS-4 has been reported to co-localize with ADAMTS mediated aggrecan cleavage in developing long bones in the rat, implying that ADAMTS-4 mediates the developmental turnover of aggrecan during long bone formation *(28).* However, ADAMTS-5 has been found to be the major aggrecanase in mice *(29).* In addition, the aggrecanase activity of ADAMTS-5 was at least 1,000-fold greater than that of ADAMTS-4 under physiological conditions *(30).* The activity of ADAMTS is regulated not only at the transcriptional level but also by post-translational modifications starting with the processing of the proprotein form by furin or MMP-9. At the cell surface, further activation takes place; glycosylphosphatidyl inositol-anchored MT-MMPs

and syndecans may collaborate on the surface of cells to maintain normal ECM homeostasis and also to respond to pro-inflammatory signals by increased activation of ADAMTS proteinases, which cleave aggregating PGs. ADAMTS-4 activation involves the coordinated activity of both glycosylphosphatidyl inositol-anchored MMP-17 and the PG form of syndecan-1 on the cell surface. *(31).* MMP-17 is the proteinase responsible for ADAMTS4 activation. Moreover, the activated enzyme form can be bound to the cell surface through the GAG chains of membrane associated syndecan-1. Another syndecan, syndecan-4, controls ADAMTS-5 activation through direct interaction with the proteinase, thereby controlling cartilage breakdown in osteoarthritis *(32).* Syndecan-4 is crucial in regulating MMP-3 expression by activating ERK1/2 and by targeting ADAMTS-5 to the cell surface of chondrocytes. Consequently, loss of syndecan-4 results in reduced expression of MMP-3 and a marked decrease in aggrecanase activity (Figure 1).

In contrast, syndecan-2 acts as a suppressor for MMP-2 activation on the cell surface *(33).* Syndecans comprise a family of cell surface HSPGs, exhibiting complex biological functions involving the interaction of HS side chains with a variety of soluble and insoluble HP-binding extracellular ligands. Munesue et al. demonstrated an inverse correlation between the expression level of syndecan-2 and the metastatic potential of clones established from Lewis lung carcinoma 3LL. Removal of HS from the cell surface of low metastatic cells by treatment with heparitinase-I promoted MMP-2 activation. In contrast, transfection of syndecan-2 into highly metastatic cells suppressed MMP-2 activation. These results indicate a novel function of syndecan-2, which acts as a suppressor for MMP-2 activation, causing suppression of metastasis in at least the metastatic system used in the present study. Another PG, testican 3, inhibits proMMP-2 processing mediated by membrane type-MMPs *(34).* However, testican 2 abolishes inactivation of membrane type MMPs by other testican families, and permits migration of glioma cells expressing MMP-14 in the presence of other

testican family proteins *(35).* The expression level of testican 2 was the highest among testican family members regardless of histological grade of astrocytic tumors. These results suggest that abundant distribution of testican 2 may contribute to glioma invasion by inactivating other testican family members, which all inhibit membrane type-MMPs.

A proteolytic cascade associated with cancer cell invasion and aimed at the degradation of the ECM, similar to the clotting and fibrinolytic cascades in serum, was proposed *(36).* This hypothesis is now broadly accepted, although not experimentally proven. The enzymes sitting at the top of the cascade may be cathepsins (lysosomal cysteine proteinases), in particular cathepsin B *(37).* Cathepsin B activity is often high in various compartments of tumors where invasion by cancer cells, endothelial cells or inflammatory cells takes place. It can activate pro-urokinase-type plasminogen activator (pro-uPA), which, by cleaving plasminogen, generates plasmin. The latter is a proteinase of broader specificity that may cleave the propeptide portion of certain MMPs. The cascade is probably more complex than that, as cathepsins may also directly activate a number of MMPs. The propeptide, which runs in an extended conformation through the active site cleft, forms an α-helical domain on top of the enzyme, which serves to anchor the prodomain to the body of the enzyme *(38).* Proteolytic removal of the prodomain, which is required for the activation of cysteine cathepsins, occurs in the acidic milieu of the endosomal/lysosomal system *(39).* Endopeptidases, such as cathepsins B, L, S, and K, can be activated autocatalytically or by other proteinases such as cathepsin D and pepsin *(40).* Procathepsin B was found to be an active species, suggesting that autocatalytic activation of cysteine cathepsins is a multi-step process, starting with a unimolecular conformational change of the zymogen, which unmasks the active site and, in the presence of negatively charged molecules or surfaces, also converts the zymogen into a better substrate. This is followed by the bimolecular proteolytic removal of the propeptide, which can be accomplished in one or more steps *(41).* In conclusion,

GAGs, which are found in the lysosomes, have the potential to interact with and regulate the activities of cysteine cathepsins. Autocatalytic activation of cysteine cathepsins can be substantially accelerated in the presence of GAGs and other negatively charged polysaccharides. GAGs facilitate procathepsin B activation through disruption of propeptide-mature enzyme interactions *(42).* In the first step GAGs bind to procathepsin B, thereby inducing a conformational change, which converts the procathepsin B molecule into a better substrate. In the next step the prodomain is removed by intermolecular proteolytic cleavage. Following propeptide dissociation, the bound GAG probably dissociates and is thus free to bind to another procathepsin B molecule. These observations suggest that the mechanism of insertion of cysteine cathepsins on the plasma membrane and its cellular traffic may depend on HSPGs present on the cell surface. In addition, the activity of cathepsin D, another lysosomal cathepsin, but pertaining to the aspartic proteinase family, which is overexpressed in breast tumors and that activates the cysteine cathepsins, is also regulated by GAG *(43).* GAGs increase the activity of cathepsin D *in vitro*. HP increases the activity of the proenzyme form and stimulates the mature (prodomain cleaved) enzyme. In addition, HP increases the limited proteolysis of procathepsin D at acidic pH, concomitant with an increased rate of substrate peptide cleavage. Like cathepsin D, GAGs induce the activation of the β-site amyloid precursor protein cleaving enzyme (BACE1) *(44).* BACE1 is a membrane-anchored enzyme that catalyzes the first step in the production of β-amyloid, the protein that accumulates in the brain of Alzheimer's disease patients *(45).* BACE1 cleavage of amyloid precursor protein (APP) may possibly occur in the endoplasmic reticulum, endosomes, the Golgi and the cell surface. HS can co-localize with BACE1 in these subcellular compartments *(46).* This suggests that HS may be able to regulate BACE1 activity in these compartments. It is also worth noting that APP (a major BACE1 substrate) can bind HP. Thus endogenous GAGs may not only activate the BACE1 zymogen during early stages of the secretory

pathway, but they could also help to orientate the enzyme with its substrate, which suggests a mechanism of HP-induced proBACE1 activation in which conformational changes in the monomer may be needed for activation.

In conclusion, interactions of proteinases with other molecules on the plasma membrane anchor and activate them at a specific location on the cell surface. Interactions of proteinases with macromolecules other than their own protein substrates or inhibitors result in changes in their activity and/or may have important biological effects.

Proteolytic activity regulation

PGs could play important roles in controlling the activation and thereby activity of proteinases. Thus, PGs, via their GAG chains, may facilitate proteolysis of certain substrates by interacting with the substrate, the enzyme, or the proteinase inhibitors.

HP promotes the binding of thrombin to fibrin *(47)* and stimulates the activity of plasminogen activator *(48).* HP acts in vitro *(49)* and in vivo *(50)* as a tight-binding, hyperbolic, competitive inhibitor of human neutrophil elastase (HNE), and this inhibition is strongly dependent upon polysaccharide chain length and degree of sulfation *(51).* HNE is an essential component of the phagocytic machinery of polymorphonuclear leukocytes. The concentration of HNE in the azurophil granules of polymorphonuclear leukocytes is thought to be in the millimolar range. An efficient anti-HNE control system must therefore be present at inflammatory sites. Healthy individuals have efficient HNE inhibitors including α1-proteinase inhibitor (α1PI), α2 macroglobulin, secretory leukocyte peptidase inhibitor (SLPI) and elafin *(52). In vitro*, the sulfated GAG has been shown to significantly increase the rate constant for the inhibition of HNE *(53),* neutrophil cathepsin G *(54)* and mast cell chymase *(55)* by SLPI. In addition, HP in combination with SLPI demonstrated *in vivo* efficacy reducing early and late phase bronchoconstriction.

α1PI is a multifunctional serpin, which circulates in blood and is implicated in a myriad of physiological functions. It is an acute phase protein and protects pulmonary tissues. α1PI depicts a high degree of sequence and structural homology towards other serpins. Serpins adopt a metastable conformation that is required for their inhibitory activity *(56).* Serpins can inhibit serine or cysteine proteinases by a remarkable conformational change-based mechanism in which the proteinase, having initiated cleavage of the serpin, is translocated 70 Å from one pole of the serpin to the other *(57).* Translocation results from the existence of a covalent acyl linkage between the proteinase active site residue serine and the P1 residue, and full insertion of the RCL into β-sheet A of the serpin *(76).* α1PI, like several other serpins such as antithrombin, proteinase nexin-I, HP co-factor II (HC-II), plasminogen activator inhibitor-I or protein-C inhibitor, is a HP binding serpin *(58).* These serpins possess a positively charged cluster at the protein surface that interacts with various GAGs. Antithrombin becomes fully effective upon activation by HP. The role of inhibition of blood coagulation proteinases by antithrombin is accelerated several thousand fold in presence of catalytic amounts of HP or related GAGs *(59)*. Binding of a specific HP or HS pentasaccharide to antithrombin induces allosteric activattion changes that mitigate the unfavorable interactions and promote template binding of the serpin and proteinase. Like antithrombin, HP brings about significant changes with respect to α1PI structure and function. The intrinsic ability of catalytic quantities of HP accelerates the inhibitory potential of α1PI. Thus, the association rate constants of α1PI in the presence of HP were found to be significantly enhanced compared to the native forms. This activation results from the specific binding of HP to a lysin rich stretch on helix-F *(60).* α1PI shows a sigmoidal transition upon activation induced by heparin binding indicative of allosteric modulation, and appears to be characterized by two steps of binding: a weak followed by a strong binding. Recently, a similar result was described with the squamous cell carcinoma antigens (SCCAs), a serpin of

cathepsin L. Higgins et al (2010) showed that the presence of HP accelerated inhibition of cathepsin L by SCCA-1 and SCCA-2 which specifically bind HP and HS but not other GAGs *(61).* In the case of SCCA-1, HP increased the second order inhibition rate constant. A templating mechanism was shown, consistent with ternary complex formation. Furthermore, SCCA-1 inhibition of cathepsin L-like proteolytic activity secreted from breast and melanoma cancer cell lines was significantly enhanced by HP.

Acceleration of proteinase inhibition also occurs when HP binds a non-serpin inhibitor such TIMP-3. TIMP-3, contrary to the other TIMPs, inhibits all known interstitial and membrane-bound MMPs as well as several key ADAMs and ADAMTSs *(62).* TIMP-3 is unique, because it is the sole TIMP regulator of one of the most versatile ADAM sheddases, ADAM-17 *(63).* Furthermore, TIMP-3 is also renowned for its ability to induce apoptosis in mammalian cells *(64).* Another extraordinary quality that distinguishes TIMP-3 from the other TIMPs is its ability to adhere to the ECM. Unlike the other TIMPs, TIMP-3 is not readily soluble *(65),* but instead is sequestered at the cell surface by association with the GAG chains of PGs, especially HP. TIMP-3 interacts with cell surface and extracellular matrix GAGs via the large number of positively charged residues in TIMP-3, and it was shown that this is the basis for its location in the extracellular matrix both *in vivo* and in cell culture. Hence, colocalization of TIMP-3 with proenzymes in the pericellular environment may be a mechanism for increasing the rate of inhibition of MMPs, such as MMP-2, and regulating extracellular matrix breakdown during morphogenetic processes *(66).*

HP and HS were found to increase the activity and stability of the lysosomal cysteine cathepsin. Cathepsin B has been implicated in a variety of diseases involving tissue remodeling states, such as inflammation, and tumor metastasis, by degradation of extracellular matrix components *(67).* The mature form of cathepsin B has been shown to be rapidly inactivated at neutral or alkaline pH and by its endogenous proteins inhibitors, mainly

from the cystatin family *(68).* On the other hand, it has been shown that membrane-bound forms of cathepsin B are very resistant to inactivation at neutral pH *(69).* The nature of the cathepsin B-GAGs interaction was sensitive to the charge and type of polysaccharide *(70).* Like papain *(71),* HP and HS bind cathepsin B specifically, and this interaction induces an increase of α-helix content, which stabilizes the enzyme structure even at alkaline pH *(70).* This coupling on the cell surface potentiates the endopeptidase activity of the enzyme by increasing 5-fold its half-life at physiological pH. The presence of cathepsin B on the plasma membrane results in focal dissolution of extracellular matrix proteins and could be enabling the tumor cell to invade the tissue.

Cathepsin K, another cysteine cathepsin, is considered as the principal proteinase responsible for the degradation of most of the bone matrix *(10).* Controlled degradation of collagen is observed in bone remodeling. Following tight attachment to the bone surface, osteoclasts secrete protons into a closed extracellular compartment enclosed by a sealing zone. This local acidification solubilizes the mineral bone and renders the organic matrix available to proteinases. Cathepsin K is abundantly and predominantly expressed in osteoclasts where it is localized in the lysosomes, in the ruffled border and in the resorption lacunae on the bone surface *(72).* GAGs can also participate in bone resorption regulation through the modulation of proteinase activity. The collagenolytic activity of cathepsin K is related to a specific complex constituted of five cathepsin K and five CS molecules. This complex has a triple helical collagen-degrading activity, whereas the monomeric form of cathepsin K can degrade noncollagenous substrates *(73).* At acidic pH, GAGs predominantly expressed in bone and cartilage, such as CS and KS, enhance the collagenolytic activity of cathepsin K, whereas DS, HS and HP selectively inhibit its activity *(74).* The structure of cathepsin K is flexible and converts between multiple conformational states with distinctive characteristics at physiological plasma pH. GAGs increase the activity of the enzyme and

promote a conformational change. GAGs act as natural allosteric modifiers of cathepsin K by affecting the distribution of a preexisting equilibrium of conformational states *(75).* GAGs, which exploit the conformational flexibility of the enzyme, regulate its activity and stability against autoproteolysis. At pH 7.40, the enzyme degrades collagen on its own and that CS/DS reduces its collagenolytic activity, whereas HP enhances it. Altogether this shows that the molecular mechanism behind the unique collagenolytic activity of cathepsin K depends on the environment.

In conclusion, PGs, through their compositional diversity, play a broad variety of roles as modulators of proteinase activities: (1) acting as crucial structural elements by the control of proteinase activities, or (2) acting as co-receptors for some inhibitors, playing important roles for the acceleration of proteinase inhibition.

Conclusion

Proteinases mediate vital processes in cells and in the homeostasis of tissues and organs. Proteinases have also been shown to participate in tumor growth and metastasis, with different proteinases exerting different functions at various stages of tumor development. These observations indicate that these enzymes are good targets for the development of anticancer therapies. However, an important question is the extent to which proteinases have structural roles in remodeling tissues versus a role in regulating access to signaling molecules. This requires a precise understanding of the roles of individual proteinases, with respect to not only ECM degradation, but also modulation of cytokine and growth factor function. Thus, a better understanding of the regulatory mechanisms that control proteinase activities in cancer cells could provide new ways for therapeutic intervention that are more specific and effective. Before inhibitors of proteinases can be applied in therapy, it is important to determine which proteinases are active in which tumor type and the exact role they have. Besides, to elucidate

the *in vivo* roles of proteinases in cellular pathways and thereby identify proteinase drug targets, it will be essential to determine their degradome that illustrates the large substrate specificity of proteinases. Once a proteinase or set of proteinases has been identified as a promising target for anticancer treatment, their production and/or activities can be modulated at each of the various steps that lead to the synthesis and activation of the mature enzyme. The modulatory effect exerted by PGs on proteinase activities is expected to lead to new insights in the understanding of some molecular systems present in pathological states, providing new targets for drug therapy.

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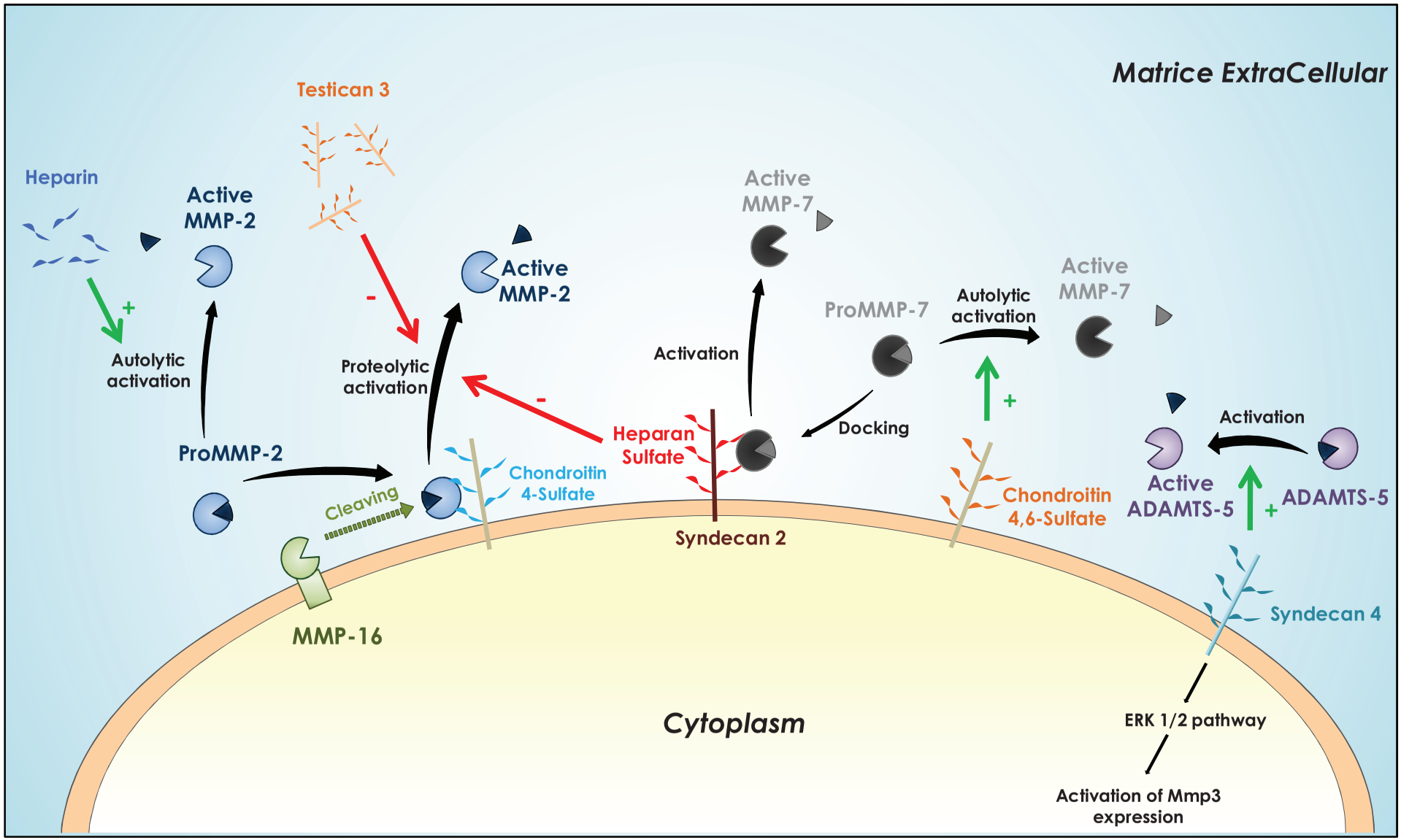
Figure legends

Figure 1: Regulation of metalloproteinases by GAGs

The activation and activity of MMP-2 is strongly regulated by GAGs. For example, Heparin enhanced the autolytic activation of proMMP-2. Furthermore, chondroitin-4-sulfate, expressed on cell surface, binds proMMP-2 and facilitates its activation by MMP-16, a membranous protease, whereas testican 3 and heparan sulfate inhibit this processing. The activity of MMP-7 is also regulated by GAGs. It's binded by sulfated GAGs like chondroitin-4,6-sulfate which acts as allosteric modulator promoting the autolytic activation. Syndecan-2 interacts directly with pro-MMP7, enhancing its processing into active MMP7. Another syndecan, syndecan-4, is able in the one hand, to control the ADAMTS-5 activation through direct interaction.

Table 1: Catalytic class and subcellar location of proteinases.

Proteinases	Catalytic class	Extracellular	Cytosolic	Plasma membrane associated	Lysosomal
Cathepsin D	Aspartate	+	-	-	+++
BACE1	Aspartate	+	-	-	+++
MMP (1-13; 19-21; 26-28)	Métallo	+++	-	+	+
MT-MMP (MMP14-17; 23-25)	Métallo	+	-	+++	-
ADAM	Métallo	+	-	-	-
ADAMTS	Metallo	+	-	+++	-
Cysteine Cathepsins (B, L, S, K)	Cysteine	-	-	++	+++
Neurophil elastase	Serine	-	-	+	+++
Cathepsin G	Serine	-	-	+	+++
Thrombin	Serine	+++	-	-	-
Plasminogen activator	Serine	-	+	+++	-


Matrice ExtraCellular
Testican 3
Heparin
Active MMP-2
+
Autolytic activation
ProMMP-2
Cleaving
MMP-16
-
Proteolytic activation
Active MMP-2
Chondroitin 4-Sulfate
-
Heparan Sulfate
Syndecan 2
Activation
Active MMP-7
ProMMP-7
Docking
Autolytic activation
Active MMP-7
+
Chondroitin 4,6-Sulfate
Activation
Active ADAMTS-5
+
ADAMTS-5
Syndecan 4
ERK 1/2 pathway
Activation of Mmp3 expression
Cytoplasm