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INVITED REVIEW

Extracellular matrix and proteolysis: mechanisms driving irreversible changes and shaping cell behavior

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The extracellular matrix (ECM) provides structural support and dynamic signaling cues, governing cellular behavior and tissue integrity. ECM remodeling, critically regulated by irreversible proteolysis, profoundly impacts development, homeostasis, and disease. This review examines the major families of ECM-degrading proteases—matrix metalloproteinases (MMPs), serine proteases, a disintegrin and metalloproteinases (ADAMs), metalloproteinase with thrombospondin motifs (ADAMTSs), and cysteine proteases—emphasizing their shared regulatory mechanisms and proteolytic activity in reshaping the tissue microenvironment. These proteases exhibit functional redundancy, particularly in the generation of matrikines, growth factors, and cytokines from common ECM substrates, all contributing to ECM softening. These overlaps in substrates and the resulting bioactive molecules amplify proteolysis within the tissue. The generated matrikines, growth factors, and cytokines further drive ECM remodeling through feedback loops, influencing the expression and activation of proteolytic enzymes. Despite these shared mechanisms, protease families demonstrate cell-specific functional specialization shaped by transcriptional programs, microenvironmental signals, and subcellular targeting, ensuring precise spatiotemporal proteolysis during processes such as development, wound healing, and immune responses. Dysregulation of this intricate proteolytic network contributes to chronic pathologies and cancer. Thus, understanding and targeting these processes is crucial for therapeutic intervention and the improved regulation of biological functions. Collectively, these insights reveal how irreversible ECM proteolysis orchestrates complex, context-dependent biological responses in both health and disease.

Introduction

Human organs and tissues are intricately supported by the extracellular matrix (ECM), a dynamic network of proteins, polysaccharides, and water. This complex structure provides both mechanical support and critical biochemical cues essential for maintaining tissue integrity and guiding cellular behavior [1]. The

Abbreviations
ADAMs, a disintegrin and metalloproteinases; ADAMTSs, metalloproteinase with thrombospondin motifs; BM, basement membrane; BMP-1, bone morphogenetic protein 1; CSPGs/DSPGs, chondroitin/dermatan sulfate proteoglycans; ECM, extracellular matrix; EMT, epithelial–mesenchymal transition; EVs, extracellular vesicles; FAP, fibroblast activation protein; Fn, fibronectin; GAGs, glycosaminoglycan chains; HPSE, heparanase; HSPGs, heparan sulfate proteoglycans; IM, interstitial matrix; KLK, kallikrein; LAP, latency-associated peptide; LOX, lysyl oxidase; LOXL1-4, lysyl oxidase-like proteins; LTBP, latent TGF-β-binding protein; NE, neutrophil elastase; OA, osteoarthritis; PGs, proteoglycans; PR3, proteinase 3; ROS, reactive oxygen species; SAPs, secreted aspartyl proteases; Serpins, serine protease inhibitors; TGF-β, transforming growth factor-β; TIMPs, tissue inhibitors of metalloproteinases; TMPRSS2, transmembrane protease serine 2; TMPRSS4, transmembrane protease serine 4; TSP-1, thrombospondin-1; TSRs, thrombospondin type 1 repeats; uPA/tPA, the urokinase-type/tissue-type plasminogen activators.

complete collection of ECM-related proteins is referred to as the matrisome, which in mammals comprises approximately 300 core proteins, classified into three major subclasses: collagens, proteoglycans (PGs), and glycoproteins [2,3]. These ECM components are synthesized intracellularly by resident cells and secreted into the extracellular space via exocytosis. The ECM plays a central role in regulating cell fate by modulating a wide range of cellular processes, including adhesion, differentiation, proliferation, migration, invasion, and signal transduction [4]. The ECM is not just a structural component; it is a dynamic signaling hub, modulating cell behavior by storing and releasing signaling molecules and by translating mechanical forces into biochemical instructions [5–10]. Importantly, the ECM is not a static scaffold but a highly dynamic and complex system, undergoing continuous modification through processes such as deposition of newly synthesized components, chemical modifications (e.g., cross-linking and glycosylation), force-mediated physical reorganization, and proteolytic degradation [11]. These remodeling events collectively shape tissue architecture, mechanics, and their interdependent roles in tissue function. Among these processes, proteolysis is particularly significant, standing out as a particularly significant irreversible degradation mechanism with profound effects on ECM structure and function [12–16]. Proteolytic degradation of ECM components is carried out by several enzyme families, including matrix metalloproteinases (MMPs), a disintegrin and metalloproteinases (ADAMs), a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs), serine proteases, and cysteine proteases such as cathepsins. These enzymes act in secreted, membrane-bound, or extracellular vesicle–associated forms, either individually or in regulated proteolytic cascades. ECM proteolysis plays diverse biological functions, including tissue remodeling, cell signaling, matrikine, growth factor, and cytokine modulation, regulation of immune response, wound healing, and disease progression. Under physiological conditions, ECM proteolysis is tightly controlled—both spatially and temporally—ensuring precise modulation of cellular processes [17,18]. In healthy tissues, this controlled activity drives repair, orchestrates development and morphogenesis, supports angiogenesis, facilitates immune cell migration, and activates signaling molecules that shape cell behavior [10,11]. In contrast, pathological states disrupt this balance, leading to excessive or mislocalized ECM degradation that causes persistent damage, altered tissue mechanics, dysregulated signaling, and disease progression [19]. In cancer, such remodeling changes the ECM's composition, structure, and mechanics, influencing every stage of tumor development—from initiation to metastasis—by affecting cell adhesion, proliferation, migration, angiogenesis, and immune modulation. This promotes tumor invasion and spread, while associated structural and signaling changes further drive malignancy [14,20]. Aberrant ECM degradation contributes to fibrosis, joint diseases, cardiovascular weakening, degenerative diseases, and chronic wounds, where ongoing matrix breakdown impairs repair and sustains inflammation [21–25]. Importantly, the biological consequences of ECM proteolysis are not purely destructive [26,27]. Rather, proteolytic cleavage events often serve as regulatory signals that influence or redirect cell behavior. These include the creation of feedback loops, amplification of local proteolytic activity, and the context-dependent generation of new bioactive molecules [28–30].

This review focuses on irreversible ECM proteolysis—its underlying enzymatic mechanisms and the downstream effects on tissue structure, cellular behavior, and disease progression. We explore how substrate and bioactive molecule redundancy affects ECM proteolysis, initiating a cascade that enhances substrate degradation and drives protease amplification loops. Finally, we highlight the application of these findings in therapeutic and bioengineering strategies.

The ECM: a molecular symphony of structure and function

The ECM is organized into two interconnected networks: the basement membrane (BM), a specialized pericellular matrix, and the interstitial matrix (IM) [3]. While sharing common properties such as providing a dynamic ECM scaffold and modulating cell signaling, the BM and IM exhibit distinct structural and compositional differences that contribute to specialized roles in tissue function [31], Fig. 1. The BM is a tightly organized, thin (50–100 nm) sheet-like structure underlying epithelial, endothelial, and muscle cells. Enriched in laminins, collagen IV, and heparan sulfate PGs (HSPGs), the BM has a stiffness of ~ 1–10 kPa, often exceeding that of the cells it supports. In contrast, the IM forms a porous 3D network in connective tissues such as the lamina propria and submucosa. Dominated by fibrillar collagens (e.g., types I, II, and III), elastin, fibronectin (Fn), and chondroitin/dermatan sulfate PGs (CSPGs/DSPGs), the IM is primarily produced by fibroblasts and mesenchymal cells. Its stiffness ranges from 5 to 50 kPa, depending on the degree and type of collagen cross-linking. The BM is critical for maintaining organ shape and is characterized by tightly bound water, whereas the IM enables tissue

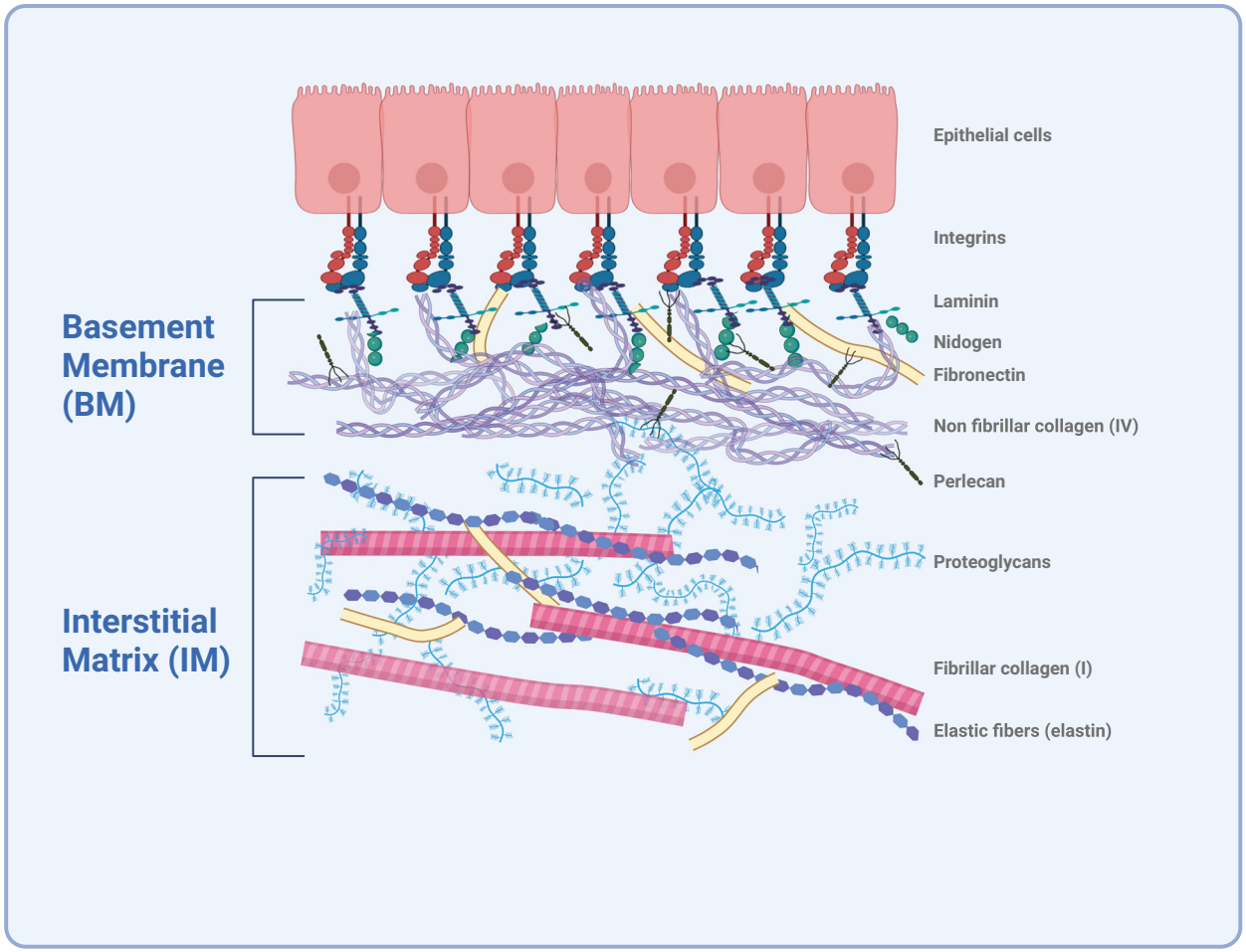

Fig. 1. Main structural components and organization of the basement membrane (BM) and interstitial matrix (IM). The basement membrane (BM) is a specialized, thin, sheet-like extracellular matrix that underlies all epithelial tissues and surrounds other cell types such as endothelial, muscle, and nerve cells. It anchors cells to the underlying connective tissue, provides structural support, establishes cell polarity, regulates filtration and permeability, and acts as a barrier to cell migration. The BM is primarily composed of laminin, collagen IV, nidogen, and the proteoglycan perlecan. Laminin forms the initial scaffold for BM assembly and directly binds integrins, facilitating cell adhesion and recruitment of additional BM components. Collagen IV provides the main structural framework and tensile strength. Nidogen acts as a key linker, connecting the laminin and collagen IV networks to ensure BM integrity. Perlecan, mainly decorated with heparan sulfate chains, further stabilizes the BM, binds growth factors, and modulates cell signaling and proliferation. Together, these components form a dynamic, multifunctional structure essential for tissue organization and homeostasis. The IM is a 3D, fibrous ECM network that fills the spaces between cells in connective tissues, providing mechanical support, elasticity, and a dynamic environment for cell signaling and migration. Its main components include fibrillar collagens (primarily types I and III), which form the principal structural scaffold and confer tensile strength; elastin, which imparts elasticity and resilience, allowing tissues to stretch and recoil; PGs, composed of a core protein with attached GAG chains that retain water, regulate hydration, and modulate signaling molecule diffusion; and fibronectin, a multifunctional glycoprotein that organizes the matrix, mediates cell adhesion, and guides cell migration by binding to integrins and other ECM components. Together, these molecules create a supportive and adaptable environment critical for tissue integrity, repair, and intercellular communication. Created in https://BioRender.com.

expansion and repair with free-flowing interstitial fluid. The BM primarily provides mechanical anchoring, regulates molecular transport, mediates cell signaling, and sequesters growth factors [9]. The IM supports tissue structure, facilitates remodeling-dependent biological processes, and participates in immune regulation as a dynamic signaling hub [10,27]. Consequently, pathological BM remodeling is associated with cancer metastasis and angiogenesis, diabetic nephropathy, and skin blistering diseases, while IM degradation is connected to fibrosis, tendinopathies, and osteoarthritis, cancer progression, immune dysregulation, and

autoimmune diseases. Remarkably, some proteins exist in both BM and IM, but they perform distinct roles. Moreover, through communication, BM and IM affect each other. For example, BM-derived growth factors (e.g., transforming growth factor-β, TGF-β) regulate IM remodeling, while IM stiffness influences BM integrity [1,10].

BM and IM contribute to mechanosensing and mechanotransduction. BM prioritizes polarity and immune regulation, while IM responds dynamically to mechanical stimuli and guides immune cell behavior [9,32]. Dysregulated ECM-immune interactions underlie diseases such as cancer, chronic inflammation, and autoimmunity. Here, we provide a brief overview of the main ECM proteins, highlighting the latest updates and advancements in understanding their roles and functions.

Collagens, the most abundant structural proteins in the ECM, comprise 28 identified types classified into fibrillar (I–III, V, XI) and non-fibrillar subgroups. Their biosynthesis, structural diversity, biomechanical properties, and tissue-specific functions have been extensively characterized, as detailed in prior reviews [33–36]. Fibrillar collagens form structural scaffolds in tissues like tendon and cartilage, while non-fibrillar collagens such as collagen IV form specialized networks in the BM. These proteins assemble hierarchically and undergo extensive posttranslational modifications, including glycosylation on hydroxylysine residues [33,37]. This glycosylation is vital for structural stability and proper folding [38]. Impaired glycosylation leads to increased degradation due to misfolding [39]. Additionally, enzymes of the lysyl oxidase family (i.e., lysyl oxidase [LOX] and lysyl oxidase-like proteins [LOXL1-4]) mediate cross-linking that is crucial for enhancing the resistance of ECM components, such as collagen and elastin, to proteolysis. However, the detailed mechanisms are beyond the scope of this review and have been discussed in other studies [40,41].

Elastin is one of the major fibrous ECM proteins that impart elasticity and resilience to tissues such as the aorta, lungs, skin, and ligaments. Its degradation contributes to pathological processes, including vascular diseases and tissue stiffening [42–44].

Fn is a multifunctional glycoprotein involved in cell adhesion, migration, proliferation, and differentiation [45]. It binds to numerous ECM components and plays pivotal roles in development, wound healing, and cancer progression [46–48].

PGs are composed of a core protein and glycosaminoglycan (GAG) chains, which vary in sulfation and length, contributing to their functional diversity [49,50]. GAGs regulate tissue mechanics and bind to growth factors, morphogens, and cytokines, where HSPGs in particular serve as growth factor reservoirs [51]. A comprehensive interactome of 827 proteins and 932 GAG-protein interactions has recently been described [52].

Laminins are trimeric glycoproteins essential for BM integrity and function. They play roles in mechanotransduction, shielding cells from deformation, and regulating cellular differentiation, shape, and survival [53–55]. They also influence cancer progression and neurobehavioral phenotypes [56–58].

Tenascins are large glycoproteins with roles in structural ECM organization, inflammation resolution, and regulation of growth factor activity [59–61].

Beyond the core matrisome, ECM-associated proteins—including ECM regulators, secreted factors, and ECM-affiliated proteins—are essential for maintaining tissue structure and stability. These components contribute to the formation of a robust, dynamic scaffold that not only provides mechanical stability and spatial organization but also anchors cells, modulates biochemical signaling, and enables tissues to adapt to physiological changes. Importantly, ECM-associated proteins serve as reservoirs for growth factors and cytokines, controlling their storage, release, and activation, which is critical for regulating cell behavior, tissue repair, and homeostasis [2,62]. The intricate interplay between ECM core proteins, which provide structural support, and ECM-associated regulatory elements enables the matrix to seamlessly integrate mechanical stability with dynamic signaling. Its ability to respond to developmental cues, mediate repair processes, and adapt to physiological demands exemplifies a highly coordinated molecular system. Through continuous remodeling and precise spatiotemporal control, the ECM preserves tissue integrity while guiding complex cellular behaviors—a true molecular symphony in which structure and function are inextricably linked.

Unmasking the ECM: the dynamic role of proteolytic enzymes

Among ECM-associated proteins, ECM regulators represent a particularly significant category within the matrisome, comprising specialized enzymes—proteases—that mediate ECM proteolysis, which is the breakdown of ECM proteins into smaller fragments. This enzymatic cleavage targets ECM components including collagens, glycoproteins, and proteoglycans and is carried out by several protease families, such as MMPs, ADAMs, ADAMTS, serine, and cysteine proteases. In this review, we focus on irreversible ECM proteolysis driven by these enzyme families, detailing their key roles in degrading and modifying ECM components and

highlighting their impact on both physiological and pathological tissue dynamics.

MMPs are zinc-dependent endopeptidases with 27 human members. They degrade a broad range of ECM proteins and collectively target over 4300 known cleavage sites within the ECM [63]. MMPs degrade both BM and IM components, playing critical roles in their degradation and influencing tissue structure and cell behavior in development, angiogenesis, inflammation, infection, fibrosis, vascular diseases, and tumor progression [21,23,64–70].

MMPs are categorized by substrate specificity and domain composition, which determine whether they are secreted or membrane-bound (e.g., MT-MMPs). Detailed information about structure, modes of regulation, and functions of MMPs in healthy states and pathologies can be found in recent reviews [14,64,65,71–75]. MMPs are secreted by various cells, including fibroblasts; immune cells such as macrophages and neutrophils; vascular smooth muscle cells; endothelial cells; bone marrow stromal cells; and cancer cells—highlighting their diverse roles across different tissue layers. Notably, their distribution is tissue- and context-specific [76].

Serine proteases, comprising ~ 30% of all known human proteases, participate in ECM degradation directly and indirectly by activating other proteases. They are grouped by substrate specificity into trypsin-like (cleaving after Arg/Lys), chymotrypsin-like (Phe/Trp/Tyr), and elastase-like (Gly/Ala/Val). Structurally, they feature a catalytic triad (Ser-His-Asp) and are classified into 13 clans and 40 families [77]. Trypsin-like ECM-targeting enzymes include plasmin, transmembrane protease serine 2 (TMPRSS2), transmembrane protease serine 4 (TMPRSS4), matriptase, and hepsin. Chymotrypsin- and elastase-like serine proteases such as chymases, neutrophil elastase, proteinase 3, and cathepsin G also contribute to ECM degradation [78,79]. These enzymes function in wound healing, immune responses, tissue repair, and embryogenesis. When dysregulated, they contribute to cardiovascular diseases, inflammation, infection, cancer development (initiation, progression, and metastasis), and altered immune responses by disrupting the BM and ECM molecules and activating MMPs [80–86]. Plasmin activates MMPs, and its own activation by the urokinase-type or tissue-type plasminogen activators, uPA/tPA, creates a positive feedback loop [82]. Another one, TMPRSS4, activates matriptase, hepsin, and TMPRSS2; hepsin activates MMP-1 and -3 [87]. Various cells produce serine proteases, including epithelial cells, endothelial cells, pancreatic- and tumor cells. They are also found in immune cells, particularly cytotoxic T lymphocytes and natural killer cells [78]. Recent reviews have comprehensively explored the structure, activation mechanisms, regulation, inhibition, and diverse functions of serine proteases [84–86,88,89].

ADAMs and **ADAMTS** are structurally related to MMPs but differ in localization and function. While ADAMs are best characterized as membrane-bound sheddases that cleave cell surface proteins, they also degrade ECM components such as laminin and collagen IV, thereby remodeling the immediate cellular microenvironment. In contrast, ADAMTSs are secreted enzymes that specialize in degrading PGs and other structural ECM molecules, enabling precise regulation of matrix integrity and biomechanical properties. Both families share a metalloproteinase and disintegrin domain, but ADAMTS enzymes additionally contain thrombospondin repeats, cysteine-rich, and spacer domains [90,91]. ADAM proteases are primarily expressed in epithelial, immune, and tumor cells, where they regulate processes such as ectodomain shedding, cell adhesion, migration, and signaling pathways critical for cancer progression and immune modulation [92–94]. In contrast, ADAMTS proteases are mainly expressed in chondrocytes, fibroblasts, and stromal cells, where they play key roles in ECM remodeling by degrading PGs like aggrecan and versican, often contributing to cartilage degradation and inflammation [95,96]. These differences in expression patterns reflect their specialized roles in tissue-specific physiological and pathological processes, despite their structural similarities. Detailed information about the structure, regulation, and functions of these protease families has been recently reviewed [97–99].

Cysteine proteases, particularly cathepsins B, K, L, and S, play crucial roles in ECM degradation. They contain a catalytic triad (Cys-His-Asn/Asp) and operate optimally intracellularly at acidic pH but can act extracellularly during bone development, processing of prohormones, cardiovascular disease inflammation, and cancer [100–104]. Interestingly, cathepsins B, L, and S mostly degrade BM, where cathepsin K shows high efficiency at degrading fibrillar collagens of the IM [105]. These enzymes are expressed by different cell types across various tissues, including macrophages, fibroblasts, osteoclasts, and epithelial cells. Comprehensive reviews about the structure, activation, and functions of cysteine proteases were published in the past decade [101,106,107].

Additional ECM-remodeling enzymes

Meprins α and β and bone morphogenetic protein 1 (BMP-1) are astacin metalloproteases capable of

degrading ECM components. Meprin α is soluble, while meprin β is membrane-bound but can be shed [108,109]. These enzymes influence wound healing, inflammation, and tumor progression [110]. Meprins are expressed in kidney, intestinal, immune, and tumor cells. They facilitate leukocyte migration and activate pro-inflammatory cytokines (e.g., IL-1β, IL-18) [111].

Secreted aspartyl proteases (SAPs) are critical virulence factors in fungal pathogens, enabling tissue invasion by degrading BM [112,113]. They facilitate immune evasion by impairing neutrophil and macrophage functions.

Heparanase (HPSE), an endo-β-D-glucuronidase, cleaves heparan sulfate chains in HSPGs, altering ECM structure, releasing growth factors, and modulating signaling. Though not a classical matrisome component, it significantly influences ECM remodeling, particularly in cancer and inflammation [114,115].

Other enzyme classes (e.g., threonine proteases) contribute marginally to ECM remodeling and are not discussed in detail here.

In summary, ECM-degrading enzymes arise from distinct protease families, each with both shared and unique structural features. MMPs, ADAMs, and ADAMTS are zinc-dependent proteases that rely on a zinc ion at their active site for catalytic activity, whereas serine and cysteine proteases utilize different catalytic mechanisms independent of zinc (Fig. 2). All these enzymes are produced as inactive zymogens with pro-domains that must be removed for activation, providing tight control over their proteolytic activity and preventing excessive ECM degradation. Additionally, these protease families exhibit common regulatory mechanisms at the levels of gene expression and enzymatic activation, as outlined in Tables 1 and 2 and discussed in the following section.

Balancing act: regulatory mechanisms of ECM proteolysis in health and disease

ECM proteolysis by different classes of remodeling enzymes is tightly regulated through both shared and distinct mechanisms that preserve tissue homeostasis and prevent pathological damage [18,138,139]. Protease expression is controlled at transcriptional and epigenetic levels, while their activation, as individual zymogens or within proteolytic cascades, can be triggered by common stimuli such as hypoxia or inflammatory signaling, reflecting a pathological shift in ECM remodeling dynamics (Tables 1 and 2).

Proteolytic cascades consist of hierarchically organized proteases that activate one another via sequential cleavage (Fig. 2). For example, plasmin, generated by uPA or tPA, not only degrades multiple ECM components but also activates MMP-1, -2, -3, and -9. Matriptase and hepsin, two membrane-bound serine proteases, also activate MMP-1 and -3; notably, matriptase contributes to pericellular matrix degradation in osteoarthritis [81,140]. These cascades amplify not only ECM degradation but also apoptosis and cell signaling [29,141].

Endogenous inhibitors

The activity of ECM-remodeling proteases is tightly controlled *in vivo* by endogenous inhibitors that ensure precise enzymatic regulation. These inhibitors play crucial roles in maintaining tissue homeostasis and preventing pathological damage. Due to the structural and functional diversity of each protease family, their respective inhibitors are specifically adapted to block target enzymes effectively (Table 2). Tissue inhibitors of metalloproteinases (TIMPs) inhibit MMPs, ADAMs, and ADAMTS [71]. Four TIMP isotypes have been described, and their detailed structure and functions have been comprehensively reviewed [142–144]. Serpins (serine protease inhibitors) inhibit serine proteases such as plasmin, elastase, and trypsin; detailed information on their mechanisms can be found in recent comprehensive reviews [145–147]. Cystatins inhibit cysteine proteases, including cathepsins, and their mechanisms of action have been structurally and biochemically characterized in detail [148–150]. While these studies primarily focus on inhibitory mechanisms and structural features, they also provide foundational insights relevant to developing cysteine protease–targeted therapeutic strategies.

Post-translational modifications further regulate protease expression, activity, and substrate specificity (Tables 1 and 2). Specifically, propeptide glycosylation of human neutrophil MMP-9 confers resistance to proteolytic activation by MMP-9 and -3, meprin α, neutrophil elastase, trypsin, and PNGase [137]. *N*-glycosylation in the SRCR domain of hepsin promotes its expression and activity on the cell surface [151]. Another example is that the binding of ADAMTS4 and ADAMTS5 to HS or to HS-containing PGs inhibits their aggrecanase activity. Whether this is a general feature of the proteoglycanase subgroup remains to be clarified [98]. Additionally, **allosteric modulations**, which refer to the regulation of enzyme activity through the binding of molecules at sites other than the active site, induce conformational changes that either enhance or inhibit enzymatic function. For example, the flexibility of catalytic and hemopexin domains of MMP-1

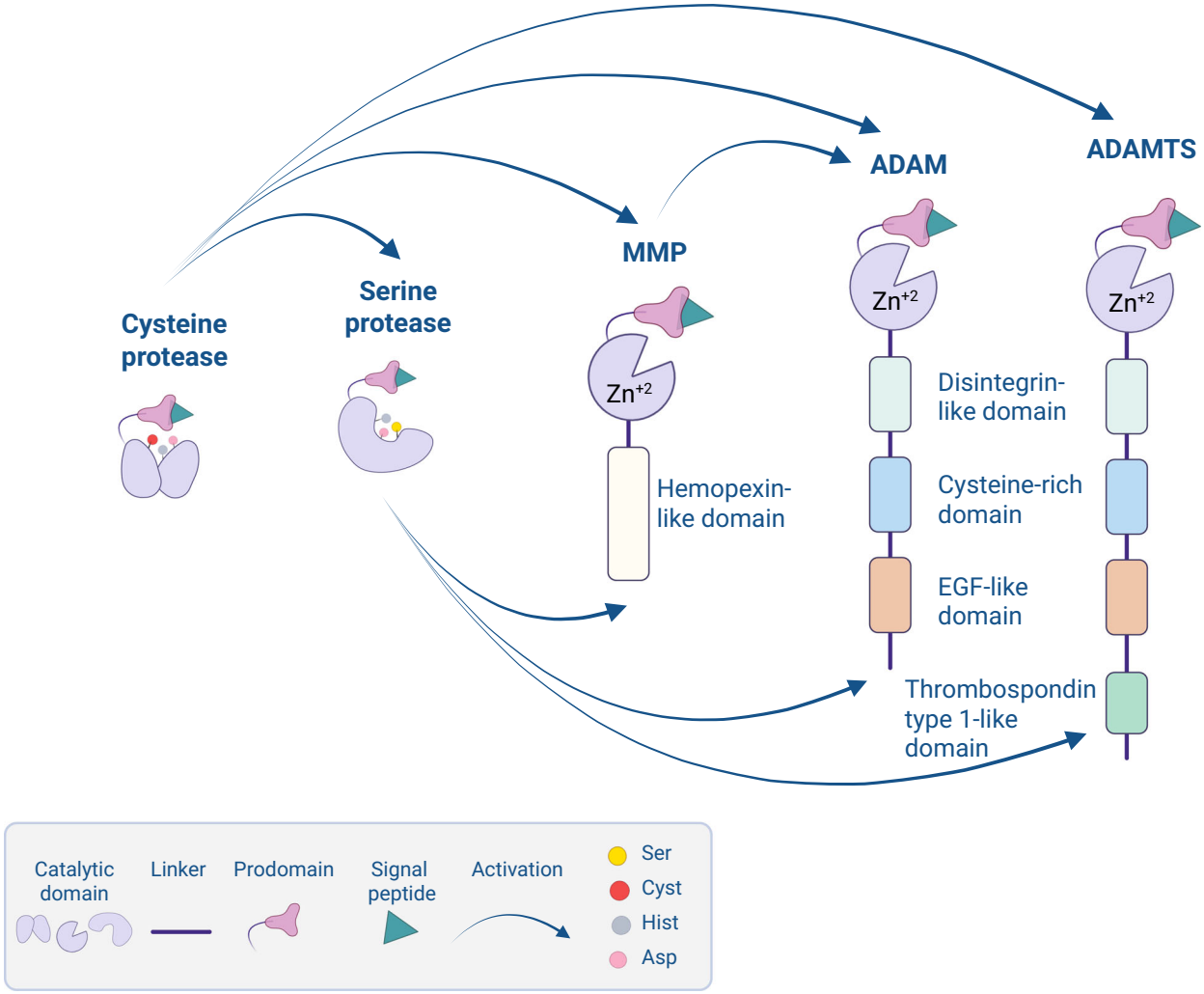

Fig. 2. Common structural features of secreted ECM-degrading enzymes and the hierarchy of proteolytic activation among protease classes. The scheme illustrates the structural organization and functional hierarchy of secreted ECM-degrading enzymes across major protease classes. Serine proteases, modeled on human neutrophil elastase (PDB: 3Q76), feature a catalytic triad (Ser/His/Asp) for proteolysis, while cysteine proteases such as cathepsins K, S, B, and L (modeled on procathepsin K, PDB: 1BY8) utilize a Cys/His/Asn triad for collagenolytic activity. Soluble MMPs (e.g., MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-13, MMP-20–MMP-28) and soluble ADAMs (e.g., ADAM-11, -12, -17, -28) share core domains, including a signal peptide that directs the nascent protein to the secretory pathway; a prodomain that maintains the enzyme in an inactive zymogen state until proteolytic removal, and a zinc-dependent catalytic domain responsible for peptide bond hydrolysis. ADAMTSs (a family of 19 human genes) also contain thrombospondin type 1 repeats (TSRs), which mediate interactions with ECM components and contribute to substrate specificity. All classes retain a conserved signal peptide and prodomain but diverge in catalytic mechanisms and accessory domains. Metalloproteinases (MMPs, ADAMs, ADAMTSs) utilize a zinc ion in their catalytic domain and typically contain a flexible hinge or linker region connecting to accessory domains, which enables precise spatial orientation and substrate recognition. In contrast, serine and cysteine proteases rely on catalytic triads, in which histidine's pH-sensitive side chain activates the nucleophile (serine or cysteine), to cleave peptide bonds. Class-specific accessory domains define enzyme functions: the hemopexin-like domain in MMPs enhances binding to collagen and other ECM proteins; disintegrin and cysteine-rich domains in ADAMs and ADAMTSs mediate cell adhesion and protein–protein interactions; EGF-like domains participate in cell signaling; and TSRs in ADAMTSs direct enzymes to specific ECM substrates. Proteolytic activation occurs through a hierarchical cascade: cysteine proteases (e.g., cathepsins) can activate serine proteases, MMPs, ADAMs, and ADAMTSs via prodomain cleavage due to their highly nucleophilic thiol group and broad substrate recognition. Serine proteases (e.g., neutrophil elastase) can subsequently activate MMPs, ADAMs, and ADAMTSs, while MMPs specifically activate ADAMs through hemopexin-mediated targeting. ADAMTSs, pre-activated by furin during secretion, function as terminal ECM remodelers due to their TSR-mediated substrate specificity and do not activate downstream proteases. This structural and functional interplay enables coordinated yet tightly regulated ECM remodeling, with enzyme activities controlled at multiple levels to prevent excessive tissue destruction and maintain tissue homeostasis. Created in https://BioRender.com.

Table 1. Factors affecting protease expression.

Factor	Description	Protease families affected	Specific examples and mechanisms
Transcriptional regulation	Gene expression directly influenced by transcription factors, modulating mRNA levels	All families of proteases	STAT3 and STAT6 synergize to promote cathepsin secretion from macrophages via IRE1α activation, enhancing the expression of genes involved in ECM remodeling [116]. MicroRNAs regulate the translation of cathepsin mRNAs [100]
Alternative splicing	Generates protein isoforms with diverse functions, affecting protein structure, secretion, and activity	All families	Alternative splicing events generate protein isoforms with diverse functions, affecting protein structure, secretion, and activity [117]. MMP-9, for instance, has multiple splice variants that can influence its activity and interaction with other molecules in the extracellular matrix [118]
MicroRNA-mediated regulation	miRNAs control the expression of genes at the posttranscriptional level	All families	microRNA targets MMP14 [119]. MicroRNA-125b regulates the expression of ADAMTS-4 in human osteoarthritic chondrocytes [120]. miR-140 reduces the expression of ADAMTS-5, which is involved in cartilage degradation in osteoarthritis [121]
External factors	Oxidative stress, hypoxia	All families	Hypoxia can increase the expression of serine proteases through the activation of hypoxia-inducible factors (HIFs). These factors bind to hypoxia response elements (HREs) in the promoters of serine protease genes, enhancing their transcription. In hypoxic conditions, serine protease activity can contribute to protection from infections, such as in hypoxic lung granulomas during *Mycobacterium tuberculosis* infection [122]
Microenvironment signals	External signals such as cytokines, growth factors, and ECM stiffness influence protease expression in a context-dependent manner	All families	Inflammatory cytokines like IL-1β and TNFα upregulate MMP expression in fibroblasts, promoting ECM degradation in inflammatory conditions [123–128]
Cell-type specificity	Proteases are expressed in specific cell types to perform specialized functions, controlled by cell-specific transcription factors	All families	Osteoclasts express high levels of cathepsin K for bone resorption [129]. Macrophages express specific cathepsins, such as Cathepsin V with potent elastolytic activity, crucial for ECM turnover [130]. Neutrophils express MMP-9 during inflammation [131]

Table 2. Factors affecting protease activation.

Factor	Description	Protease families affected	Specific examples and mechanisms
Zymogen activation	Synthesized as inactive precursors (zymogens); activation occurs via proteolytic cleavage or environmental changes	All families	Pro-MMPs are activated by proteolytic cleavage of the prodomain by other MMPs or serine proteases in the extracellular space [16]
Metal Ion dependency	Metal ions, particularly Zn^{2+} in the catalytic domain, are essential for the catalytic activity of MMPs, ADAMs and ADAMTSs	MMPs, ADAMs, ADAMTSs	Zn^{2+} in the active site is crucial for substrate binding and hydrolysis by MMPs and ADAM/ADAMTS proteases. Metal ion chelation by inhibitors suppresses their activity [71]
pH sensitivity	pH conditions modulate protease activity and stability, with acidic pH often promoting activation	All families	Cathepsins B, K, and L are activated in acidic lysosomes and extracellular environments [100]. MMP8 shows optimal catalytic efficiency in acidic conditions (pH: 4.8–6.0). MMP-2 shows broad tolerance (pH: 6.4–9.0), with peak activity near neutral pH (7.0–7.2). MMP-9 displays a narrow, bell-shaped pH-dependent activity profile, with optimal activity at pH 7.0 [132]
Endogenous regulators	Endogenous inhibitors are crucial for keeping ECM-degrading proteases in check, ensuring that tissue remodeling and protein breakdown happen only when needed and do not damage healthy tissues	All families	Endogenous inhibitors for different families of ECM remodeling proteases show both specificity and some degree of overlap in their targets, depending on the inhibitor class and the protease family. TIMP-3 inhibits MMPs and several members of the ADAM and ADAMTS families
GAGs	GAGs influence protease activation by disrupting propeptide-enzyme interactions or altering protease conformation	All families	Depending on the type of GAG and the specific MMP, GAGs can either inhibit or enhance the activity of MMPs [133]. The facilitation of autocatalytic activation of cysteine cathepsins is influenced by various GAGs and other negatively charged surfaces [134–136]
Posttranslational variations of proteolytic activity	Glycosylation	All families	*N*- and *O*-linked glycosylation can modulate the catalytic activity of MMPs, affecting their ability to cleave extracellular matrix (ECM) components [137]

regulates collagen degradation [152]. This mechanism allows for precise control over enzyme activity, making it crucial in drug design as it offers advantages such as improved specificity, reduced side effects, and the ability to target proteins considered undruggable through traditional orthosteric approaches [153].

Cell adhesion-related signaling

Beyond intrinsic regulatory mechanisms, ECM proteolysis is influenced by integrin- and cadherin-mediated signaling. Migrating cells coordinate adhesion and proteolysis through these pathways. Integrin clustering can induce uPA and MMP-9 expression via ERK1/2 signaling [154]. Integrins co-localize with proteases, modulate their signaling, and serve as scaffolds for uPA and plasminogen, amplifying ECM remodeling. For example, integrin α3β1 regulates BMP-1, MMP-3, and MMP-9 expression in keratinocytes; α2β1 integrin mediates MMP-14 activation in fibroblasts following MMP-2 activation; α5β3 is partially correlated to MMP-2 activation in pancreatic carcinoma [155,156]. In contrast, E-cadherin-mediated adhesion suppresses uPA and MMP-9 expression via PI3K-Akt and EGFR-MEK/ERK pathways. Loss of E-cadherin correlates with increased invasion, as shown in bronchial tumor cell models [157].

By maintaining zymogen expression and latency, cells ensure precise control over ECM remodeling, balancing tissue repair with the risk of pathological degradation. Dysregulation of these control systems underscores their therapeutic potential in diseases like cancer and fibrosis.

Outlook and future tools

There is substantial overlap in protease substrates and degradation products, as discussed throughout this review. Understanding the common and unique regulatory mechanisms governing ECM proteolysis is essential for advancing drug development, tissue engineering, and biomarker discovery. Emerging tools such as degradomics and computational approaches not only improve the identification of cleavage sites and activity signatures in complex biological systems [158,159], but also accelerate the entire research process by enabling high-throughput analysis, predictive modeling, and more targeted experimental design.

The cutting edge: uncovering the complexities of ECM proteolysis

ECM proteolysis is mediated by remodeling enzymes that function through two primary spatial modes: (a) secreted enzymes that act within the extracellular space and (b) cell surface-associated enzymes that facilitate pericellular proteolysis [12].

Proteolytic enzymes, including MMPs, ADAMTs, serine, and cysteine proteases, degrade core matrix proteins and can release embedded cytokines, chemokines, and growth factors. Due to extensive substrate redundancy, ECM degradation by these enzymes facilitates physiological processes such as tissue repair, angiogenesis, and development. In a pathological context, however, excessive proteolysis contributes to conditions such as cancer, arthritis, and aortic aneurysms [11,19], Fig. 3. The release and activation of growth factors (e.g., TGF-β and FGF) and other bioactive molecules further support critical cellular processes such as proliferation, migration, and differentiation. Notably, membrane-bound proteases such as MMP-14, ADAM-12, ADAM-15, and PRSS21, alongside secreted pericellular proteases like MMP-2, MMP-9, and uPA, are uniquely adapted to function in the immediate cellular environment. These enzymes not only degrade ECM molecules but also regulate local signaling by shedding membrane proteins' ectodomains, a process beyond the scope of this review but discussed in depth elsewhere [12,87,160–162]. Through modulating growth factor receptors, adhesion molecules, and chemokines, pericellular proteolysis orchestrates cell adhesion, migration, and signal transduction. This spatially confined degradation is essential in angiogenesis, tumor invasion, wound healing, and immune cell functions and is often localized to dynamic cellular protrusions such as invadopodia and podosomes, which concentrate proteolytic activity at the leading edge of migrating cells [162,163].

Proteolytic cascades amplify these processes by sequentially activating additional proteases, thereby broadening both the substrate range and intensity of ECM degradation. For example, plasmin, generated from plasminogen by tPA or uPA, degrades Fn, laminin, aggrecan, and decorin, and activates MMP-1, -2, -3, and -9, amplifying matrix degradation spatially and quantitatively. Similarly, matriptase, upregulated in osteoarthritis (OA), activates MMP-1 and -3, promoting cartilage degradation [164]. Hepsin, another type II transmembrane serine protease, also activates MMP-1 and -3 and contributes to the breakdown of collagen and aggrecan in OA [165]. Neutrophil elastase, from immune cells, further drives collagenolysis in OA cartilage activating MMP-13 [166]. These cascades demonstrate that ECM degradation can be driven by multiple, spatially and functionally distinct proteases from both tissue resident and infiltrating cells.

A novel mechanism of ECM remodeling involves extracellular vesicles (EVs)—membrane-bound particles

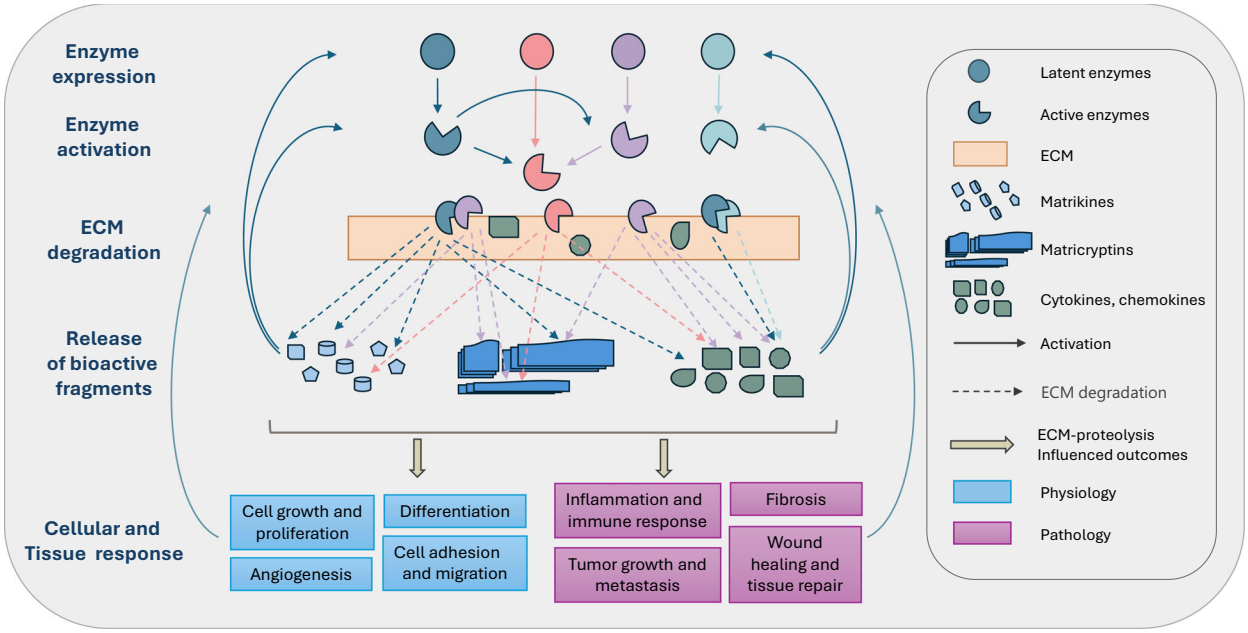

Fig. 3. ECM degradation by proteolytic enzymes and its effects on cell behavior. In response to various stimuli (see main text), latent proteases embedded within the ECM can become activated. Once active, these proteases may initiate proteolytic cascades that amplify both the number of active ECM-degrading proteases and the extent of ECM breakdown. Many ECM-degrading proteases exhibit functional redundancy, targeting both overlapping and distinct ECM components. This coordinated proteolysis results in the release of increased amounts of bioactive fragments including matrikines, cytokines, and chemokines—some functionally redundant, others unique. These factors can further modulate the expression and activation of additional ECM-degrading enzymes, creating a feed-forward loop that accelerates matrix remodeling. Simultaneously, the released factors and newly exposed matricryptins influence cellular behavior, affecting tissue homeostasis, repair responses, and progression of various pathologies. Notably, in pathological conditions this process can become self-perpetuating, as disease-associated signals drive further protease expression and activation, sustaining a chronic cycle of ECM degradation and dysfunctional tissue remodeling.

functioning as mediators of intercellular communication with significant roles in maintaining homeostasis and pathological progression. EVs can carry active proteolytic enzymes including MMPs, serine proteases, ADAMs, and ADAMTSs, enabling localized ECM remodeling in diverse settings such as tissue repair, cancer, cardiovascular disease, and infection [167–171]. Tumor-derived EVs, for instance, promote invasion and migration by delivering proteases to the ECM or to recipient stromal cells, stimulating additional protease production. Melanoma-derived exosomes enriched in MMP-2 and MMP-9 induce a pro-invasive fibroblast phenotype [172], while cancer-associated fibroblast-derived exosomes low in miR-29c-3p upregulate MMP-2 in ovarian cancer, promoting metastasis [173]. Furthermore, EVs' cargo contributes to angiogenesis by modulating diverse signaling pathways across pathological contexts [174–177]. Although much of the current evidence for EV-associated proteases derives from *in vitro* models, growing *in vivo* data supports their relevance, underscoring the need for further investigation into EV-mediated proteolysis.

Breaking boundaries: the cellular consequences of irreversible ECM proteolysis

Decoding matrikines: the ECM's bioactive language

Proteolytic cleavage by ECM-remodeling enzymes generates matrikines, which can be classified as either soluble bioactive molecules released during ECM protein degradation or as matricryptins—latent sequences within ECM proteins that become exposed and active only after protease-mediated cleavage. Once released, both types of matrikines can exert diverse biological effects such as cell signaling, gene expression, and enzymatic activity [30,178]. As collagen is the most abundant ECM protein in mammals, it serves as the primary source for the generation of matrikines. Matrikines derived from the non-collagenous domains of type IV collagen from BM include arresten, canstatin, and tumstatin (from α1, α2, and α3 chains, respectively). More intriguingly, these matrikines are not specific to a single

type of protease and can be generated by the activity of multiple proteases. Arresten and canstatin are generated by MMP-3, -9, and -15, with MMP-2 contributing specifically to arresten production [179,180]. In contrast, tumstatin is specifically released by MMP-9 cleavage [181,182]. This redundancy amplifies the production of matrikines within tissues, enhancing their regulatory impact on inhibition of angiogenesis and tumor progression. Despite their shared functions, these matrikines engage different mechanisms: arresten binds integrin α1β1, suppressing endothelial cell adhesion and migration and promoting cell–cell contacts [182]; canstatin induces mitochondrial apoptosis through αvβ3 and αvβ5 integrins [183]; tumstatin also targets αvβ3 integrin, inhibiting pro-survival signaling via the FAK/-PI3K/Akt/mTOR pathway. Internalization of all three occurs via clathrin-mediated endocytosis, representing a protective mechanism against pathological angiogenesis and tumor progression and offering insights into both cancer biology and potential therapeutic approaches in preventing pathological ECM remodeling. Similarly, endorepellin, a C-terminal fragment of perlecan, may be produced by BMP-1, chymases, and tryptases, and exerts antiangiogenic effects by interacting with receptors like α2β1 integrin and VEGFR2.

Conversely, matrikines from IM collagen types I, II, III, and V tend to promote fibrosis and inflammation. Examples include the tripeptide Pro-Gly-Pro (PGP), procollagen type I carboxy-terminal propeptide PICP, and the N-terminal telopeptide of collagen II, which stimulate endothelial cell migration and increase cathepsin B, K, and L expression in chondrocytes [180]. Notably, PGP can also be generated from BM collagen IV by various proteases. While IM fibrillar collagens are mainly degraded by MMPs, BM collagen IV is processed by enzymes like neutrophil elastase and cathepsin K. Despite their different origins, PGP consistently acts as a chemotactic matrikine, regulating neutrophil recruitment and inflammation.

In addition, a macrophage-collagen fragment axis mediates adipose tissue remodeling in mice. In obesity, this mechanism fails, leading to collagen fragment accumulation and promoting macrophage proliferation and fibroinflammatory responses in fibroblasts. This suggests that both collagen-degrading macrophages and the fragments they generate could be therapeutic targets for conditions like type-2 diabetes [184].

When an ECM protein is degraded by various proteases, the resulting matrikines may exhibit similar or distinct functions. For instance, the proteolytic cleavage of Fn by MMPs and uPA generates fragments with distinct functions. Certain Fn fragments, such as those containing the RGD motif—a key recognition site for integrins—promote cell migration by interacting with integrins like α5β1. Conversely, a 29-kDa Fn fragment exhibits pro-inflammatory activity, upregulating cytokines (e.g., IL-1β, TNFα) as well as inflammatory enzymes [185]. On the other hand, neutrophil elastase and MMP-12 cleave elastin, generating elastokines with distinct sequences but similar functions, including the regulation of processes such as inflammation, angiogenesis, cell migration, and tissue repair.

Finally, there is a feedback loop whereby matrikines regulate protease expression, further amplifying ECM remodeling. In submandibular gland morphogenesis, MT2-MMP-dependent release of collagen IV NC1 domains (non-collagenous region located at the C terminus of collagen IV) was shown to promote branching morphogenesis. Recombinant NC1 domains restored morphogenesis after MT2-MMP silencing, increasing expression of MT-MMPs and proliferative genes via β1 integrin and PI3K-AKT signaling [186].

These examples highlight three key points: (a) Matrikine generation is largely nonspecific to a single enzyme, as multiple enzymes can produce the same matrikines; (b) conversely, a single ECM-remodeling enzyme can generate different matrikines depending on the context and the substrate it cleaves; and (c) matrikines from distinct ECM sources can perform similar functions through distinct pathways. Numerous studies have demonstrated that matrikines influence cell behavior by engaging ECM receptors, growth factors, and cytokine pathways, making them valuable in drug development [28,178]. Additionally, a recent study developed a comprehensive pipeline for discovering and characterizing matrikines for skin rejuvenation, combining *in silico* peptide cleavage prediction, *in vitro* testing on human dermal fibroblasts, and *in vivo* clinical studies. This approach led to the identification of ECM peptide mimics, which were found to rejuvenate photoaged skin [30]. Additionally, advancements in the application of MMP-sensitive peptides have introduced novel strategies for managing OA and rheumatoid arthritis, focusing on MMP activity modulation [187].

ECM's secret arsenal: cryptic sites and matricryptin diversity

MMPs and other proteases can expose cryptic sites within ECM proteins via proteolytic cleavage. In addition to enzymatic activity, mechanical forces can expose cryptic sites within ECM proteins, although this is beyond the scope of this chapter [188]. Proteases such as MMPs and elastases selectively expose matricryptins within ECM proteins during tissue remodeling or diseases like tumor growth, angiogenesis, and viral infection

progression, underscoring their critical role in regulating biological activity. For example, MMP-2 cleavage of laminin-5 exposes a cryptic site that promotes cell motility; MMP-9 and -2 cleave collagen IV to reveal a hidden epitope (HUIV26 epitope) that is crucial for angiogenesis due to switching integrin binding from α1β1 to αvβ3, facilitating endothelial cell migration and tumor growth [189]. Cryptic sites are often exposed by multiple proteases. For example, collagen type I contains cryptic sites that are hidden within its triple-helical structure and become exposed through proteolytic cleavage or mechanical stress. These cryptic sites are not identical, as their exposure depends on the specific protease involved and the cleavage context. MMP-1, a collagenase able to degrade fibrillar collagens, cleaves collagen type I at specific sites within the α1(I) and α2(I) chains, generating fragments that expose cryptic binding sites, such as Fn-binding domains [190]. MMP-2 and -9 further degrade collagen-specific fragments [191], revealing additional cryptic sites that regulate cell adhesion and migration [192]. Remarkably, proteases from other families degrade collagen differently, releasing different cryptic motifs with different functions. For example, Cathepsin K degrades collagen type I during bone resorption, exposing cryptic integrin-binding sites that facilitate osteoclast adhesion [192]. In contrast, meprins (α and β) cleave procollagen I at unique sites within the C-terminal and N-terminal propeptides, releasing mature collagen molecules that expose cryptic binding motifs that affect collagen organization and contribute to the integrity of connective tissue in the skin [110]. The biological activity of Fn matricryptins depends on exposed cryptic sites (specifically, the RGD motif in type III repeats). Proteolysis exposes these sites, enabling unique interactions with integrins or other receptors that are not accessible in full-length Fn [193,194]. Thus, the sequence and functional properties of cryptic sites depend on the specific acting protease and the cleavage location. This diversity allows cryptic sites to serve distinct roles in processes like cell adhesion, migration, angiogenesis, tissue repair, and bone remodeling.

The recognition and functional characterization of protease-exposed cryptic sites has advanced significantly, thanks to improved computational predictions and experimental validation techniques [195–197]. Interest in these sites continues to grow due to their therapeutic potential and their role in complex biological processes. Materials that mimic the dynamic interplay between cells and their environment by incorporating cryptic sites could harness this endogenous signaling mechanism in synthetic ECM hydrogels. Recently, researchers developed synthetic ECM hydrogels with depsipeptides ("switch peptides"), which can undergo trypsin-triggered changes in their primary sequence, rearranging it into the bioadhesive form capable of supporting endothelial cell growth [198].

Protease-driven awakening: unlocking cytokine and growth factor function

ECM-remodeling enzymes modulate immune responses and tissue repair by activating cytokines, chemokines, and growth factors through cleavage of precursor forms, or binding proteins [84,199]. A well-characterized example is TGF-β, which is stored in the ECM or tethered to the cell surface. Although non-proteolytic mechanisms also exist [200], this section focuses exclusively on protease-mediated activation. TGF-β is secreted in a latent inactive complex consisting of the latency-associated peptide (LAP) and the latent TGF-β-binding protein (LTBP). This complex anchors TGF-β to ECM proteins such as Fn and fibrillins until activated [201–203]. Of the three primary isoforms (TGF-β1, -β2, -β3), all are encoded by separate genes and bind ECM via LTBP, though they differ in ECM residence time, activation pathways, and tissue roles [204,205]. TGF-β1 is the most extensively studied isoform and can be proteolytically activated by MMP-2, -9, and plasmin [206]. Thrombospondin-1 (TSP-1) selectively activates TGF-β2 via its KRFK motif, which is absent in TSP-2 [207]. Interestingly, MMP-9 preferentially activates latent TGF-β2 and TGF-β3 at the cell surface [208]. Additionally, other MMPs, such as MMP-13 and MMP-14, contribute to the activation of TGF-β isoforms, either by directly cleaving LTBP and LAP or indirectly through the generation of reactive oxygen species (ROS).

MMP-mediated TGF-β activation is observed in various processes: liver wound healing, fibrotic diseases, and cancer [203,209–211]. Despite extensive research, the contribution of ECM-bound TGF-β versus cell surface tethered pools remains unclear. Only a few studies have directly addressed the effects of ECM-stored TGF-β activation [212,213], and often, the cytokine tethered to the cell surface or both pools appears to contribute in a context-dependent manner.

TGF-β signaling and ECM remodeling form a two-way regulatory loop. Activated TGF-β promotes fibroblast and myofibroblast differentiation through SMAD signaling, stimulating secretion of MMPs and TIMPs [202]. This loop is critical in tissue homeostasis but, when dysregulated, contributes to fibrosis. In the tumor microenvironment, abundant active TGF-β1 upregulates MMPs and uPA/uPAR expression, further amplifying its own activation and promoting epithelial–mesenchymal transition (EMT) [214].

Beyond MMPs, several other proteases can activate TGF-β by cleaving LAPs or indirectly through MMP activation. These include fibroblast activation protein (FAP), neutrophil elastase (NE), proteinase 3 (PR3), plasmin, and kallikreins (KLK-12, -14, -15). However, the *in vivo* specificity of these proteases remains to be fully established. Recent findings suggest that the biological outcomes of TGF-β activation depend on the protease involved and the surrounding microenvironment. MMP-2, -9-mediated activation is linked to wound healing and fibrosis, whereas plasmin-mediated activation aligns more with inflammation. Cathepsin-mediated TGF-β activation has been associated with tumor progression. These examples highlight the spatial and temporal specificity of protease action in TGF-β release and function.

In immune cell-rich environments, the release of growth factors and cytokines through ECM degradation is more pronounced, as immune cells serve as major sources of ECM-degrading enzymes. Neutrophils are key sources of PR3, NE, and cathepsin G, which cleave ECM-bound IL-1β and progranulin, thereby increasing cytokine bioavailability [215]. HPSE, expressed by T cells and other immune cells, degrades HSPGs in both the ECM and on cell surfaces [216]. This releases several key signaling molecules such as (a) FGF-2, promoting angiogenesis and tissue repair; (b) VEGF, essential for angiogenesis; (c) TGF-β, regulating differentiation and immune balance; (d) EGF, enhancing proliferation and survival [216,217]. Notably, HPSE-induced FGF-2 release can be further amplified by MMPs and plasmin, which cleave the HSPG core proteins [202], thereby boosting tumor growth and EMT. The ECM, in turn, modulates immune responses during inflammation; enzymatic modification of the ECM liberates cytokines that influence leukocyte behavior. These examples highlight the bidirectional relationship between ECM remodeling and immune function.

ECM stiffness is another important factor regulating proteolytic activity. Increased matrix stiffness can reduce MMP accessibility and alter their conformation, hindering effective ECM degradation [218,219]. At the same time, stiff matrices promote cell contractility, which increases TIMP expression and reduces the proteolytic activation of growth factors such as TGF-β by fibroblasts [220].

Matrix mechanics: stiffness, pores, and patterns in action

Stiffness, porosity, and topography are key determinants of ECM biomechanics, shaped by degrading and cross-linking enzymes, as discussed in detail elsewhere [11,20,221,222]. Here, we focus on biomechanical changes induced by proteolytic enzymes, specifically matrix softening through collagen degradation, which is a key factor in various pathologies [191,223–225]. ECM softening disrupts tissue homeostasis by weakening structural support and altering cell signaling, which impairs cell adhesion, migration, proliferation, and repair [191,221]. Excessive ECM softening is linked to OA and Alzheimer's disease through loss of tissue integrity and disrupted signaling [226,227]. In cardiovascular diseases, MMPs, cathepsins, serine, and cysteine proteases degrade vascular ECM, weakening vessel walls and promoting aneurysm and atherosclerosis [228,229]. In cancer, softer ECM enhances glycolytic metabolism (Warburg effect) and cancer cell proliferation via pathways like YAP activation. It facilitates metastasis and immune cell infiltration by degrading structural barriers, though excessive softening may also reawaken dormant cells, paradoxically heightening metastatic risk [218,230,231].

Notably, in response to such changes, cells secrete additional remodeling enzymes, creating a feedback loop that perpetuates changes in the tissue architecture. For example, in collagen-rich tumor environments, activated fibroblasts and tumor cells upregulate MMPs (particularly MMP-14), facilitating cancer invasion and wound repair by degrading structural barriers [232,233]. Moreover, exosomes enriched with MMPs support the maturation of invadopodia and amplify ECM degradation [72,234,235].

Porosity, another key determinant of ECM biomechanics, is defined by pore size and density, influencing how cells move, proliferate, and interact with their environment. MMP-2 and -9 degrade collagen IV in BMs, increasing porosity and facilitating immune cell infiltration as well as cancer cell invasion and proliferation by providing less physical resistance and more space for movement [224,236]. In the IM, cathepsins B and K, together with MMPs, further enhance ECM degradation and porosity. For example, cathepsin B activates pro-uPA to uPA, which then activates plasmin and MMPs, amplifying ECM remodeling in a proteolytic cascade [237–239]. Increased porosity thus promotes cell migration by lowering mechanical constraints. Conversely, in the absence of proteolytic activity, dense, linearized collagen and fibronectin in the tumor stroma prevent T-cell infiltration, which is associated with poor patient survival [240].

Topography is a third parameter that defines tissue mechanics. When proteolytic enzymes degrade ECM components such as collagen, they generate new surface features—like grooves, disturbed fibers, or fragmented structures—that alter the topographical cues sensed by

cells [11,191,225]. These changes can modulate cell identity and gene expression, influence cell adhesion and morphology, affect cell migration and invasion, and regulate signaling pathways [191,225,241,242]. Importantly, the biomechanical properties of the ECM are highly dependent on the specific type and activity of the degrading enzyme, resulting in distinct cellular behaviors and impacting tissue homeostasis, development, and disease progression.

Summary

ECM proteolysis is fundamental for shaping tissue architecture, regulating biochemical signaling, and mediating immune interactions. Recent advances in experimental and computational methods have greatly improved our ability to study proteolytic processes, offering unprecedented resolution for dissecting protease activity, substrate specificity, and their biological outcomes in health and disease [30,243–249].

Protease families–such as MMPs, ADAM/ADAMTS, serine, and cysteine proteases–share overlapping substrates and generate diverse protein forms through cleavage, yet their functions are highly cell- and context-specific. This specificity is governed by transcriptional programs, microenvironmental cues, and subcellular localization. For instance, osteoclasts secrete cathepsin K for bone resorption, while tumor-associated fibroblasts produce MMP-14 to degrade collagen in desmoplastic tumors. On the other hand, the same protease can have different roles depending on its cellular source. For example, neutrophil-derived MMP-9 promotes tumor angiogenesis, whereas macrophage-derived MMP-9 supports tissue repair. Overall, precise spatial and temporal regulation of proteolysis is essential for normal development, wound healing, and immune responses.

During pathological remodeling, the co-expression and, more importantly, the proteolytic activation of multiple proteases—along with the degradation of diverse substrates, which is accompanied by the exposure of new cryptic sites and the release of various soluble bioactive molecules—provide multiple potential avenues for therapeutic intervention (Fig. 3). While early approaches using broad-spectrum protease inhibitors have largely failed due to off-target effects, recent strategies focus on selective targeting with therapeutic antibodies, small molecules, peptides, and CAR-T cells [14,250–256]. Combination therapies that simultaneously target multiple proteases, their substrates [257], or their resulting degradation products may be required to effectively halt disease progression, as indicated in Fig. 3. Additionally, combining substrate inhibition with protease inhibitors may provide a more comprehensive blockade of pathological ECM degradation.

Beyond inhibition, the broad substrate specificity of individual proteases can be harnessed therapeutically. For example, administration of collagenase-1 in mouse uteri enhances embryo implantation by remodeling ECM proteins and releasing matrix-bound VEGF, thereby promoting angiogenesis and immune cell infiltration [225]. Similarly, proteolytic nanoparticles have been applied to targeted periodontal remodeling, reducing tissue damage and accelerating recovery [258]. Novel technologies now exploit cryptic ECM sites exposed by proteases, such as synthetic hydrogels with switch peptides that undergo protease-triggered rearrangements to reveal bioadhesive sequences supporting endothelial cell growth [198]. Protease-sensitive hydrogels are also engineered to release growth factors during wound healing and cardiac repair, or for controlled drug delivery in response to local enzymatic activity, mimicking native tissue dynamics [259,260]. Matrikine-derived peptides are under investigation as therapeutic agents for skin aging and degenerative joint diseases, with accumulating evidence supporting their roles in tissue repair, anti-inflammatory effects, and regeneration [30]. Recent bioengineering efforts leverage these insights to construct ECM-mimetic systems enabling controlled, protease-mediated cytokine release. For instance, heparin/HSPG mimetics can bind and release FGF-2 or VEGF upon degradation by MMPs or plasmin, enhancing dermal healing in diabetic models. Similarly, MMP-cleavable hydrogels delivering TGF-β, IGF-1, or SDF-1α have shown promise in cardiac repair by promoting stem cell recruitment following infarction [261]. In solid tumors, strategies aimed at degrading dense ECM barriers or targeting protease-expressing cells are advancing cancer immunotherapy by improving immune cell infiltration and treatment efficacy. Additionally, inactivated proteases, such as MMP-1 decoys functionalized with gold nanorods, have been developed for advanced targeted photoacoustic imaging of fibrillar collagen in desmoplastic tumors. This innovative approach enables sensitive, noninvasive diagnostics and risk stratification by specifically visualizing tumor-associated collagen [262]. Collectively, these examples underscore the growing importance of understanding ECM proteolysis and its multifaceted impact on cell behavior.

Future directions for the field include mapping tissue-specific protease activities, developing biosensors and imaging systems for real-time monitoring of proteolysis, engineering ECM-targeted therapeutics with spatiotemporal precision, and creating disease-specific ECM atlases to guide personalized medicine.

In conclusion, ECM proteolysis represents a central and irreversible mechanism in tissue remodeling, offering unique diagnostic and therapeutic opportunities across oncology, regenerative medicine, and immunotherapy. Continued innovation, transdisciplinary collaboration, and the integration of emerging technologies—including, but not limited to, artificial intelligence—will be essential to fully harness the clinical potential of these advances.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

ISa, ISo, and OK conceptualized and wrote the review.

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