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A GUIDE TO...

A guide to the composition and functions of the extracellular matrix

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Extracellular matrix (ECM) is a dynamic 3-dimensional network of macromolecules that provides structural support for the cells and tissues. Accumulated knowledge clearly demonstrated over the last decade that ECM plays key regulatory roles since it orchestrates cell signaling, functions, properties and morphology. Extracellularly secreted as well as cell-bound factors are among the major members of the ECM family. Proteins/glycoproteins, such as collagens, elastin, laminins and tenascins, proteoglycans and glycosaminoglycans, hyaluronan, and their cell receptors such as CD44

Abbreviations
ADAM, a disintegrin and metalloproteinase; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; AML, acute myeloid leukemia; BM, basement membrane; CAF, cancer-associated fibroblast; CD44, cluster of differentiation 44; CNS, central nervous system; CR-CSCs, colorectal cancer stem cells; CS, chondroitin sulfate; DDRs, discoidin domain receptors; DES, desmosine; DS, dermatan sulfate; EBP, elastin-binding protein; ECM, extracellular matrix; EDPs, elastin-derived peptides; EGFR, epidermal growth factor receptor; EMILINs, elastin microfibril interfacers; EMT, epithelial-to-mesenchymal transition; ERC, elastin receptor complex; ERM, ezrin-radixin-moesin; FACITs, fibril-associated collagens with interrupted triple helices; FGFR, fibroblast growth factor receptor; GAG, glycosaminoglycan; GFs, growth factors; GPC, glypican; GPI, glycosylphosphatidylinositol; HA, hyaluronan; HAS, hyaluronan synthase; HB-EGF, heparin-binding EGF; Hep, heparin; Hh, Hedgehog; HPSE, heparanase; HS, heparan sulfate; Hyal, hyaluronidase; IGFIR, insulin-like growth factor receptor I; LacCer, lactosylceramide; LOX, lysyl oxidase; LRP, lipoprotein receptor-related protein; MACITs, membrane-associated collagens with interrupted triple helices; MET, mesenchymal-to-epithelial transition; MMPs, matrix metalloproteinases; Neu-1, neuraminidase-1; OPN, osteopontin; PEGF, pigment epithelium-derived factor; PG, proteoglycan; RHAMM, receptor for hyaluronan-mediated motility; RIP, regulated intramembrane proteolysis; ROS, reactive oxygen species; RTK, receptor tyrosine kinase; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; SLRPs, small leucine-rich proteoglycans; SRGN, serglycin; TE, tropoelastin; TIMP, tissue inhibitor of metalloproteinases; TLR, toll-like receptor; TMEM, transmembrane protein; TN, tenascin; TSP, thrombospondin; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor.

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and integrins, responsible for cell adhesion, comprise a well-organized functional network with significant roles in health and disease. On the other hand, enzymes such as matrix metalloproteinases and specific glycosidases including heparanase and hyaluronidases contribute to matrix remodeling and affect human health. Several cell processes and functions, among them cell proliferation and survival, migration, differentiation, autophagy, angiogenesis, and immunity regulation are affected by certain matrix components. Structural alterations have been also well associated with disease progression. This guide on the composition and functions of the ECM gives a broad overview of the matrisome, the major ECM macromolecules, and their interaction networks within the ECM and with the cell surface, summarizes their main structural features and their roles in tissue organization and cell functions, and emphasizes the importance of specific ECM constituents in disease development and progression as well as the advances in molecular targeting of ECM to design new therapeutic strategies.

The extracellular matrix architecture

ECM main structural and functional components at a glance

Extracellular matrices (ECMs) are multiplicate well-organized 3-dimensional architectural networks with critical structural and functional roles in tissue organization and remodeling and in the regulation of cellular processes [1,2]. The building blocks of these ultrastructures are the collagens, proteoglycans (PGs) and glycosaminoglycans (GAGs), elastin and elastic fibers, laminins, fibronectin, and other proteins/glycoproteins such as matricellular proteins [3,4]. ECMs operate as communication liaisons between the cells in organs and tissues by coordinating multiple signaling inside-out or outside-in commands [1,2]. As a consequence, ECMs guide tissue morphogenesis, development and homeostasis, through the regulation of cellular physiology, growth, survival, differentiation, and adhesion. ECMs undergo extensive remodeling during pathological conditions acting as key players driving disease progression [5–8]. Specific ECM phenotypes configure the different tissues (i.e., epithelial, nervous, muscle, and connective tissues) to meet the requirements for optimal tissue functions [8–13]. Nevertheless, the formulation of ECMs can constantly be adapted according to biochemical or mechanical signals, resulting in a fine-tuned vivid ECM remodeling procedure [14]. Schematic representations of the main ECM networks and their composition in the various tissues are given in Fig. 1.

A short introduction to each one of the main macromolecular components that construct the core of the ECM networks is given below.

Proteoglycans consist of a protein core decorated with negatively charged GAGs including heparan sulfate (HS), heparin (Hep), chondroitin sulfate (CS), keratan sulfate (KS), and dermatan sulfate (DS) [15]. PGs hold both structural and biological roles, as they are responsible for the mechanical resistance to compression and hydration of the tissues and also serve to trap growth factors (GFs) in the ECM [15]. PGs are divided into four groups depending on their localization and homology. Extracellularly secreted PGs include the hyaluronan (HA) and lectin-binding PGs, know as hyalectans, and the small leucine-rich PGs (SLRPs). SLRPs control the tissue spatial properties in development and homeostasis since they immobilize GFs in the ECM, and also regulate collagen fibrillogenesis [16]. The hyalectan versican can, in addition to its binding to HA, can also regulate numerous signaling pathways and cellular functions [17]. Pericellular PGs, perlecan and agrin, interact with various cellular receptors and can modulate the cardiovascular and musculoskeletal systems [18]. The multiplexin collagens XVIII and XV, which bear GAG chains, also belong to the pericellular PGs and are expressed in all vascular tissues and basement membranes (BMs) [16]. Proteolytic degradation of the protein core of perlecan by chymase, matrix metalloproteinases (MMPs) and cathepsins can generate multiple fragments, with the most important being endorepellin, which has an anti-angiogenic activity [19]. Further cleavage of endorepellin by proteases and cathepsin L releases a laminin G-like domain which binds α2β1 integrin [20]. Endostatin, an anti-angiogenic and antitumoral fragment, is released from collagen XVII by several proteases including cathepsin L and elastase [21]. Cell surface

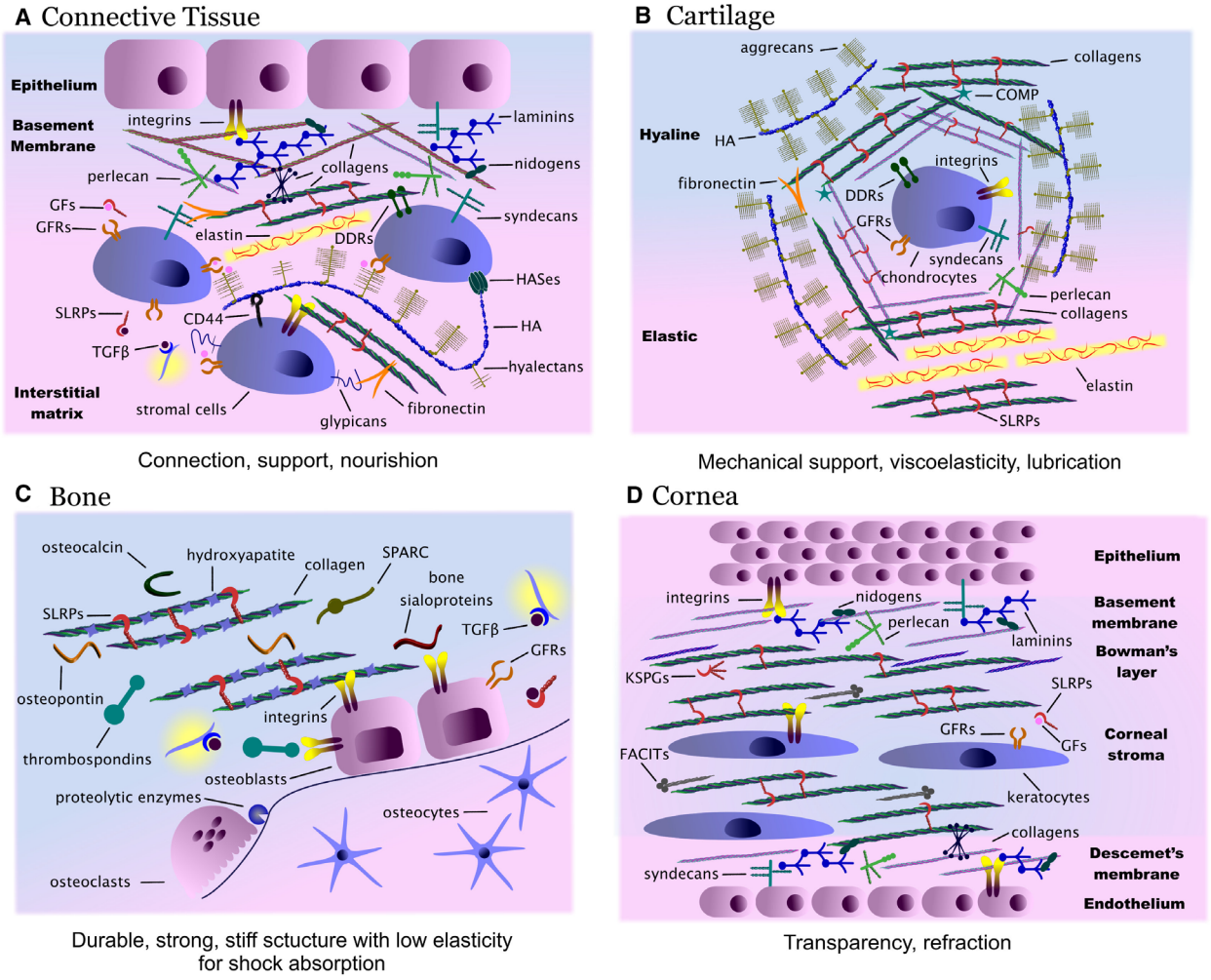

Fig. 1. Schematic representation of the composition and structure of ECMs loose connective tissue (A), and specialized connective tissues such as cartilage (B), bone (C) and cornea (D). (A) Epithelial cells create an adhere line to define a barrier in which tissue cells can prosper and function. BM is an underlying matrix in contact with epithelial cells composing mainly from interconnected networks of collagens IV and laminins, nidogens and HSPGs such as perlecan. BM anchors underlying connective tissue with tethering collagens such as VI and VII. Collagen and elastin networks, PGs and glycoproteins such as fibronectin act as scaffolding and functional biomacromolecules communicating with cells via cell surface receptors such as integrins, DDRs and syndecans. HA is synthesized by HASes and interact with CD44 to regulate tissue repair and homeostasis. GFs are attached to various molecules which regulate their bioavailability and presentation to GF receptors. (B) Cartilage is a thin hydrated tissue with tensile strength. Chondrocytes are surrounded from a pericellular matrix abundant in collagen VI and perlecan. Hyaline cartilage matrix is composed mainly from collagen II and large aggregates of HA with aggrecans. SLRPs aid the inter connection of collagens as well as the assembly of aggrecans with collagens. Additionally, the presence of elastin defines the elastic cartilage providing tissue elasticity. (C) Bone is a specialized strong connective tissue with an ECM rich in mineralized collagen with hydroxyapatite. PGs and specialized bone proteins regulate collagen assembly, while bone sialoproteins, OPN and thrombospondins control bone metabolism and collagen mineralization. Cellular components control bone development and homeostasis through secretion of GFs and consists of bone-forming osteoblasts and bone-resorption osteoclasts, where mature bone tissue encloses osteocytes. (D) Cornea is a unique organ due to its transparency and refraction capacity achieved by the finely assembly of ECM components. Cornea is protected from an outer epithelium with its unique BM, which is in contact with Bowman's layer rich in KSPGs, collagen I and V. The underlying corneal stroma provides biomechanical stability and shape. Corneal fibroblasts (keratocytes) are embedded in an ultrastructure of collagens I and V, FACITS and SLRPs. An inner epithelium with a BM called Descemet's membrane nourishes and control the homeostasis of the corneal stroma. Both corneal BMs consist of the collagens IV and VII, laminins, nidogens and HSPGs.

PGs, syndecans and glypicans (GPCs), play a more direct role in signaling coordination, as they act as coreceptors via the binding of ligands [22,23]. For example, GPC-6 seems to be essential for the proper length of intestines during embryonic development as it regulates the bioavailability of Wnt5α and presents Patched1 to Hedgehog (Hh), thus controlling the two more crucial signaling cascades connected to this elongation process [24]. Serglycin (SRGN) was first discovered as an intracellular PG, but later it was found as a secreted complex in the matrix. SRGN participates in the storage and bioavailability of important molecules, and in plethora of functions such as maturation of granules and apoptosis of mast cells and immune regulation [25,26].

A GAG with distinct functions is HA. It is not linked to a protein core and it is not esterified with sulfate groups. HA contributes to water retention in tissues and to their structural integrity. It plays a pivotal role in embryogenesis and tissue repair, regeneration, and homeostasis. HA has a versatile function depending on its size, concentration, and interaction with cell receptors and its ECM binding partners. HA can regulate signaling in a context- and tissue-specific manner. HA synthesis occurs through specific hyaluronan synthases (HASes) and is controlled epigenetically at the post-transcriptional level [27,28]. HASes are also tissue-specific and can generate HA of different sizes. The catabolism of HA is controlled by hyaluronidases (HYALs; mainly HYAL1, HYAL2, PH20), reactive oxygen species (ROS), and nitric oxide synthase (NOS). HA interacts with several receptors such as cluster of differentiation 44 (CD44), HARE (also known as stabilin-2), LYVE-1, the receptor for hyaluronan-mediated motility (RHAMM), also known as CD168, and layilin, which can also bind other ECM molecules and activate function-targeted signaling pathways [29]. Interaction of HA with CD44 can generate receptor clustering, for instance with Toll-like receptor 4 (TLR4) [30]. HA binding to CD44 and RHAMM guide the muscle development via the regulation of migration and growth of myogenic progenitors [31]. Articular cartilage acquires an archetypal ECM rich in hydrated aggrecan-HA aggregates contributing to its architecture. HA aids aggrecans to interact with cell membrane, while concomitantly is connected to HAS or bound to CD44 [32].

Collagens are the main occupants, over 30%, of ECMs and especially the collagen type I, II, and III, which make up 80–90% of the overall body collagen. Collagens possess a characteristic triple-helix morphology consisting of homo- or hetero-trimeric α-chains. Collagens operate as supportive tissue material, while at the same time bringing elasticity and stability. The collagen family consists of 28 members and depending of their supramolecular structure and function are classified into several subfamilies [33–35]. Some types of collagens are found widespread in tissues, such as type I and III, which are often co-distributed, as well as types VIII and VI. Collagen IX is abundant in connective tissues and often is co-distributed with collagen II. Collagens such as XI, XXIV, XXVII, XII, XIV, XX, and X are present mainly in connective tissues such as tendons and cartilage. On the other hand, collagens XIII and XVII are found in epithelial tissues. Some of them, such as collagens IV, VII, XV, XVII, and XIX, are constituents of BM and collagen type XXVIII is more tissue-specific as it is present in the BM of glial cells in the peripheral nervous system. One collagen, type XXII, has a very specific localization, in myotendinous junctions [34].

Organs such as arteries, lungs, and skin are abundant in elastic fibers. In the circulatory system, the elastic property is crucial for the even blood flow and pressure produced by the heart. Tropoelastin (TE), which is secreted from specific elastogenic cells, creates the elastin meshwork via cross-linking upon a scaffold of fibrillins and other microfibrils proteins [36]. Fibrillins additionally aid binding proteins to harbor elastin, but also participate in cell signaling through interaction with syndecans and integrins, and the storage of transforming GF β (TGFβ) family of GFs in the matrix [37]. Elastin is shaped during development and childhood and gradually decomposes during adulthood and aging [38]. Upon proteolytic action of elastases, the elastin-derived peptides (EDPs) can manipulate signal transduction and as a consequence the physiologically maintenance of arteries and the prevention of skin photoaging, among other events [39].

Lysyl oxidase (LOX) and LOX-like (LOXL) proteins initiate the covalent cross-linking of TE and collagen fibrils, which stabilizes the corresponding networks [40]. Moreover, they can act as signaling manipulators due to the interaction with various GFs such as fibroblast GF 2 (FGF2) and TGFβ, or due to the oxidation of platelet-derived GF β (PDGFRβ), for example. LOX and LOXL participate in development, tissue repair, and remodeling and their expression levels are altered in pathological situations [40]. Their regulation can be controlled by ECM proteins, inhibitors and PGs. For instance, fibromodulin and syndecan-4 facilitate the interaction of LOX proteins with collagens [41]. Recently, thrombospondin-2 arose to be a modulator of skin elasticity, as its knockdown resulted in reduced collagen fibrillogenesis and LOX levels [42].

Another macromolecule forming supramolecular assemblies is fibronectin which regulates mechanical properties, such as tension due to conformational changes of its fibers; active-stretching versus relaxed fibronectin fibers. Fibronectin also interacts with integrins regulating cellular adhesion, as well as with GFs, cytokines, and ECM molecules [43,44].

The laminin family contains more than 16 members and each molecule consists of three chains: α, β, and γ. The distribution of laminins is tissue- and cell-specific, as laminin-111 can be found mainly in embryos and laminins 521 and 511 in adult tissues. Laminins 211 and 221 have a more specific distributions, and they are present in the BM of skeletal and cardiac muscles, whereas laminins 411 and 421 are found in the BM of endothelial cells and laminin-332 in the BM of the epithelium [45,46].

The tenascin (TN) family members belong to the group of matricellular proteins and comprise four members: TN-C, TN-R, TN-W, and TN-X. TNs include three different domains, EGF-like domains, fibronectin-type III domains and a fibrinogen-like globe. Many of these domains interact with several other ECM proteins, like collagens, fibronectin, fibrillins, PGs, GFs, chemokines, and other soluble factors. Moreover, TNs modulate cell adhesion through their interaction with integrins. They play important roles during embryonic development and pathogenesis but probably also in tissue homeostasis [47]. TN-C is involved in tissue morphogenesis, and its presence in adult tissues is limited to some stem cell niches, lymphoid organs, and tendons [48]. TN-C becomes re-expressed upon tissue injury serving as endogenous danger associated molecular pattern molecule (DAMP) orchestrating tissue repair but also promoting pathologies such as chronic inflammation, fibrosis, myocarditis, and cancer when TN-C expression remains high. TN-X is involved in organogenesis as it is ubiquitously expressed in late embryos [49]. TN-W is implicated in osteogenesis and is abundant in specific stem cell niches and dense connective tissues [50,51], while TN-R is expressed in the central nervous system (CNS) and is mostly connected to neurogenesis [52].

In the following parts of the article, a more detailed description of the types, structure variability, and main biological functions of the ECM components is presented. These parts involve the following: In the section of the ECM as tissue-distinctive functional meshwork, the structural organization and functions of BM and loose connective tissue, cartilage, bone, and cornea are presented. The section of the PGs as key players in ECM organization and cell properties includes the main types of the extracellularly secreted, the cell surface, the intracellular, and the BM/pericellular PGs with reference in their GAG moieties, the tissue distribution, the matrix phenotype as well as the biological role and prognostic value in malignancies. In following section, the matrisome, that is, the ECM databases and interaction networks, a modern field with significant impact to understand the matrix components interactomes is presented. The GAGs, the sweet regulatory partners of ECM, constitute the branches of the PG tree with enormous structural variability and distinct biological roles. Here emphasis is given to hyaluronan and particularly to its biosynthesis, degradation, and functions. The hyaluronan receptors, multifaceted cell-matrix interaction partners, are presented separately as they play key roles also triggering intracellular signaling. Particular emphasis is given in CD44 and its role in malignancies and human disease; the collagen family as the major structural components of the ECM networks with numerous functions and close association with human diseases as well as the main collagen rectors are presented and discussed. The next sections involve important types of ECM macromolecules that also play critical roles in structural architecture and function of ECM, and involve the elastic fibers that preserve tissue elasticity; the laminins as the three-armed ECM adhesion proteins; the integrins as adhesion and signaling mediators between ECM and cells, their ligands and activation as well as their roles in pathophysiology. The last part of this guide is dedicated to the physiological and pathological functions of the critical proteolytic and glycolytic enzymes implicated in tissue remodeling and human disease.

ECMs as tissue-distinctive functional meshworks

Structural organization and functions of basement membrane and connective tissue

Depending on the topographical position, ECMs are divided into (a) a pericellular matrix enhancing cellular attachment and (b) an interstitial matrix providing tissue integrity (Fig. 1A). The first one is a tightly organized network which is in contact with the cells creating cross-junctions via integrins, discoidin domain receptors (DDRs) and PGs such as syndecans [3]. A typical example of pericellular ECM is the BM, which is rich in laminin isoforms and collagen IV. Both of them create self-assembled networks that are connected through ninogens and the HSPGs, perlecan and agrin. These components create an adhesive microenvironment for the resident cells, providing support via

the tethering role of laminins with the cytoskeleton [8,53]. Laminins possess dual roles in scaffolding and in signaling. Their N-terminal domain interacts with several BM biomacromolecules, defining the architecture of BM. Their C-terminal domain binds to cell-surface receptors, such as α3β1, α6β1, α6β7, and α7β1 integrins, creating a link between the biochemical signals and the cells facilitating adhesion, migration, survival, differentiation, and apoptosis [54,55]. Integrins create a bridge between the outside ECM and the inside, in particular the cytoskeleton through which they affect cell signaling [56,57]. A recent study revealed that the cooperation of α3 and α6 integrin subunits is responsible for crucial functions such as adhesion, proliferation, and migration of kidney epithelial collecting duct cells [58]. The simultaneous deletion of these α subunits results in a bigger reduction of these properties and a greater impairment of signaling cascades and minimized cellular reaction to FGF-10 and glial cell line-derived neutrophilic factor, in contrast with the milder alterations observed upon the deletion of a single subunit [58]. BM also contains matricellular proteins such as secreted protein acidic and rich in cysteine, cartilage oligomeric matrix protein (COMP), thrombospondins (THBSs), TNs, pigment epithelium-derived factor (PEGF), and osteopontin (OPN) contributing to tissue-specific functions and play a role in tissue homeostasis.

Underlying the BM is the so-called interstitial connective tissue that encloses, separates, and supports tissues. Its ECM composition can be variable to create specific environments for distinct tissue function and loose, dense, and specialized connective tissues can be distinguished. Collagens, mainly type I, but also type III resist to tensional loads and elastin may further support additional tissue stretching. On the contrary PGs, also abundant in these ECMs, opposite compression forces. Moreover, specialized matrix components such as PGs and proteins boost the tissue mechanochemical properties [8]. Bone and cartilage consist of specialized ECM. Cartilage ECM is composed mainly of a viscous gel enriched by mostly avascular PG aggregates, while bones consist of rigid mineralized and vascularized ECM.

ECM composition in cartilage

The distribution, concentration, and ratio of the various ECM molecules, together with their post-translational modifications such as glycosylation and cross-linking, determine the unique biomechanophysical properties of distinct tissues (Fig. 1B). Cartilage, a specific type of connective tissue, depending on its composition can be divided in hyaline, elastic and fibrocartilage. Hyaline cartilage is a thin, translucent matrix found on rids, nose, larynx, and trachea, whereas articular hyaline cartilage can be found at diarthrodial joints. The main components of hyaline matrix are collagen II, SLRPs such as decorin, biglycan, and fibromodulin and aggrecans aggregates mediated by HA. The SLRP decorin, as expected from its engagement in collagen II fibrils alignment, is crucial for the assembly of aggrecans with collagen II [59]. In this way, decorin participates more directly to the tissue integrity and mechanical operation [60]. Elastic cartilage is found in the middle part of the ear and the external one as well as in the epiglottis. Its main role is the tissue shape maintenance and flexibility, due to the presence of elastic fibers. Collagens IX and XI are also found in these cartilages [61]. Chondrocytes are the responsible cells for the ECM homeostasis of these cartilages preserving the matrix compositions. Chondrocytes are in fiber-free and PG-rich cavities called lacunae and their function can be controlled by pro-inflammatory cytokines and GFs [62]. Nutrients are diffused to the majority of hyaline and elastic cartilage via the perichondrium surrounding cartilage or the synovial fluid in the case of articular hyaline cartilage. Perichondrium is a dense irregular collagenous protective connective tissue, rich in fibroblasts, chondrogenic cells and chondroblasts [63]. Recently, collagen III and collagen I emerged to be a crucial mediator of collagen II fibrillogenesis in articular cartilage and meniscus, respectively. Especially, the cross-linking of collagen III to collagen II seems to contribute to the mechanical resistance of collagen II fibrils and the integration of aggrecan-HA complexes within the collagen II network [64]. All types of cartilages have a low intrinsic regeneration capacity, due to the poor proliferation rate of chondrocytes. Changes occur in cartilage during aging. For example, old articular cartilage is less hydrated and contain less PGs aggregates, and undergoes a swift of GAG context to favor KS than CS and more HA fragments [65]. Fibrocartilage is found in menisci, intervertebral disk, tendons, ligaments and temporomandibular joint and consists of fibrocartilage cells in lacunae, and a dense fibrous matrix of collagen and elastic networks. This matrix type is composed of collagens I, II, III, V, XI, XVI, and XIX, fibril-associated collagens with interrupted triple helices (FACITs), the characteristic cartilage PGs, as well as versican and in addition TN-C [66]. MMPs of the family of collagenases, gelatinases, and stromelysins, cathepsin B and D as well as ADAMTSs are responsible for the cartilage turnover [65,66]. Bone and cartilage consist of ECM adapted to bear loads, although they differ in

their appearance and in vascularization, cartilage being avascular and bone mineralized and vascularized.

ECM in bone

Bone is also a type of connective tissue that is mainly composed of ECM, characterized by organic biomacromolecules and inorganic compounds (i.e., hydroxyapatite [$Ca_5(PO_4)_3OH$] and trace elements; Fig. 1C). Collagen I is the main collagen type found in bone but collagen III and V are also present and altogether act as a scaffold and contribute to the bone biomechanical properties. PGs such as decorin, biglycan, keratocan, and asporin maintain bone homeostasis through promotion of the collagen assembly [67]. Moreover, biglycan and CS chains retain water, which is essential for the proper bone toughness [68]. Bone glycoproteins include osteonectin, THBSs, and R-spondins. The first one affects the collagen mineralization, the second ones the differentiation of the cellular bone components and the regulation of bone metabolism and the third ones are regulators of Wnt/β-catenin pathway controlling bone development in embryos and bone remodeling in adults. Specialized proteins containing γ-carboxyglutamic acid such as osteocalcin, periostin and matrix Gla protein contribute to bone formation via regulation of mineralization and fibrillogenesis. SIBLINS, the small integrin binding ligands *N*-glycosylated proteins, include bone sialoprotein (BSP2) and OPN, which play a role in bone formation and mineralization, and also dentin matrix protein-1 (DMP1), dentin sialophosphoprotein (DSPP), and matrix extracellular phosphoglycoprotein (MEPE), which additionally conduce to phosphate metabolism [67,69]. Bone ECM is continuously changing for example due to aging and disease, and it seems like that several MMPs and cathepsin K oversee this remodeling [70]. For example, the cooperation of MMP-9 and MMP-14 secreted by osteoclasts is likely to play a crucial role in bone catabolism [71]. Bone development and homeostasis are controlled by TGF-β, BMP, Wnt, FGF, Hh, and PTHrP signaling [72]. TGFβ and BMPs are abundant in bone matrix, and their bioavailability and activity can be modulated by THBSs, SLRPs, and MMPs [67,69,70].

ECM organization in cornea

Cornea is another distinct tissue due to its opacity. Cornea consists of the outer epithelium and its BM, which is in contact with the Bowman's layer. The middle layer is the stroma and the inner the endothelium, separated by a BM, called Descemet's membrane (Fig. 1D). The epithelium is a multilayer of cells facilitating the flow of tears and acting as a barrier to external bacteria [73] and viruses [74]. The corneal epithelial BM is a very thin ECM allowing thereby continuous refraction and consists of collagens IV and VII, laminin-322, nidogens, and HSPGs, creating an adhesive microenvironment for the epithelium. The Bowman's layer is a noncellular dense collagens I and V, enriched with KSPGs [75]. The corneal stroma occupies 80% of the tissue thickness and is mainly responsible for its transparency. This connective tissue is rich in collagen I giving the cornea biomechanical stability and shape. Collagen fibril diameter is controlled by collagen V, which is present in nucleation sites of collagen fibrils, while FACITs regulate the inter lamellar interactions and lastly SLRPs synchronize the linear and lateral collagen assembly. Keratocytes are residents cells of the stroma and are responsible for the homeostasis of its components, either by synthesizing them or by controlling their proteolytic degradation due to MMP production [76,77]. Crystallins are also present in stroma ECM to reinforce refraction [78]. The Descemet's membrane consists of collagens IV and VIII, laminins 332, 411, and 511, perlecan, KSPGs, DSPGs, and nidogens [79]. Its thickness depends on the age and the composition of ECM molecules. Some biomolecules such as TN-C, fibrillin-1, and fibronectin-1 are reexpressed in pathological conditions [80]. Corneal endothelium supports homeostasis, hydration, and nutritional supply of the stroma [81]. The cornea is an avascular tissue, and this property is controlled by the expression of anti-angiogenic and the inhibition of pro-angiogenic molecules, including vascular endothelial GF (VEGF), thrombospondins 1–2, MMPs, bFGF, and PEGF [82].

The matrisome: ECM databases and interaction networks

The matrisome has been defined as the ensemble of genes encoding ECM and ECM-associated proteins [83,84]. Specific features of ECM proteins (signal peptide, presence of protein domains, motifs, or repeats) have been used to determine the matrisome of various species using automated machine learning-based algorithms [84]. The human matrisome comprises 1027 genes, encoded by 4% of the human genome, and the murine matrisome 1110 genes [84]. The matrisome of model organisms, namely zebrafish [85], *Caenorhabditis elegans* [86], *Drosophila melanogaster* [87], quail [88], and planarians [89] has been predicted too. The matrisome is divided into the core matrisome, comprised of collagens, glycoproteins, and matrisome-

associated proteins, which are categorized into ECM-affiliated proteins, secreted proteins (e.g., cytokines, TGFβ) and ECM regulators such as ECM-degrading and ECM cross-linking enzymes [84]. A machine learning model, ECMPride, has also been developed to predict ECM proteins [90].

The matrisome definition is very useful (a) to identify matrisome components in large transcriptomic, proteomic, and multi-omic datasets collected in normal and diseased cells and tissues, and to analyze ECM organization, functions, and remodeling in physiological processes (e.g., development and aging) and diseases using bioinformatic tools and dedicated databases [91], and (b) to design new therapeutic strategies [92]. The matrisome has been experimentally characterized by mass spectrometry in various healthy and diseased tissues including skin [93], the cerebrovascular system [94], normal and fibrotic human liver [95–97], and normal and fibrotic human lung [98]. The matrisome has also been investigated in numerous cancer types [99–103], leading to the definition of a tumor matrisome index measuring deregulated matrisome associated with tumor progression [104], to determine cancer-induced changes and cancer markers [105]. Proteomic data from 17 studies on the ECM of 15 normal tissue types, six cancer types, and other diseases including vascular defects and lung and liver fibrosis have been curated and compiled in MatrisomeDB, the ECM-protein knowledge database (http://www.pepchem.org/matrisomedb) [106]. Furthermore, the glycomes and glycoproteomes of the matrisome have been studied in order to understand how glycosylation is involved in matrisome functions with a focus on brain diseases [107]. The term 'matreotype' has been coined to describe 'the composition and modification of ECM or matrisome proteins associated with or caused by a phenotype, such as longevity, or a distinct and acute physiological state, as observed during aging or disease' [108].

High-throughput techniques have been developed to collect glycosaminoglycomic [109], proteoglycanomic [110], and ECM interactomic data [111]. The interaction networks formed *in vivo* between matrisome components are crucial to decipher the molecular mechanisms connecting and regulating ECM molecular functions and biological processes. Biomolecular interactions involving at least one matrisome component are available in the interaction database MatrixDB (http://matrixdb.univ-lyon1.fr) [112–114] and can be used to build interactomes of ECM proteins and their receptor such as integrins [115]. Interaction networks have been built for several matrisome proteins and PGs including procollagen C-proteinase enhancer-1 [116], thrombospondin-1 [117], LOX family [118], decorin [119] and syndecans [23] and also for matricryptins [120,121], and GAGs [122–124]. The matrisome definition has been used to categorize binding features of Hep partners [125] and will be crucial to determine how matrisome interaction networks are rewired in different tissues in physiological and pathological contexts.

Proteoglycans: key players in ECM organization and cell properties

Proteoglycans are ubiquitously expressed by all cell types and ECMs and can sometimes be a dominant component (i.e., in the vertebrate cartilage matrix) [15,16]. Given that PGs are present in multicellular animals and in all mammalian ECM phenotypes, it is not surprising that PGs possess an array of functions. PGs are rapidly emerging as dynamic modulators of normal states (i.e., ECM hydration, supramolecular assembly, homeostasis, development, wound healing, tissue repair, and senescence) and pathobiological conditions (i.e., inflammation, autophagy, fibrosis, osteoarthritis, atherosclerosis, and cancer) [126,127].

They do not only regulate matrix structural organization and mechanics but also act as integrators of major signaling cascades governing cell behavior (Table 1) [128,129]. Acting as coreceptors for GFs, facilitating chemokine signaling through G protein-coupled receptors, interacting with matrix remodeling enzymes and ECM effectors, PGs are attributed with peculiar features in cell behavior and signaling [130,131]. Integral cell properties including adhesion, migration, proliferation, angiogenesis and survival are closely correlated with PG expression, while altered PG expression and post-translational modifications in cancer cells and tumor stroma critically affects cancer progression and response to therapeutics [132,133].

Mammalian genome decoding has disclosed four major PG classes: extracellular, pericellular, cell surface associated, and intracellular. PG classification is based on their cellular and subcellular localization; however, they can be further classified in subcategories as reported by gene homology, modular arrangement, structural properties, and biological functions (Table 1) [16].

There is only one intracellular PG, SRGN, best known as hematopoietic cell granule PG. This unique PG carrying HS chains is packed in the granules of mast cells and participates in the formation of mast cell secretory granules and mediates storage of GFs and cytokines in secretory vesicles [26,134]. It is expressed in inflammatory, endothelial, smooth muscle

Table 1. Intracellular, extracellular, pericellular/BM and cell surface PGs: GAG moieties, tissue distribution, matrix phenotype, biological role and prognostic value in malignancies. Sources: [8,15,16,135,147]. PDZ, postsynaptic density 95/disk-large/zona occludens-1; PTN, pleiotrophin; TRPC7, transient receptor potential cation channel subfamily C member 7.

PG	GAG chains	Localization	Matrix phenotype	Interactions	Functions	P2rognostic marker in malignancies
Intracellular PGs						
SRGN	HS/CS/DS	Bone marrow; lung; blood cells; inflammatory cells; endothelial cells; smooth muscle tissue	Interstitial connective tissue	pro-MMP9/ MMP13; Ca^{2+}; chemokines; histamines; proteases	Secretion of inflammatory regulator; formation of mast cell secretory granules; storage of GFs & cytokines in secretory vesicles; tumor growth	Colorectal; breast; lung; prostate; liver; myeloma; lymphoma; thymoma; glioma
Extracellular PGs						
Versican	CS	Hippocampal formation of brain; adrenal gland; lung; gallbladder; urinary bladder; placenta; heart; smooth muscle tissue; adipose tissue; cecum; lymph node; bone marrow; blood	Interstitial connective tissue (fibrocartilage)	Ca^{2+}; HA; lectin; fibrillin	Cell motility; growth; differentiation; tissue morphogenesis & maintenance; heart/ cartilage/dermal development	Stomach; renal; melanoma; pancreatic; breast
Aggrecan	CS/KS	Cerebral brain cortex; heart; lung; seminal vesicles; cervix; soft tissue in chondrocytes	Interstitial connective tissue (hyaline cartilage); Soft connective tissue	HA; link protein	Cartilage integrity; neuron protection against oxidative stress; cell adhesion	Renal; breast; laryngeal squamous cell carcinoma
Brevican	CS	Cerebral brain cortex; adrenal gland; small intestine; male tissues; skin; blood cells	Interstitial connective tissue	HA; lectin	Formation of the brain ECM; Growth & motility of brain tumor cells	Glioma; melanoma
Neurocan	CS	Cerebral brain cortex; cerebellum	Interstitial connective tissue	Ca^{2+}; HA; NG-CAM; N-CAM	Neuronal adhesion; neurite growth during development; cell adhesion & migration	Glioma; breast
Decorin	CS/DS	Female & male tissues; lung; esophagus; adipose tissue	Interstitial connective tissue (hyaline cartilage); bone	EGFR; Met; VEGFR2	Growth inhibition & angiogenesis; endothelial cell autophagy; inflammation; tumor cell mitophagy	Renal; pancreatic; lung; breast; ovarian
Lumican	KS	Cornea; placenta; eye; lung	Interstitial connective tissue; cartilage	MMP14; integrins; collagen type I	Endothelial cell invasion; cell proliferation	Pancreatic; melanoma; breast; lung; colorectal; renal
Fibromodulin	KS	Skin; gallbladder; thyroid gland; heart; female & male tissues; pancreas	Interstitial connective tissue; cornea	Collagen type I & II; LOX	Collagen fibrillogenesis	Renal; urothelial; prostate
Biglycan	CS/DS	Skeletal & heart muscle; kidney;	Interstitial connective tissue; bone	Collagen type I & II	Bone & muscle regeneration; osteoblast	Pancreatic; renal; lung; breast

Table 1. (Continued).

PG	GAG chains	Localization	Matrix phenotype	Interactions	Functions	P2rognostic marker in malignancies
		lung; placenta; spleen			differentiation; inflammation; innate immunity	
SPOCK/testican	CS	Brain; lung; endocrine tissues	Interstitial connective tissue	Ca^{2+}	Neuronal function	Head & neck; renal; colorectal; ovarian; lung; stomach
Pericellular/BM PGs						
Perlecan	HS/CS	Endometrium; urinary bladder; adipose tissue; smooth muscle tissue	BM; cornea	VEGFR2; α2β1 integrin	Cell adhesion; articular cartilage formation; endochondral ossification; inflammation; endocytosis; peripheral node assembly; lipid catabolism; cardiovascular development; autophagic regulation	Breast; stomach; renal; thyroid; colorectal
Agrin	CS/HS	Kidney; gallbladder; skin	BM	Ca^{2+}; laminin	Postsynaptic differentiation; depolarization at CNS synapses	Liver; hepatocellular; thyroid; colorectal; ovarian
Collagen XV	CS/HS	Placenta; esophagus; adrenal gland; heart; adipose tissue	BM	Fibronectin; α1β1 integrin; DDR1; E-cadherin	Adhesion of the BM to underlying connective tissue	Colorectal; liver; urothelial; pancreatic
Collagen XVIII	HS	Liver; male tissues	BM	Zn	Inflammation; cell adhesion	Liver; endometrial; ovarian; testis
Cell surface PGs						
Syndecan-1	HS/CS	Breast; kidney; liver; skin	Interstitial connective tissue; cornea	HER2; α6β4, αvβ3, αvβ5 integrins	Angiogenesis; sound healing; cell invasion & survival	Lung; hepatocellular; breast; head & neck; multiple myeloma; ovarian; colorectal
Syndecan-2	HS/CS	Thyroid gland; liver; ovary	Interstitial connective tissue; cornea	α-tubulin; c-Src-cortactin; ERM	Actin cytoskeleton; cell adhesion	Head & neck; thyroid; liver
Syndecan-3	HS/CS	Brain; colon; smooth muscle; spleen	Interstitial connective tissue; cornea	heparin-binding growth-associated molecule	Neurite outgrowth	Glioma; melanoma
Syndecan-4	HS/CS	Liver; breast; skin; kidney	Interstitial connective tissue; cornea	EGFR; α6β4, α5β1 integrins; PKCα; α-actinin; TRPC7; Ca^{2+}	Wound healing; cell invasion & survival; focal adhesion & stress fibers	Breast; thyroid
GPC-1	HS	Brain; adrenal gland; esophagus; gastrointestinal tract; pancreas; skeletal muscle; skin; tonsil	Interstitial connective tissue; cornea	FGF2; Wnt/β-catenin; Hh; IGF; VEGF; TGFβ	Neurodegeneration; cell growth; immune response	Breast; ovarian; prostate; pancreatic; esophageal; liver; endometrial; thyroid
GPC-2	HS	Brain; testis; skin; thymus	Interstitial connective tissue; cornea	Wnt; PTN	Neuronal cell adhesion & motility; neurite outgrowth	Neuroblastoma; urothelial; testis

Table 1. (Continued).

PG	GAG chains	Localization	Matrix phenotype	Interactions	Functions	P2rognostic marker in malignancies
GPC-3	HS	Lung; placenta; adipose tissue	Interstitial connective tissue; cornea	Frizzled (Wnt receptor); SHH & PTC1 (Hh protein and receptor, respectively); CD81	Apoptosis; limb patterning; skeletal development; renal morphogenesis; controlling cell movements during gastrulation	Stomach; hepatocellular carcinoma; breast; ovarian
GPC-4	HS	Brain; lung; gallbladder; kidney; adipose tissue; placenta	Interstitial connective tissue	MMP14; IGF; FGF2	Development of kidney tubules and of the CNS; cell division; growth regulation	Renal; lung
GPC-5	HS	Brain; kidney; testis; lung; endocrine tissues	Interstitial connective tissue	Hh	Cell division; growth regulation	Glioma; lung
GPC-6	HS	Brain; retina; liver; gallbladder; colon; male & female tissues; smooth muscle tissue; adipose tissue	Interstitial connective tissue	Frizzled; PTC1	Cell growth; cell division	Renal; liver; pancreatic; stomach
CSPG4/NG2	CS	Skin; colon; esophagus; smooth muscle tissue; adipose tissue	Interstitial connective tissue	Integrins; collagen type V & VI; FGF; PDGF; PDZ-domain proteins	Cell proliferation; cell migration; angiogenesis; tissue remodeling; endothelial cell motility during microvascular morphogenesis	Melanoma; renal; urothelial; glioma
Betaglycan	HS/CS	Testicular cells; breast; ovary; urinary bladder; adipose tissue	Interstitial connective tissue	TGFβ; β-arrestin	EMT; reproductive functions; fetal growth	Thyroid; endometrial; head and neck; renal; breast
Phosphacan	CS/DS	Brain cerebral cortex	Interstitial connective tissue	N-CAM; fibronectin	Implicated in developmental processes in CNS; antiapoptotic role; cell differentiation; oligodendrocyte precursor proliferation	Urothelial; glioma

[135], and many tumor cells, including among others breast, prostate, colon, hepatocellular, myeloma, and glioma [26]. SRGN is required for the storage of proteases in both connective tissue and mucosal mast cells and for storage of granzyme B in T-lymphocytes, while it plays a role in cytotoxic cell granule-mediated apoptosis [136]. It is well established that this PG promotes the secretion of inflammatory regulators, tumor growth, and development [25,137,138].

The extracellular PGs, hyalectans, small LRPs (SLRPs) can be found in interstitial ECMs, and they interact with several ECM components and stabilize interactions between HA and other PGs through HA binding in their C-terminal domain. Hyalectans consist of four members: versican, aggrecan, brevican, and neurocan. Versican is composed of different isoforms (V_0, V_1, V_2, V_3), and it has high tissue distribution. It is mainly expressed in brain, lung, female tissues, heart, smooth muscle, and adipose tissue, and it is secreted into blood [135]. This PG interacts with calcium and HA, while it is involved in cell adhesion, proliferation, migration, and angiogenesis and plays a central role in tissue morphogenesis and maintenance [139]. Aggrecan is an integral part of the ECM in cartilagenous tissue and it withstands compression in cartilage. It is detected in many tissues, including cerebral

brain cortex, lung, seminal vesicles, cervix, and soft tissue in chondrocytes [135]. This PG is heavily glycosylated by CS and KS and may hold hundreds of GAG attachment sites. Brevican and neurocan are CS PGs that are specifically expressed in the CNS, in the cerebral cortex of the brain and interact with HA [135].

Small leucine-rich proteoglycans designate the largest class of PGs; the most abundant matrix components (18 members) in terms of distinct gene products with very vast biological functions. This family interact with GFs, cytokines, receptor tyrosine kinases (RTKs) and TLRs, thereby regulating vital processes as embryonic development, homeostasis, migration, proliferation, angiogenesis, innate immunity, apoptosis, and autophagy [126]. SLRPs are ubiquitously expressed in all interstitial matrices and may act both as structural constituents and as signaling molecules, especially during ECM remodeling in cancer, diabetes, inflammation, and atherosclerosis. Among the most studied members of this family, decorin, lumican, fibromodulin, and biglycan have been implicated in cell signaling, mesenchymal-to-epithelial transition (MET), inflammation, autophagy, collagen fibrillogenesis, and matrix organization [140–143].

The third class of extracellular PGs includes SPOCK/testican family, which encompasses three calcium-binding HSPGs and can be found in interstitial ECMs. SPOCK1 is exclusively expressed in cerebral cortex of the brain, SPOCK2 is expressed in brain, lung, and endocrine tissues, while SPOCK3 expression is located exclusively in parathyroid gland [135]. SPOCKs are associated with various neuronal mechanisms in the CNS [144].

The subfamily of pericellular/BM PGs include the modular PGs perlecan and agrin, and collagens XVIII and XV. This group of PGs is mostly HSPGs, which are closely associated with the cell surface anchored via integrins or other receptors, but they can also be a part of most BMs [145]. Their structure contains large protein cores with diverse structural motifs. Intriguingly, the C-terminal domains of perlecan and collagen XVIII (endorepellin and endostatin, respectively) exert autophagic and anti-angiogenic properties following proteolytical cleavage [21,146]. These pericellular/BM PGs interact with RTKs and other cell surface receptors as well as with other matrix molecules to promote cell signaling cascades that control migration, angiogenesis, autophagy, matrix assembly, and vascularization [16]. Perlecan carries up to three HS/CS chains and is mainly located in smooth muscle tissue, but also in endometrium, urinary bladder, and adipose tissue [135]. Three HS chains are attached to agrin, which is located in kidney, gallbladder, and skin [135]. Both collagens XVIII (liver, brain) and XV (female tissues, heart muscle, adipose tissue [135]) have a wide tissue distribution but the highest expression is detected in BM zones, so they may function to adhere BMs to the underlying connective tissue stroma.

The family of cell surface PGs enclose two main subfamilies: the HS/CS PGs syndecans and GPCs. CSPG4, phosphacan, and betaglycan complete this family. Syndecans are type I transmembrane (TM) glycoproteins that consist of four members (syndecan-1 to syndecan-4) and are ubiquitously expressed in many human tissues (i.e., immune cells, brain, lung, breast, testis, skin) with the exception of syndecan-3 that is mainly expressed in the nervous and lymphoid systems [135]. Three distinct domains determine their architecture and biological functions; the C-terminal cytoplasmic domain, the N-terminal polypeptide where GAGs are attached to serine residues of the protein core and a single TM domain. Syndecans have key roles in many biological processes, such as development, wound healing, stem cell differentiation, inflammation, and cancer progression. Studies focusing on structure-function relationships revealed that via their HS/CS chains syndecans bind GFs (i.e., HGF, FGF, EGF, VEGF) and cytokines, as they also interact with cell receptors [i.e., epidermal GF receptor (EGFR), HER2, TGFβRI, TGFβRII, insulin-like growth factor receptor I] and integrins (αvβ3, αvβ5, α3β1, α6β4, α5β1) to promote cell signaling [147–149]. Notably, the function of cell surface PGs can be altered by ectodomain shedding, which converts the membrane-bound coreceptors into soluble paracrine effectors that in the case of tumor has a huge impact on cancer cells and their surrounding stroma [132,150].

Glypicans are modified with HS chains near the juxtamembrane region and are anchored to the plasma membrane through a C-terminal glycosylphosphatidylinositol (GPI) linkage, which is cleavable by the lipase Notum. Six GPCs have been identified in mammals (i.e., GPC1–6) that are mainly expressed in epithelial and mesenchymal cells and control cell division and growth regulation. Moreover, GPCs have been characterized as putative cell surface coreceptors for GFs [such as Wnt/β-catenin, Hh, fibroblast GF (FGF), insulin-like GF (IGF), VEGF, and transforming GF-β (TGFβ)] and matrix modifying enzymes (proteases and lyases) through their HS chains in many cancer cells, thus being involved in cell signaling and the control of tumor growth, angiogenesis, and metastasis [151–153]. For instance, GPC1 is mainly expressed in the CNS, skin, and skeletal system, whereas studies have shown increased expression in breast [154], ovarian [155], prostate [156], pancreatic

[157], and esophageal cancer cells [158]. GPC2 has a key role in neuronal cell adhesion and neurite outgrowth and has been associated with poor overall survival of neuroblastoma patients [159,160]. GPC3 positively regulates the canonical Wnt signaling pathway by binding to the Wnt receptor Frizzled [161] and the subsequent tumor growth in hepatocellular carcinoma [162], breast [163], and ovarian cancer [164].

Other cell surface PGs that are involved in cell adhesion and signaling are the single-pass TM CSPG4, betaglycan, and phosphacan. CSPG4/NG2 expression is detected in skin, colon, esophagus, smooth muscle, and adipose tissue [135], as regulates cell proliferation and migration which stimulates endothelial cell motility during microvascular morphogenesis [165] and is involved in the origin and progression of human gliomas [166]. Moreover, it is an integral membrane CS PG expressed in human malignant melanoma cells [167]. Betaglycan/TGFβ type III receptor is a single-pass TM PG with one HS/CS chain that belongs to the TGFβ superfamily of coreceptors and its extracellular domain contains several potential GAG attachment sites and protease-sensitive sequences near the plasma membrane [168]. It is a ubiquitously expressed cell surface PG and most abundant in testicular cells, in breast and ovary [135]. Phosphacan/receptor-type protein tyrosine phosphatase β is exclusively expressed in the cerebral cortex of the brain [135] and it has up to five CS/DS chains. It has been proposed that phosphacan is involved in the regulation of specific developmental processes in the CNS, it is required for normal differentiation and has antiapoptotic role [169].

GAGs: the sweet regulatory partners of ECM

At the structural level, PGs consist of a core protein into which from one to more than a hundred GAG chains are covalently attached. Variations in the structure of disaccharide unit and sulfonylation degree define six GAG members: CS, DS, Hep, HS, KS, and HA [170,171]. GAGs themselves are unbranched, negatively charged, linear heteropolysaccharides with a repeating disaccharide structure of hexuronic acid (D-glucuronic or L-iduronic acid) or galactose only in KS, and *N*-acetylated hexosamines (*N*-acetyl-D-glucosamine or *N*-acetyl-D-galactosamine). GAG biosynthesis, with the exception of HA, takes place in the endoplasmic reticulum and Golgi apparatus via discrete biosynthetic cascades orchestrated by several enzymes [15]. HA is the simplest GAG in nature, the only GAG produced on the cell membrane and immediately extruded to the extracellular space without any covalent linkage to proteins. The unique structure of each GAG chain risen from differences in saccharide composition, size, and the positions in which the sulfonylation takes place in both disaccharides of the repeating unit, together with the numerous combinations that GAGs are attached to the core proteins, explain the extremely structural and functional diversity of PGs [171,172].

Glycosaminoglycan chains regulate various signaling pathways in normal and pathological processes through their interactions with different classes of matrix proteins. Hep and HS demonstrate high sequence heterogeneity and variable sulfation patterns; therefore, the majority of GAG-binding proteins interact with these GAGs [173]. Compared to Hep, HS demonstrates a lower sulfation and C-5 epimerization of D-glucuronic to L-iduronic acid and has key roles in homeostasis, embryonic development, and disease progression through its interactions with GFs (i.e., FGF, TGFβ, IGF, VEGF, PDGF), cell surface receptors [i.e., CD44, fibroblast GF receptor (FGFR)], and matrix enzymes, thus modulating major signaling cascades [172,174]. The effects of HS on GF signaling are tightly regulated by the actions of heparanases (HPSE), sulfotransferases, and sulfatases [175,176]. In addition to its anticoagulant activity, there is accumulating evidence highlighting various anticancer activities of Hep and nano-Hep derivatives in several cancer types [177,178]. Importantly, Hep and nano-Hep derivatives significantly reduce breast cancer cell proliferation and metastasis *in vitro* and *in vivo* as well as regulates the expression profile of major ECM macromolecules, providing strong evidence for therapeutic targeting [179,180]. Intriguingly, it has been recently discovered that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral attachment and infection involves HS-dependent enhancement of binding to angiotensin-converting enzyme 2 (ACE2) through its receptor-binding domain [181]. Moreover, exogenous Hep and nonanticoagulant derivatives inhibit viral adhesion and cell infection, presenting novel therapeutic approaches for COVID-19 [181].

Chondroitin sulfate and its C-5 epimerization counterpart, DS, are the prevalent GAGs in many tissues, as in brain and cartilage [182]. GFs including FGF, interleukin-10, and EGF interact with CS in a CS structure-specific manner [183]. CS/DS have key roles in axonal growth via the TM receptor protein tyrosine phosphatases and the receptor for advanced glycation end products (RAGE) that have been identified as functional CS/DS receptors [184,185]. Moreover, CS/DS expression in the tumor niche affects tumor

progression and metastasis in many cancer types including breast, lung, colon, and prostate [186,187].

The disaccharide units of KS can be nonsulfated, mono-sulfated or di-sulfated, like HS. KS is expressed in cartilage and a number of epithelial and neural tissues, playing a role in wound healing, embryogenesis, collagen fiber assembly, and corneal organization [188,189]. KS is also present in neurosecretory vesicles, suggesting its roles in vesicle formation and neuronal activity [190].

Hyaluronan: biosynthesis, degradation, and functions

When Carl Meyer in 1934 described for the first time the hyaluronan (hyaluronic acid, HA) chain in substantia ialoidea in the eye, he might never have imagined the incredible future that polymer would have [191]. HA is a high molecular size unbranched GAG found intracellularly, on the cell surface, but predominately in the ECM. In fact, in ECM, a milieu of several macromolecules interacting specifically each other exerting extraordinary properties, HA plays a unique role for different reasons [15]. The repeating unit of the HA possesses one carboxyl group and is therefore a negatively charged polyelectrolyte at neutral pH. The unit can be repeated up to 25 000 times, generating a molecular mass of millions of Daltons (5×10^5 to 5×10^6 Da; Fig. 2) [192,193]. HA exhibits superior hydrophilic properties that underlie most of its biomechanical properties since every HA disaccharide unit interacts with 25 water molecules [194].

Due to its biological properties, HA emerged among the most biologically active relevant molecules in the body. HA plays a key role in several physiological processes including development [195], wound healing [196], cell migration [197] and proliferation [198]. This GAG is found in high amounts during embryogenesis, as well as when rapid tissue turnover and repair are taking place. HA is involved in several pathologies, such as inflammation [28,199–202], cancer [203–207], vascular diseases [208–210], diabetes [210], and virus infections such as dengue virus and SARS-CoV-2 [211–214]. Vital cellular functions, such as division and motility, of either normal or transformed cells are highly dependent on HA. A boost in HA synthesis occurs just prior to mitosis, enabling cells to lose adhesion from their surrounding ECMs as well as to dissociate from neighboring cells in preparation for division, a feature also important for tumor cell metastatic capacity [203].

The enzymes involved in HA biosynthesis are structurally organized to extrude the polymer outside of the cell in the ECM picking in the cytoplasm the necessary UDP sugars. It has been suggested that the cytoplasmic domain of the enzymes could be involved in the regulation of the polymers size, influencing the chain stability during the synthesis [215].

HASes use the UDP sugars precursors (UDP-GlcA and UDP-GlcNAc) due to the presence of a double catalytic domain, which interacts with these two different substrates. This generates the disaccharide units necessary to create the polymer. The kinetics of the HASes have been extensively studied, even though, without crystallography information, all kinetic explanations are still hypothetical [216]. The presence in mammals of three different enzymes to produce HA underlines the possibility that each specific enzyme has unique kinetic properties. In fact, it has been proposed that the different enzymes can produce polymers of different length and at different rates. In particular, HAS1 and HAS2 produce longer HA polymers ($\sim 2 \times 10^6$ Da) than HAS3 ($\sim 2 \times 10^5$ Da), while HAS1 synthesizes lower amounts of HA compared to HAS2 and HAS3 [193,217–220].

Beside the different size of the polymer produced, HASes differ also in the regulation of their catalytic activity. It has been demonstrated that HAS2 has several covalent regulations, such as the activating phosphorylation by PKC or the inhibitory phosphorylation by AMP-activated protein kinase [221,222], as well as *O*-GlcNAcylation [223] and mono-ubiquitination [224] that both increase the stability and activity of the enzyme. On the other hand, HAS3 is regulated by its sorting to the cell membrane by interaction with Rab10 [225]. These regulations are mainly related to the energy content of the cells [226]. In addition, several studies have demonstrated the presence of HA intracellularly under cellular stress conditions, such as hyperglycemia, virus infection, inflammation, and cancer, revealing also a paradoxical topology of HASes inside the cells [202].

HA has a rapid turnover in the tissues, and one-third of the total body HA is turned over every day. In mammals, HA synthesis and degradation rates regulate HA concentration. A large body of literature showed that the HA fragments can be recognized by specific receptors triggering specific inflammatory pathways. As mentioned above, HA digestion in mammalian tissues is carried out by six different HYALs (HYAL-1, HYAL-2, HYAL-3, HYAL-4, P1, and PH20). The HYALs are classified as endo-beta-*N*-acetylglucosaminidases according to their hydrolytic mechanisms [227,228]. HYAL-1 and HYAL-2 are the major HYALs found in tissues. HYAL-1 is found in lysosomes, and its deficiency leads to a genetic disease called mucopolysaccharidosis type IX [229]. HYAL-2 is a GPI-anchored protein with extracellular activity that co-localizes with

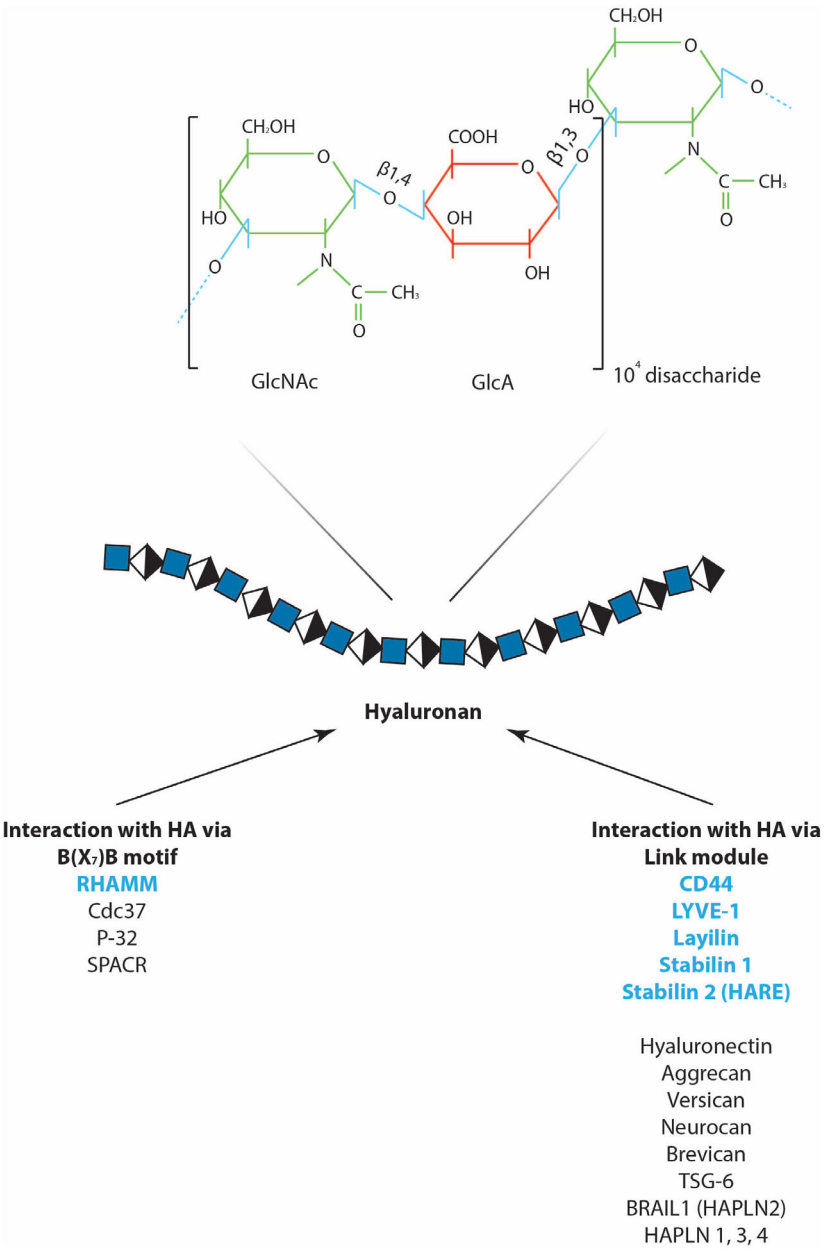

Fig. 2. Structure and interactions of HA. HA is a polymer constituted by a repeating disaccharide units composed of D-glucuronic acid (GlcA) linked to *N*-acetyl-D-glucosamine (GlcNAc) with a glucuronic beta 1–3 linkage between GlcA and GlcNAc and a hexosaminidic bond beta 1–4 between GlcNAc and GlcA. HA interacts with hyaladherins that include receptors (depicted in blue) as well as PGs and other ECM molecules via the LINK module or the BX_7B motif.

CD44 within specialized microdomains (i.e., lipid rafts) and together exert the coordinated functions of binding and degrading HA [227]. In mammals, HYAL-1 and HYAL-2 activities are synergic: HYAL-2 degrades HA to fragments of 20 kDa corresponding to about 50 disaccharide units, and HYAL-1 degrades these fragments into smaller fragments of about 800 Da (~ 2 disaccharide units; Fig. 3). The role and activities of HYAL-3 are still elusive [230]. HA polymer degradation could also be due to ROS produced by inflammation, while UV radiation breaks HA polymer in fragments without specific size [231].

The human *HYAL4* expression is restricted to placenta and skeletal muscle [232]. There is evidence indicating that HYAL-4 is a chondroitinase with no activity against HA making this enzyme peculiar in vertebrate tissues. *HYALP1* is a pseudogene transcribed, but not translated, in the human, and PH-20 is the enzyme that digests oocyte-surrounding HA, facilitating ovum fertilization [233]. The HYALs (−1 to −4) can work in acidic environment (about pH 3 and 4), whereas PH-20 and other HYALs from insects and snakes' venoms are active at neutral pH [234]. A new HYALs has been described in

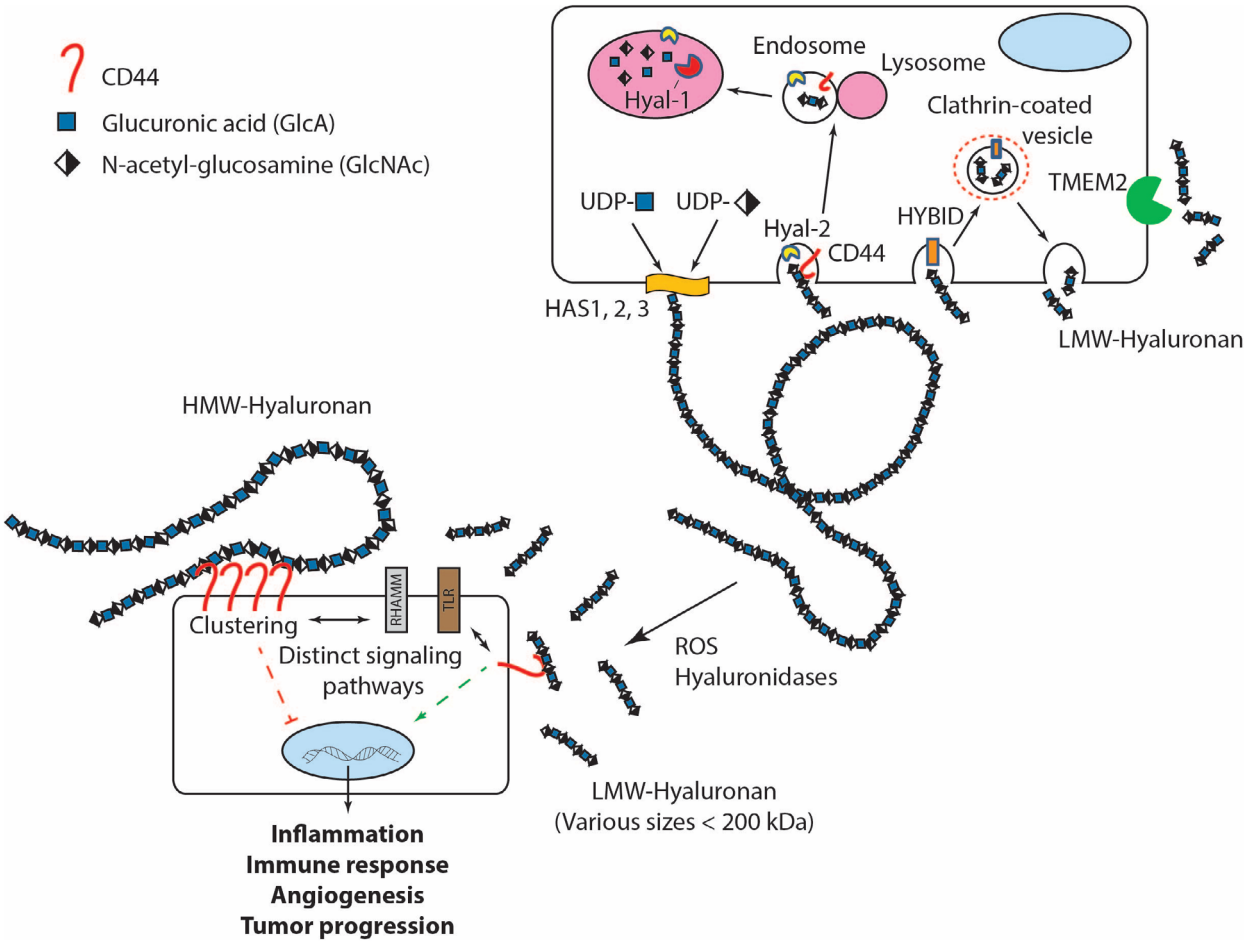

Fig. 3. Synthesis, degradation and functions of HA. HASes (HAS1, 2 and 3) use UDP sugar precursors to produce the HA polymer that is extruded to the extracellular space. HMW-HA induces the formation of CD44 clusters on cell membrane. In contrast, LMW-HA derived from the action of HYALs (i.e., Hyal-1, Hyal-2, HYBID, TMEM2) and/or ROS cannot induce receptor clustering thereby driving distinct signaling pathways and cellular functions.

mammals: TMEM2, which is a TM protein with strong HYAL activity [235] that specifically degrades high molecular weight HA (HMWHA) into ~ 5 kDa fragments. TMEM is a potent modulator of ER-stress resistance and innate immunity [236]. Another HYAL called CEMIP/KIAA1199 (also known as HYBID) has been recently identified with HA-degrading activity [237,238]. Interestingly, this enzyme has a key role in cancer development, in skin biology and cell senescence [238] (Fig. 3). In addition, bacteria produce several HYALs which act as lyases [239].

The degradation of HA produce fragments that have important biological functions. HA minimal size able to trigger cell response has been extensively addressed. It has been reported that 4–6 disaccharide units (4–6 mers) are responsible for NF-κB signaling and MMP synthesis, while HA oligomers ranging from 4 to 16 disaccharides are able to activate dendritic cells via TLR receptors [240,241].

HMWHA shows anti-angiogenic, immune suppressive, and anti-inflammatory activities, and induces tissue reparative processes as described in wound healing [242]. In an opposite fashion, the fragments of HA, called oligosaccharides when < 200 kDa, show the capacity to modify the expression of a variety of genes (such as MMPs, syndecan-4, HPSE) to induce inflammatory processes as well as angiogenesis throughout their interactions with specific receptors [29,243–245] (Fig. 3).

Hyaluronan receptors: the multifaceted cell-matrix interaction partners

HA requires specific receptors that regulate cell-ECM interactions triggering also intracellular signaling. In

general, all proteins that interact with HA are defined 'hyaladherins' including not only receptors but also PGs and other ECM molecules. PGs as aggrecan, neurocan, brevican, and versican can be included among hyaladherins interacting with HA creating networks that form an architectural scaffold [194]. Hyaladherins include receptors (i.e., CD44, RHAMM, LYVE-1, Layilin, Stabilin1 and HARE) that can trigger specific intracellular signaling [246] or mediate endocytosis and degradation of HA. Proteins can interact with HA by using the LINK module or BX_7B motif (where B is either lysine or arginine and X can be any amino acid other than acidic residues; Fig. 2) [215,246]. HA and PG Link protein family (hyaluronan and PG link protein 1–4) have been described to interact with HA and PG, stabilizing such multicomponent complexes [246]. Some HA-binding proteins do not contain a link module (RHAMM, ITI, SPACR, SPACRCAN, CD38, CDC37, HABP1/P-32, Siglec-9, and IHABP4), and most of these are unrelated to one another by amino acid sequence. Even though some of these proteins contain clusters of basic amino acids (BX_7B motifs), the actual HA docking site of the chain with this motif has not been established yet.

The most common HA receptor is CD44, a TM glycoprotein widely distributed in different cells and particularly concentrated on the membranes of inflammatory and cancer cells [203,247]. The human CD44 gene comprises 19 exons, 10 of which (designated v1–v10) can be subjected to alternative splicing. Although alternative splicing of the CD44 gene could in theory generate more than 700 different isoforms, only around 20 isoforms have been identified so far. Splicing together exons 1–5 and 16–19 of the transcribed mRNA led to the translation of the standard CD44 (CD44s) that is the conserved form and has a molecular mass of about 85–90 kDa as mature protein [204,247]. Besides alternative splicing, other post-translational modifications add to the heterogeneity of CD44 proteins, such as N- and O-linked glycosylations or the addition of CS and other GAGS [248,249]. The exon of CD44 variant 3 (CD44v3) product can also be modified by HS. A splicing switch, resulting in either the expression of CD44v isoforms or the expression of CD44s, is controlled by extracellular cues, cues that still need better characterization [250]. However, the splicing of CD44 clearly takes place in pathological conditions and is determinant for tumor progression. In breast cancer for example, the switch from CD44 variant isoforms to CD44s was shown to be involved in the determination of cancer stem cell fate [251]. In colorectal cancer, a switch from CD44s to CD44v4-v10 occurs in $Lgr5^+$ cancer stem cells [252].

CD44 is a TM glycoprotein that bind with its extracellular domain (ectodomain) components of the ECM, including HA, fibronectin, and laminin, but also to various GFs and cytokines, and senses stimuli from the external cell microenvironment [204,253]. The TM domain provides a docking site for cofactors and adaptor proteins and seems to be involved in lymphocyte homing [253,254]. The intracellular domain can be cleaved and translocated to the nucleus where it seems to mediate transcription [254]. Although the cytoplasmic tail of CD44 is relatively short (contains only 72 amino acid residues) and devoid of any enzymatic activity, it contains structural motifs implicated in interactions with multiple cytoskeletal and signaling proteins [255].

Notably, the CD44 interactions with HA are molecular size-dependent and lead to receptor clustering when the HA chains are long enough (HMWHA ~ 2 MDa). This clustering does not occur in the case of low molecular weight (LMW) HA molecules. The binding induces different signaling pathways and cell functions (Fig. 3). One of the effects of CD44 engagement by HA is the activation of Rho GTPase signaling, which controls cytoskeletal organization, chemoresistance, cell growth and proliferation. Rho activation induces PI3K which triggers the serine/threonine kinases (Akt) and, in turn, the phosphorylation of substrates involved in cell proliferation, survival, and motility [256]. The relationship between size and biological functions is confirmed by literature describing a very HMWHA (vHMWHA > 6 MDa) in naked mole rat, an animal with an incredible longevity and cancer resistance [257,258]. Unexpectedly, vHMWHA masks and suppresses CD44 clustering, interactions and signaling of CD44 proteins, in contrast to HMWHA, and thereby exhibits distinct cytoprotection through modulating the p53 pathway [258]. The involvement of CD44 in tumor progression and metastasis is further discussed in the next section.

Another important HA receptor related to cell motility is RHAMM (also known as CD168). Several structural variants of RHAMM arise from either alternative splicing of RHAMM pre-mRNA or post-translational mechanisms or alternative start codon usage [259,260]. RHAMM is a hydrophilic helical protein that binds both fragmented and HMWHA via positively charged amino acid clusters within its C terminus that are structurally distinct from the LINK module responsible for the binding of HA to CD44 [261,262]. RHAMM also binds to microtubules via its N and C termini [259]. In addition, it can self-associate as dimers or trimers as predicted by the presence of

leucine zippers within the protein together with its potential to form a coiled coil [260].

The multifaceted functions of RHAMM are related to its complex subcellular localization. Typically, it is an intracellular protein that is exported to the cell surface in response to specific stimuli. Cell surface RHAMM functions as a coreceptor by affecting signaling through HA and GF receptors, while intracellular RHAMM binds to a number of protein partners that mediate its functions as a regulator of microtubule dynamics, structure/function of centrosomes and gene expression affecting cell polarity, directed cell movement, and mitotic spindle integrity [259,263].

RHAMM is present in several cell types, including cancer and endothelial cells [264,265]. Interaction between HA and RHAMM triggers a signaling pathway not completely described yet which includes RAS-oncogene activity [263,266,267]. Several studies support a correlation of RHAMM expression to malignancy, while it cooperates with CD44 in HA endocytosis and signaling [268]. Indeed, CD44 is responsible for HA uptake in adherent cells, while in nonadherent cells RHAMM is the main HA endocytic receptor [269]. It is also known that RHAMM and CD44 share ERK1/2 phosphorylation cascade activation [263].

CD44: a promiscuous family of molecules affecting tumor growth and metastasis through signaling control

CD44 proteins are involved in several cellular responses including proliferation, differentiation, survival, and migration (reviewed in [247]). Ample evidence points to a role of CD44 in signaling [270]. However, as mentioned above, CD44 cannot signal on its own. To control signaling it associates to other cell surface receptors such as RTKs, hepatocyte GF receptor (MET), VEGFR-2, EGFR, and GPCRs. These multiple collaborations, showing that CD44 proteins are promiscuous molecules, enables them to have an impact on many diseases, the most prominent one being cancer. The pleiotropic effect of CD44 and the fact that expression of CD44 variant isoforms is limited to a restricted number of tissues are potentially advantageous for therapeutic targeting.

Consequences of CD44-dependent signaling on tumor progression and metastasis

The diversity of signals that can be generated from RTKs and other cell surface receptors is in part due to the association with other molecules such as cell adhesion molecules at the cell surface [270]. The coreceptor function of CD44 for RTKs and thereby its function in signaling seems to be ligand-dependent (Fig. 4). In the case of HGF/MET and VEGF/VEGFR-2, isoforms containing the exon v6 are involved [270]. CD44v6-containing isoforms control the activation of MET and VEGFR-2, as indicated by the inhibition of RTK phosphorylation upon treatment with CD44v6 inhibitors, such as the CD44v6 peptides or CD44v6 antibodies [271,272]. Signaling from both of these receptors requires the recruitment of Ezrin-radixin-moesin (ERM) to the CD44 cytoplasmic tail. The collaboration between CD44v6 and these RTKs is involved in the progression of pancreatic cancer (Fig. 4) [272]. Indeed, in several mouse models of pancreatic cancer, inhibition of CD44v6 by a CD44v6 peptide led to decreased tumor growth and decreased metastasis. In the KPC (*LSL-KRas*^{G12D}, *LSL-Tpr53*^{R172H} *and Pdx1-Cre*) model of pancreatic cancer, treatment with the CD44v6 peptide led to an increase in life span of 40 days compared to mice treated with a control peptide. A modified version of the CD44v6 peptide used in the above-mentioned studies, is currently being used in a clinical trial. An interesting study in pancreatic cancer, showing that expression of CD44v6 and Tetraspanin on exosomes plays a role in metastasis [273,274], added to the function of CD44v6 in pancreatic cancer. In colorectal cancer, the CD44v6/MET pair has also been shown to be involved [275,276]. Other isoforms of CD44 collaborate with other RTKs. The ligand dependency of the coreceptor function of CD44 was demonstrated using the EGFR family of RTK as a model (Fig. 4) [277]. In several breast cancer cell lines, the authors showed that CD44v6 was recruited by EGF-induced and Epiregulin-induced ErbB1, and that the same isoform was also recruited by neuregulin-induced ErbB3 and ErbB4. In contrast, Hep-binding GFs such as HB-EGF needed the CD44v3 heparan-sulfated isoform of CD44 instead of CD44v6. Transforming GF (TGFα) was independent of all CD44 isoforms. These *in vitro* studies were supported by *in vivo* data showing that the CD44v6 peptide led to smaller metastases in a 4T1 model of breast cancer. FGF is another GF that recruits the heparan-sulfated form of CD44v3, whereby CD44v3 is required for the presentation of FGF to its authentic receptor FGFR during limb development [278]. In contrast to the activating role of CD44 described above, HA inhibited PDFGR activation upon recruitment of a tyrosine phosphatase by CD44 [279].

The involvement of CD44 isoforms in these signaling pathways suggest that CD44 molecules are able to

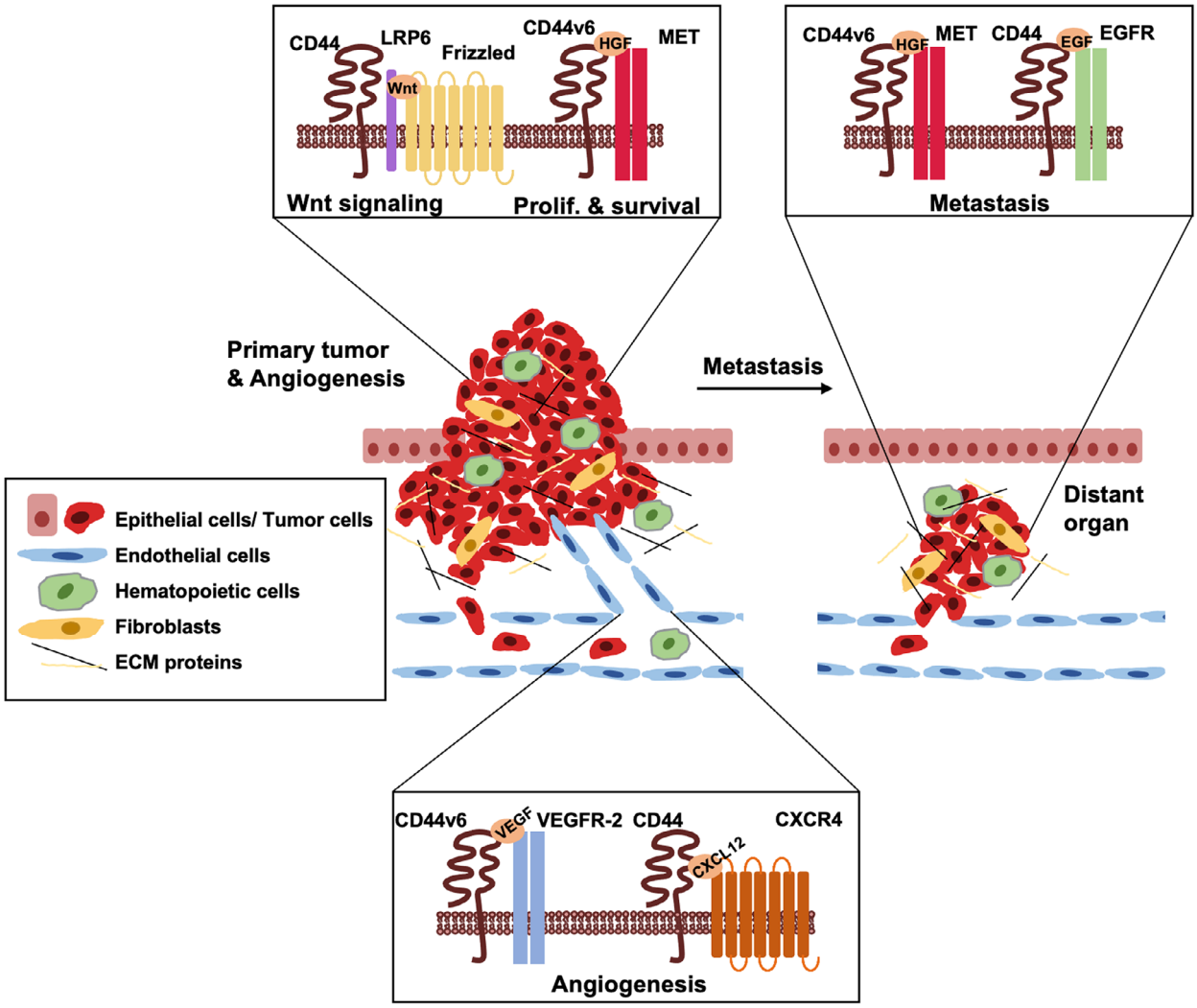

Fig. 4. CD44 isoforms associate with various cell surface receptors including RTKs, G protein-coupled receptors and Wnt receptors during tumor development and metastasis. The MET/CD44v6 pair has been shown to promote tumor growth and metastasis in pancreatic cancer, EGFR/EGF/CD44v6 are involved in initiation of metastasis in breast cancer, and CXCR4/CD44/HA/CXCL12 take part in angiogenesis [270].

organize platforms of signaling, which lead to pathological outcomes after they are deregulated. The examples above also suggest that the function of CD44 in RTK activation is in part due to its ability to recruit GFs among which HGF or VEGF [280], but also HB-EGF and bFGF [281].

As previously described, HA is involved in angiogenesis, tumor progression and metastasis [282] through its binding to various receptors of which CD44 is the major one. Once HA binds CXCL12 (SDF1alpha) [283], the additional binding to CD44s boosts CXCL12-induced activation of CXCR4. However, Fuchs *et al.* [284], showed that small HA fragments block this activation. The authors also showed that this CD44/CXCR4/CXCL12/HA complex is involved in angiogenesis. The interplay between CD44 and CXCR4 could also be instrumental for the development of acute myeloid leukemia (AML) [285] and for sustaining leukemic stemness [285] and our own unpublished results]. A possible explanation of the function of HA/CD44 in CXCL12/CXCR4 signaling is that HA acts as an adhesive, collecting CXCL12 in the vicinity of CXCR4 and CD44 and thereby unleashing the signaling cascade (Fig. 4).

Besides GFs and HA, CD44 isoforms also bind cytokines like OPN [248]. The binding of OPN to CD44 is involved in the expression of CD44v6 in colorectal cancer stem cells (CR-CSCs) [275]. This

interaction also takes place in gastrointestinal cancer cells, thus enhancing their survival [286].

To the multiple pathways recruiting CD44, we can add the Wnt signaling pathway as an additional signaling route for CD44 (Fig. 4). In a positive feedback loop, CD44 influences the Wnt pathway upon association to one of the main components of the Wnt signalosome, LRP6 [287]. To date, however, there is no indication that CD44 binds to Wnt.

CD44 contribution to tumor progression is not restricted to the tumor cells

CD44 plays a role at several steps in tumor progression and metastasis. However, most studies on the function of CD44 in cancer have focused their attention on cancer cells. Although CD44 is present on several cells of the tumor stroma, few reports have shown an involvement of CD44 on stromal cells in tumor progression. Expression of CD44 on cancer-associated fibroblasts (CAFs) was shown to increase in hypoxic conditions and in the avascular region. This expression of CD44 on CAFs contributed to sustaining the stemness of colorectal cancer cells [288]. SRGN, another partner of CD44, is a PG secreted by human CAFs. In non-small-cell lung cancer, the SRGN-CD44 interaction was shown to promote aggressiveness by inducing epithelial-to-mesenchymal transition (EMT) [289].

The MET/CD44v6 and VEGFR-2/CD44v6 pairs were targeted on endothelial cells in pancreatic tumors by means of the C44v6 peptide [271]. A decreased angiogenesis and decreased tumor growth and metastasis were observed.

In breast cancer, the expression of CD44 on fibroblasts promoted survival of tumor cells and resistance to paclitaxel [290]. Other studies in breast cancer have shown that the absence of CD44 in bone marrow cells decreased the contribution of these cells to tumor stroma and led to decreased migration of mesenchymal stem cells and weak angiogenic support [291].

Using the *Cd44*-floxed mice [292], a role for CD44 in pancreatic stellate cells was revealed [Heneka, Treffert and Orian-Rousseau, unpublished data]. Crossing of these *Cd44*-floxed mice with other stromal-specific promoter-driven Cre mice should help to further elucidate the role of CD44 in cancer. Moreover, targeting CD44 on tumor cells and on the tumor stroma will probably have a bigger impact.

CD44 as a promising target in human disease

Altogether, targeting CD44 is necessary and urgent [293]. Given the pleiotropic effects of CD44 not only in cancer but also in other inflammatory diseases—including nonalcoholic liver diseases [294], multiple sclerosis [295], or arthritis [reviewed in [296]]—it appears to be a potentially important therapeutic target. The ubiquitous expression of CD44s makes this targeting difficult, but isoforms have been targeted successfully as in the case of CD44v6. Indeed, the use of shRNA against CD44v6 led to a decreased growth of adenoma in $Apc^{Min/+}$ mice [297]. Moreover, the aforementioned CD44v6 peptide [272] is currently being used in a clinical trial. T cells targeted to CD44v6 showed a potent antitumor effect against AML and multiple myeloma without targeting hematopoietic stem cells and CD44v6-expressing keratinocytes [298]. In chronic lymphocytic leukemia this time, a humanized mAb against CD44 (RG7356) was shown to have potential therapeutic effects in patients expressing both CD44 and ZAP-70 [299]. In any case, it is clear that targeting CD44s might induce side effects that could prevent the use of these inhibitors in patients. Therefore, the more we learn about the interactions between CD44 isoforms and their partners, the more precise and successful the targeting will be.

The collagen family: from tissue design to the regulation of biological processes

Collagens form a family of ECM proteins, which share a structural motif, the triple helix. Twenty-eight collagen types numbered by Roman numerals (I–XXVIII) have been identified [300 IX, X, XII, XIV, XVI, and XIX, 301–303]. They act as scaffolds providing ECMs and tissues with their structural organization and mechanical properties. In addition to their role as tissue designers, collagens interact with cell surface receptors, and regulate numerous biological processes either as full-length proteins or via their bioactive fragments, called matricryptins or matrikines, released by limited proteolysis (Table 2). Changes in collagen expression, deposition, cross-linking, and/or degradation occur in many diseases where stromal collagen organization is altered including cancer [304–307] and fibrosis [308–313]. Furthermore, mutations in the genes encoding collagens are associated with a variety of genetic disorders [314,315].

Collagens are trimeric proteins comprised of three identical or different polypeptide chains called α chains, which form a triple helix. The triple helix of collagens—an ancient protein structure that enabled animal multicellularity and tissue evolution. The characteristic structural feature common to all collagen

Table 2. The collagen subfamilies. The collagen alpha chains listed in the table are those containing identified protein domain signatures in addition to the triple helix. The major matricryptins released from these collagen chains are indicated. For further information on collagen domains and matricryptins see [402,637]. C1q, complement C1q, EMI, emilin, FNIII, fibronectin III, NC, noncollagenous, TSP, thrombospondin.

Collagen subfamilies	Collagen chains	Domains	Matricryptins and their parent collagen chains
Fibril-forming collagens	α1(I)		Proline-glycine-proline
I, II, III, V, XI, XXIV, XXVII	α1(V), α3(V), α1(XI), α2(XI), α1(XXIV), α1(XXVII)	TSP	Iα1 C-1158/59 fragment
FACITs	α1(IX), α1(XVI), α1(XIX)	TSP	NC1(XIX)
IX, XII, XIV	α1(XII), α1(XIV), α1(XX)	FN, TSP, vWFA	
XVI, XIX, XX, XXI, XXII	α1(XXI), α1(XXII)	TSP, vWFA	
Beaded filaments	α1-α2-α5-α6(VI)	vWFA	Endotrophin
VI	α3(VI)	vWFA, Kunitz	
	α4(VI)	Kunitz	
Anchoring fibrils	α1(VII)	vWFA, FN, Kunitz	
VII			
Network-forming collagens	α1-α6(IV)	7S & NC1 domains	
IV	α1(IV)		Arresten
	α2(IV)		Canstatin
	α3(IV)		Tumstatin
	α4(IV)		Tetrastatin
	α5(IV)		Pentastatin-1
	α6(IV)		Hexastatin
Hexagonal networks	α1(VIII)	C1q domain	Vastatin
VIII, X	α1(X)	C1q domain	
Multiplexins	α1(XV)	TSP	Restin
XV, XVIII	α1(XVIII)	TSP	Endostatin
Membrane collagens	α1(XIII), α1(XVII),	TM	
MACITs (XIII, XXIII, XXV) & XVII	α1(XXIII), α1(XXV)		
Collagen XXVI	α1(XXVI)	EMI	
Collagen XXVIII	α1(XXVIII)	vWFA, Kunitz	

types is the presence of at least one triple-helical domain of variable length, which is interrupted by noncollagenous domains in several collagen types. Recent studies on the mechanics and structural stability of the triple helix pointed out the influence of the local sequence and raised the question of its sequence-specific mechanical stability and function [33]. Other proteins including the so-called soluble defense collagens and membrane proteins such as ectodysplasin and the macrophage receptor MARCO also contain a triple helix and belong to the collagen superfamily. Several collagen types contain noncollagenous domains such as fibronectin III, von Willebrand factor A (vWF), thrombospondin, and Kunitz inhibitor domains (Table 2) [303].

Most collagen types form supramolecular assemblies, which define collagen subfamilies, in association with other ECM components. Collagens form fibrils [316], anchoring fibrils (collagen VII), beaded filaments (collagen VI), networks (collagens IV, VIII and X). In contrast, some collagens did not form supramolecular assemblies on their own but associate with existing ones such as the FACITs, which are located at the surface of collagen I and II fibrils. No supramolecular assemblies have been identified so far for the multiplexins (multiple triple-helical domains and interruptions, collagens XV and XVIII), and for the four membrane collagens (XIII, XVII, XXIII, and XXV) but the shed ectodomain of collagen XIII associates with the fibrillar fibronectin matrix and may interfere with its assembly *in vitro* [317]. Supramolecular assemblies may also depend on the context as shown for collagen XVI, which associates with collagen fibrils in cartilage and with beaded filaments in skin [318]. Few data are available for collagen XXVI, which is specifically expressed in testis and ovary and has been identified by its ability to bind to heat shock protein 47 [319].

Fibrillar collagens

Collagens I, II, III, V, and XI are the most extensively characterized fibrillar collagens both structurally and functionally, whereas few studies focus on collagens

XXIV and XXVII. Collagens I, III, and V are found in many tissues, whereas collagen II is mainly restricted to cartilage in association with collagen XI [320]. Collagen XI also regulates collagen fibril assembly, organization and functional properties in tendon [321]. Collagen XXIV is a specific marker of osteoblast differentiation and bone formation [322], and promotes osteoblastic differentiation and mineralization through TGFβ/Smads signaling pathway [323]. Overexpression of *COL24A1* in hepatocellular carcinoma is a predictor of poor prognosis [324]. Collagen XXVII organizes the pericellular matrix in the growth plate [325], and mutations of COL27A1 cause Steel syndrome [326].

Collagens I, II, III [327], V [308], XI, XXIV, and XXVII self-assemble into fibrils of various diameters forming different three-dimensional structures (i.e., orthogonal lattices in cornea, basket weaves in skin and blood vessels, and parallel bundles in tendon, ligament, and nerves), depending on tissues and on collagen types [328]. Collagen fibrillogenesis is orchestrated at the cellular level in relation to the tissue and stage of development [329], and is regulated by the FACITs, the ECM protein fibronectin and SLRPs [316]. Disabling the circadian clock causes abnormal collagen fibrils and collagen accumulation [330]. Indeed, a circadian clock mechanism of protein homeostasis has been identified, with nocturnal procollagen synthesis and daytime collagen fibril assembly [330]. Collagen fibrils are stabilized by covalent cross-links, which provide ECM and tissues with their mechanical properties (i.e., resistance to traction). Covalent cross-linking of fibrillar collagens is initiated by the enzymes of the LOX family [40]. These copper-dependent amino acid oxidases catalyze the oxidative deamination of specific lysine and hydroxylysine residues into aldehydes, which spontaneously react with other lysine and lydroxylysine residues to form first reducible cross-links, and then mature cross-links [331]. A nonenzymatic process, glycation, leads to the formation of several glycation end products, which form cross-links in collagens [332].

Collagens are post-translationally hydroxylated on proline and lysine residues [333] by prolyl and lysyl hydroxylases [334,335]. Hydroxylated lysine residues can be O-glycosylated. Collagens are glycosylated to various extent, collagen type IV being more glycosylated than fibrillar collagens [336]. Post-translational modifications of collagens can be identified by mass spectrometry [337]. The heat shock protein Hsp47 acts a molecular chaperone to prevent local unfolding or aggregate formation of procollagen in mammalian cells [338], and belongs to the network, which regulates collagen I proteostasis (folding, quality control, and secretion) [339]. Mutations in genes encoding collagens I, III and V are associated with Ehlers-Danlos syndrome, an heterogeneous group of hereditary disorders 'with common features including joint hypermobility, soft, and hyperextensible skin, abnormal wound healing and easy bruising' [340], and mutations in *COL2A1* are associated with skeletal disorders [341].

Fibril-associated collagen with interrupted triple helices

The FACITs include collagens IX [342], XII [343], XIV, XVI [318], XIX [344], XX [345], XXI [346], and XXII, a marker of tissue junction [347]. Collagens IX and XII carry GAG chains and can be thus also considered as PGs. Collagen IX is found in cornea and in cartilage where it is associated with the surface of collagen II fibrils, the interaction between both collagens being stabilized by covalent cross-links [320,342]. Collagen IX mutations caused skeletal dysplasias [314,315]. Collagen XII is associated with the surface of collagen I fibrils, and protects bone and muscle integrity by organizing collagen fibrils [343]. Recessive and dominant mutations in COL12A1 cause an overlap syndrome involving muscle and connective tissue in humans and mice [348]. Collagen XIV plays a role in growth and structural integrity of the myocardium [349]. Collagens XII and XIV collagens interact with COMP (also called thrombospondin-5) in skin and are colocalized in the superficial papillary dermis of normal skin, and in anchoring plaques [350]. Collagen XVI is expressed in various tissues including skin and cartilage, where it connects and organizes fibrillar networks [318]. It also promotes tumor cell adhesion and glioma cell invasiveness. Collagen XIX, which is present in the BM zone, is involved in the differentiation of muscle cells and in the CNS development [344]. It is also a prognostic biomarker of amyotrophic lateral sclerosis progression [351].

Collagen XX is a minor component of sternal cartilage, cornea, and tendon in chick embryo [345]. *COL20A1* is, with *COL14A1*, a potential candidate gene for striate palmoplantar keratoderma [352,353]. Collagen XXI is expressed in numerous tissues (e.g., heart, stomach, kidney, skeletal muscle, and placenta) [354]. There is a significant decreased *COL21A1* copy number in large extended Malay families with nonsyndromic cleft lip and/or palate [355]. Collagen XXII is expressed in the myotendinous junction [347], where its expression level influences muscle injury risk in athletes [356], and at the articular surface of joint cartilage. *COL22A1* maintains vascular stability in

zebrafish and mutations in *COL22A1* could be associated with intracranial aneurysms in humans [357].

Collagen forming beaded filaments

Collagen VI forms 100-nm periodic end-to-end beaded filaments [358] and contains vWF A and Kunitz family of serine protease inhibitors domains. It plays a key role in skeletal muscle and mutations in the *COL6A1*, *COL6A2*, and *COL6A3* genes encoding collagen VI chains lead to congenital muscular dystrophies [359]. Collagen VI is required for the structural and functional integrity of the neuromuscular junction [360] and is a component of the peripheral and CNS [358].

Like collagen VI, collagen XXVIII contains vWF A and Kunitz family of serine protease inhibitors domains [361]. It is located in the BMs around Schwann cells and is associated with nonmyelinated regions of the peripheral nervous system such as the nodes of Ranvier [362] but it has not been reported to form specific supramolecular assemblies so far. The C-terminal Kunitz domain is collagen XXVIII is often proteolytically processed in zebrafish [363]. The lack of collagen XXVIII in mice causes age-related insufficiency in retinal pigment epithelium proteostasis in mice [364]. *COL28A1* gene is one of the prognostic feature genes identified in glioblastoma multiforme, the most common type of brain cancer [324].

Collagen forming anchoring fibrils

Collagen VII forms anchoring fibrils located at the dermal-epidermal junction. Loss of collagen VII induces inflammation that promotes keratinocyte-driven, progressive fibrosis [365]. Collagen VII plays a dual role in skin wound healing by promoting wound closure, supporting fibroblast migration and regulating their production of cytokine in the granulation tissue [366]. It is also expressed in podocytes of normal human kidney and in endothelial cells of the glomerular filtration barrier [367]. Mutations in *COL7A1* result in epidermolysis bullosa [368], and autoantibodies directed against collagen VII triggers epidermolysis bullosa acquisita [369].

Network-forming collagens

Collagens IV, VIII, and X form networks (Table 2). The three molecular isoforms of collagen IV occur in BMs. They associate via their N and C termini to form networks, which are stabilized by covalent cross-linking mediated by LOX-2 at the N terminus [370] and peroxidasin at the C terminus [371]. The sulfilimine cross-link, formed by peroxidasin, contributes to kidney tubular BM stiffness [372], and halogens play a role in building collagen IV scaffold [373]. Mutations in *COL4A3*, *COL4A4* and *COL4A5* induces the Alport syndrome, a hereditary kidney disease associated with alterations in the glomerular BM [374]. Mutations in *COL4A1* causes hereditary angiopathy, nephropathy, aneurysms, and muscle cramps syndrome [375].

Collagen VIII is found in a specialized BM, the Descemet's membrane, where it forms hexagonal lattices. Collagen VIII is synthesized by endothelial cells and vascular smooth muscle cells, and *COL8A2* regulates the fate of corneal endothelial cells [376]. Collagen VIII expression is upregulated in atherosclerosis [377], and lack of collagen VIII reduces myofibroblast differentiation and fibrosis in mice with heart failure [378]. Collagen X is mainly restricted to the hypertrophic zone of the growth plate, where it regulates endochondral ossification of articular cartilage [379], and is required for proper hematopoietic development [380]. *COL10A1* expression is increased in a number of tumors and is associated with tumor vasculature [381]. Mutations in the *COL10A1* gene are associated with Schmid-type metaphyseal chondrodysplasia [382], a very rare inherited disorder characterized by short stature with abnormally short arms and legs (short-limbed dwarfism) and bowed legs.

Membrane-associated collagens

Four collagens (XIII, XVII, XXIII, and XXV) are membrane proteins. With their triple-helical domains are located in the ECM. The membrane-associated collagens with interrupted triple helices (MACITs) include collagens XIII, XXIII, and XXV [383,384]. Collagen XIII is involved in the development, differentiation and maturation of musculoskeletal tissues and vessels and in maintaining tissue integrity [385]. It also plays a role in the formation and function of the neuromuscular system at the neuromuscular synapses. Its correct expression and localization are crucial for motor synapse formation and function [386,387]. Furthermore, collagen XIII promotes cancer metastasis and enhances anoikis resistance [388]. Collagen XXIII is localized at the surface of basal keratinocytes, and plays a role in cancer cell adhesion, anchorage-independence and metastasis [389]. Collagen XXV is synthesized by neurons and is found in Alzheimer amyloid plaques where it binds to the amyloid β-peptide [390]. Furthermore, *COL25A1* triggers Alzheimer's disease-like pathology *in vivo*. Collagen XXV promotes myoblast fusion into myofibers during

myogenic differentiation and muscle formation [391]. The other membrane collagens are also expressed in the brain [392].

Collagen XVII is mainly expressed by basal keratinocytes, and is located in hemidesmosomes. It plays a key role in epidermal-dermal junction and serves a niche for hair follicle stem cells [393]. Changes in collagen XVII expression have been reported in many epithelial cancers, and the shedding of its ectodomain is strongly associated with tumor invasiveness in squamous cell carcinoma [394].

Multiplexins

This collagen subfamily includes collagens XV [301] and XVIII [395,396], which both comprise several triple-helical domains interspersed with noncollagenous domains, and bear GAGs chains. Collagen XV connects striated collagen fibers subjacent to the BM [397], and regulates cell adhesion and migration [398]. The deficiency in collagen XV predisposes to cardiomyopathy [399], but the lack of collagen XV is protective after ischemic stroke in mice [400]. Collagen XVIII is required for the maintenance of BM integrity and regulates cell survival, stem or progenitor cell maintenance and differentiation and inflammation [396]. It also plays a role in the development of the eye, and mutations in the *COL18A1* gene cause the Knobloch syndrome associated with encephalocele and vitreoretinal degeneration [382]. Both collagens XV and XVIII release a bioactive C-terminal fragment, called restin and endostatin, respectively, upon limited proteolysis [401,402].

Collagen bioactive fragments

Collagens are degraded by secreted and membrane-bound MMPs, secreted gelatinases, and lysosomal cysteine proteases [403]. Numerous collagen types (e.g., collagens I, IV, VI, XV, XVIII, and XIX) release bioactive fragments called matrikines or matricryptins [401,402] (Table 2) upon limited proteolysis by zinc metalloproteinases (matrixins, adamalysins and astacins), cysteine proteinases and serine proteases [404,405]. The biological activities of matricryptins are mediated by the interaction network they form with integrins and GF receptors [121]. Their release can be modulated in diseases, for example, in those affecting BM turnover [406]. They regulate numerous biological processes such as autophagy, angiogenesis, adipogenesis, fibrosis, tumor growth, metastasis, and wound healing. A number of collagen fragments (e.g., arresten, canstatin, endostatin, restin, tumstatin) have anti-angiogenic and/or antitumoral properties [402,407]. In contrast, endotrophin promotes tumorigenesis and is a target for anticancer therapy [408]. It is also involved in the development of liver diseases (i.e., nonalcoholic steatohepatitis and hepatocellular carcinoma) [409], whereas endostatin is antifibrotic [410]. Canstatin is anti-lymphangiogenic and modulates voltage-dependent calcium channel activity in rat cardiomyocytes [411], and endostatin-derived peptides are antifibrotic [410]. ECM bioactive (matricryptins and matrikines) have potential applications in therapeutics, tissue engineering [402,412], and cosmetics [413]. Recombinant endostatin has been approved by the State Food and Drug Administration of China as a treatment for late stage non-small-cell lung carcinoma [414].

Collagen receptors

Collagens interact with several receptor families without (e.g., integrins) or with (DDRs) tyrosine kinase activities [415,416]. Collagens bind to α1-integrins (i.e., α1β1, α2β1, α10β1, and α11β1 integrins) [415]. α11β1 integrin is a major collagen receptor on fibroblastic cells [417], and α10β1 integrin is a critical collagen receptor critical in skeletal development [418]. DDR1 and DDR2 can be activated by collagens I, II, III and V, whereas collagen IV is also able to activate DDR1 [419,420]. Other collagen receptors include Tumor Endothelial Marker 8, which regulates cell-collagen interactions [421], G protein-coupled receptor 56 (GPR56), which binds to collagen III, its major ligand in the developing brain [422,423]. Furthermore, three members of the immunoglobulin superfamily of receptors bind to collagens. They are glycoprotein VI, which is the major collagen binding-site on native platelets [424], the leukocyte-associated immunoglobulin-like receptor-1 [425,426], and the osteoclast-associated receptor [427], which interacts with collagens I and II and promotes osteoclastogenesis.

Full-length DDRs are multidomain type I TM glycoproteins, comprising an extracellular discoidin domain, a TM region, and an intracellular segment of variable length that included a kinase domain [428,429]. The DDR1 gene comprises seventeen exons that are alternatively spliced to form five different isoforms, DDR1a to DDR1e, while only one isoform for DDR2 has been identified so far [430].

The reason of DDR1 isoform diversity is still unknown, although it seems that structural differences may be necessary to activate distinct signaling pathways. It should be noted that the extracellular and TM regions of DDR1 isoforms are identical, and that

alternative splicing affects the intracellular catalytic domain leading to length modification (DDR1a to DDR1c) and, in some isoforms (DDR1d and DDR1e) to kinase inactivation. Accumulated evidence suggests that the most frequently expressed isoforms are DDR1a and DDR1b. DDRs can bind native fibrillar collagens I and III with similar affinities but differ in their binding to nonfibrillar collagens. It has been observed that DDR1 can bind collagens type IV, VIII and XV, whereas DDR2 binds collagens II and X, the binding occurring through their globular discoidin domain.

Collagen binding to DDRs induces their autophosphorylation like other RTKs [431,432]. As shown for other RTKs, DDRs regulate key cellular processes like cell migration, proliferation, differentiation, and survival. Moreover, DDRs control remodeling of ECMs through the control of MMPs' expression and activity, which is a key functional consequence of DDR binding to collagen and results mainly in the induction of MMPs expression and/or activation and have overlapping functions with collagen binding integrins by both DDR1 and DDR2.

The ECM-based tissue elasticity: elastic fibers and elastin

The extracellular assemblies that account for the necessary elasticity and extensibility are the elastic fibers. They are essential for the physiological function of many organs such as arteries, skin, tendons, or lungs, which undergo reversible and repetitive deformation. Elastic fibers consist of two morphologically distinguishable components: a mantle of longitudinally aligned fibrillin-based microfibrils and a dense core of cross-linked elastin, which accounts for over 90% of the fiber content. The microfibrils are 10- to 12-nm-wide filaments, which have a beads-on-a-string appearance [433]. They provide the tissues with long-range elasticity, especially with participation of elastin when it is deposited on a microfibrillar scaffold. Microfibrils are formed mainly from fibrillins [434], but several other proteins are known to be associated with microfibrils [433]. Among them are the microfibril-associated glycoproteins (MAGPs) [435], elastin microfibril interfaces [436], a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) and ADAMTS-like proteins [437] as well as latent TGFβ binding proteins (LTBPs, such as LTBP-4) [438].

The other major component, elastin, is an insoluble biopolymer made up of units of its soluble precursor TE. TE's primary structure is characterized by alternating hydrophobic and hydrophilic domains, which are encoded by distinct exons, and thus, TE's domain structure maps the exon organization of the gene. The hydrophilic domains contain either Lysyl-Alanine (KA) or Lysyl-Proline (KP) motifs [439] and are involved in covalent cross-linking induced by LOX or and LOXL enzymes, leading to mature elastin. The hydrophobic domains, however, are responsible for elasticity and are involved in cell interactions [440]. Elastin's primary transcript undergoes extensive alternative splicing resulting in numerous isoforms without affecting the reading frame. The expression of several of these isoforms in human tissues has been proven in some studies [441].

Under healthy conditions, mature elastin is metabolically stable over the species' lifespan. Its half-life in humans has been determined to be > 70 years [442]. One of the reasons for this exceptional durability is elastin's high resistance to proteolysis, which is mainly caused by its vastly cross-linked nature and the extremely dense packing of the molecules. In its mature form, elastin is hydrophobic and completely insoluble, but its hydrophobic hydration is required for its elastic properties [443].

Elastogenesis (Fig. 5) is initiated with the expression of fibrillins and their assembly into microfibrils, which serve as a scaffold for the subsequent TE deposition [444]. The fibrillin network can undergo cross-linking, which further stabilizes the three-dimensional bundle structure. The cross-links reported to date are intermolecular disulfide bonds [445] and ε(γ-glutamyl)lysine cross-links that are catalyzed by members of the transglutaminase family [446].

The expression of the TE monomer takes place in elastogenic cells. TE undergoes rapid self-association in an endothermic, entropically-driven process referred to as coacervation [447] leading to the formation of distinct globular aggregates on the cell membrane [448]. PGs may interact with this domain facilitating the correct alignment of the TE monomers [449]. The alignment of TE and the subsequent cross-linking is further promoted by fibulin-4 and fibulin-5, which mediates the association between TE and the extracellular Cu^{2+}-dependent amine oxidase, LOX [450]. LOX and LOXL enzymes catalyze the oxidative deamination of the ε-amino group of Lys residues to the highly reactive α-aminoadipic acid-δ-semialdehyde, termed allysine [440] leading to the formation of a variety of cross-links including desmosine and its isomer isodesmosine [441].

Due to its long half-life, elastin is subjected to various chemical reactions, which induce a molecular aging responsible for progressive alterations of its

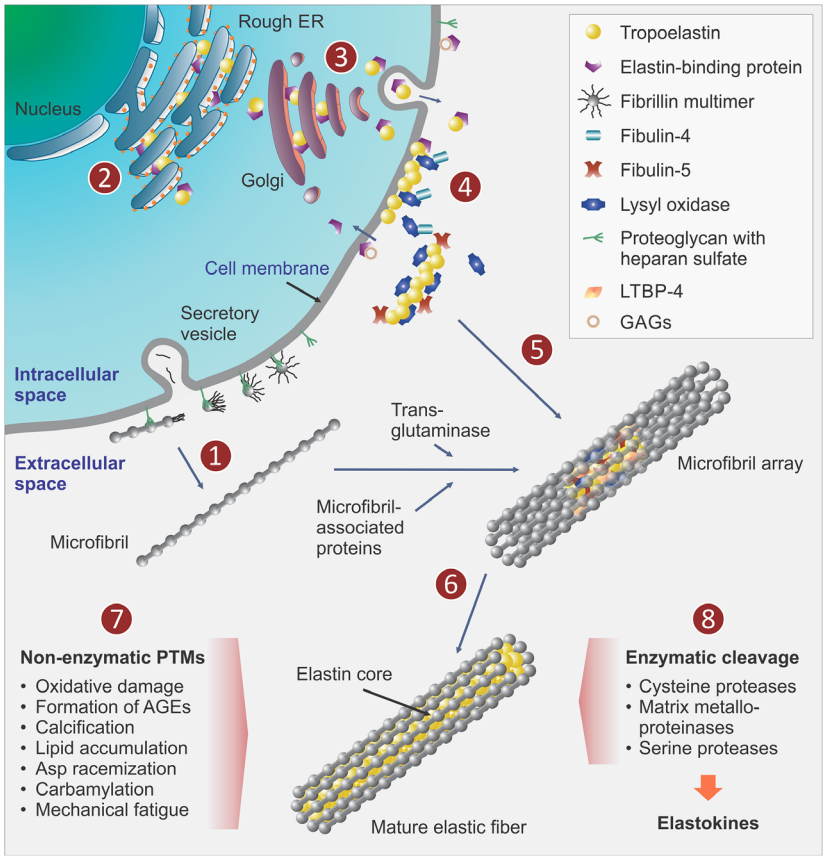

Fig. 5. The elastic fiber assembly. (1) Fibrillin and microfibril-associated proteins are secreted into the extracellular space, multimerize and form the microfibrillar array. (2) TE is synthesized on the rough endoplasmic reticulum where it binds to the chaperone EBP. (3) The EBP-TE complex is transported through the Golgi apparatus and secreted to the cell membrane. (4) TE is released from the chaperone and forms globules at the cell surface, while EBP dissociates as result of the interaction with GAGs. Fibulin-4 is important for the chain alignment of TE mediating the interplay with LOXs. The oxidation of Lys residues is followed by various condensation reactions leading to the formation of covalent intra- and intermolecular cross-links. (5) After the cluster of TE molecules reaches a critical size, it is moved from the plasma membrane through the extracellular space. Fibulin-5 is thought to direct the premature elastin to fibrillin microfibrils. (6) The elastin aggregates fuse into larger assemblies with support by LTBP-4 and are subsequently further cross-linked. (7) Throughout life, elastin undergoes various alterations caused by nonenzymatically processes and (8) proteolytic cleavage. The latter leads to the release of bioactive peptides, so-called elastokines. EBP, Elastin Binding Protein; ER, Endoplasmic Reticulum; LTBP, Latent TGFβ Binding Protein; PTMs, Post-Translational Modifications.

structural and functional properties including oxidation, aspartic acid racemization, glycation or carbamylation [451–453]. This molecular aging of elastin is concomitant to organism chronological aging but can also be intensified during chronic diseases such as diabetes mellitus, end-stage renal disease, or atherosclerosis [454,455]. The consequences of elastin's molecular aging are multiple, ranging from direct impacts on structural and mechanical properties of this matrix protein to inappropriate effects on cells [456–458].

Beside these chemical modifications, elastin suffers processes of mechanical fatigue [459,460]. The impairments or even ruptures of elastin lead to a decrease of elastic fiber function, and to a transfer of mechanical stress to other extracellular components such as collagen fibers, drastically altering tissue mechanics [461]. Moreover, the failure of elastic fibers is further induced by the action of members of several classes of extracellular proteases, elastases. They belong to three classes of families: serine proteinases with cathepsin G, proteinase 3 and neutrophil elastase [462–464], MMPs including MMP-2, -7, -9, -12 and -14 [465–467] and the cysteine proteinases cathepsins K, L, S and V [468,469]. Besides the functional impairment, elastin degradation leads to the release of bioactive peptides named elastokines and belonging to the matrikine family [470,471].

The released bioactive EDPs [470,472] are known to induce a variety of biological effects. These include cell adhesion, chemotaxis, migration, proliferation, proteinase activation, angiogenesis, and apoptosis [473]. They have been clearly shown to be involved in several pathophysiological processes such as cancer progression [474,475], emphysema and vascular diseases progression and are now considered as central modulators of the cardiovascular continuum [476].

The human elastin receptor was originally identified as a 67 kDa peripheral membrane protein called elastin-binding protein (EBP) that binds to elastin and laminin fragments [477]. EBP interacts with the 55 kDa cathepsin A/protective protein and the 61 kDa membrane-bound neuraminidase-1 (Neu-1) to form a ternary complex called elastin receptor complex (ERC) [478]. The EBP subunit [479] displays two functional-binding sites comprising (a) the elastin site on which EDPs binding is directly involved in the generation of intracellular signal transmission, and (b) the galactolectin site whose occupation by galactosugars leads to EDPs release and ERC dissociation [480]. EDPs binding to EBP leads to Neu-1 activation, which locally catalyzes the conversion of the GM3 ganglioside into lactosylceramide (LacCer), an essential second messenger of ERC signaling pathways [481,482]. EDPs have been shown to induce in fibroblasts the activation of multiple tyrosine kinases including MEK1/2 and ERK1/2 through PKA and PI3K-dependent mechanisms [483]. In endothelial cells, this MAPK signaling has been shown to be triggered through PI3K/Akt/endothelial nitric oxide synthase/nitric oxide/protein kinase G pathway module [484]. Finally, it has been shown in smooth muscle cells that EDP binding on ERC triggers the activation of Gi proteins, the opening of L-type calcium channels, and then cell proliferation [485]. Neu-1 plays a key role in these signaling pathways and several studies have been conducted to further elucidate its involvement in ERC signaling in different pathophysiological processes [481,486–488]. In addition to its role in the production of the second messenger LacCer, it has been demonstrated that a distinctive dimerization process is required for its catalytic activity. Indeed, two potential dimerization sequences, corresponding to two TM domains (148–168 and 316–333 residues), have been found within human Neu-1. Point mutations in the 316–333 TM domain inhibit significantly dimerization and sialidase activity of Neu-1 [489]. Moreover, an increasing number of studies also indicate that Neu-1 plays an important role in modulating the activation of numerous membrane receptors such as the insulin receptor, c-Met, IGFR, PDGFR, or CD36 by desialylation [488,490–492]. As a whole, these data imply that Neu-1 may be one of the crucial factors of the membrane signalosome from the lipids but also the protein points of view (Fig. 5). All these signaling mechanisms are responsible for the deleterious influence of elastokines on the precited pathologies and research efforts are oriented toward the definition of a specific way to inhibit the processes in which they are involved.

Laminins: the three-armed ECM adhesion proteins

Laminins are HMW (400–900 kDa) heterotrimeric adhesion proteins found in BMs, which are thin sheets of highly specialized extracellular protein structures that surround muscle, fat and Schwann cells. In essence, laminins self-polymerize into a cell-associated network and are crucial for the formation and function of BMs. Laminins are found in worms, flies, and mammals and are composed of an α, a β and a γ chain, each encoded from a distinct gene. Five α, three β, and three γ chains have been identified in vertebrates, and they can assemble into more than 16 different isoforms (Fig. 6). The laminins are named according to their chain composition and the prototype laminin-111 (composed of an α1, β1, and γ1 chain) was the first laminin isoform to be discovered more than 40 years ago. Hence, laminin-111 is the most extensively studied laminin isoform but at the same time it has a relatively restricted expression pattern in adult humans. Overall, laminins are expressed in a tissue-specific manner and BMs contain at least one laminin isoform (and some several; Fig. 6) [145,493–498].

The laminin structures have been elucidated by rotary shadowing electron microscopy, cDNA sequencing, and protein crystallographic studies. All laminin chains share a common domain structure with globular and rod-like domains. The α1, α2, and α5 chains assemble into the characteristic cross-shaped structure with three short arms and a long arm, but laminins also form T-, Y-, or I-shaped structures (Fig. 6). The three short arms contain disulfide-rich domains and the N-terminal globular domains (LN domain). These globular domains are found in a majority of the laminin polypeptides and are required for laminin polymerization. Hence, laminin isoforms devoid of the N-terminal globular domains (α4, α3A, and γ2 chains) are not able to polymerize but are nevertheless incorporated into BMs, possibly by interactions with other BM components. The long arm of the laminin molecule is an α-helical coiled coil of all three

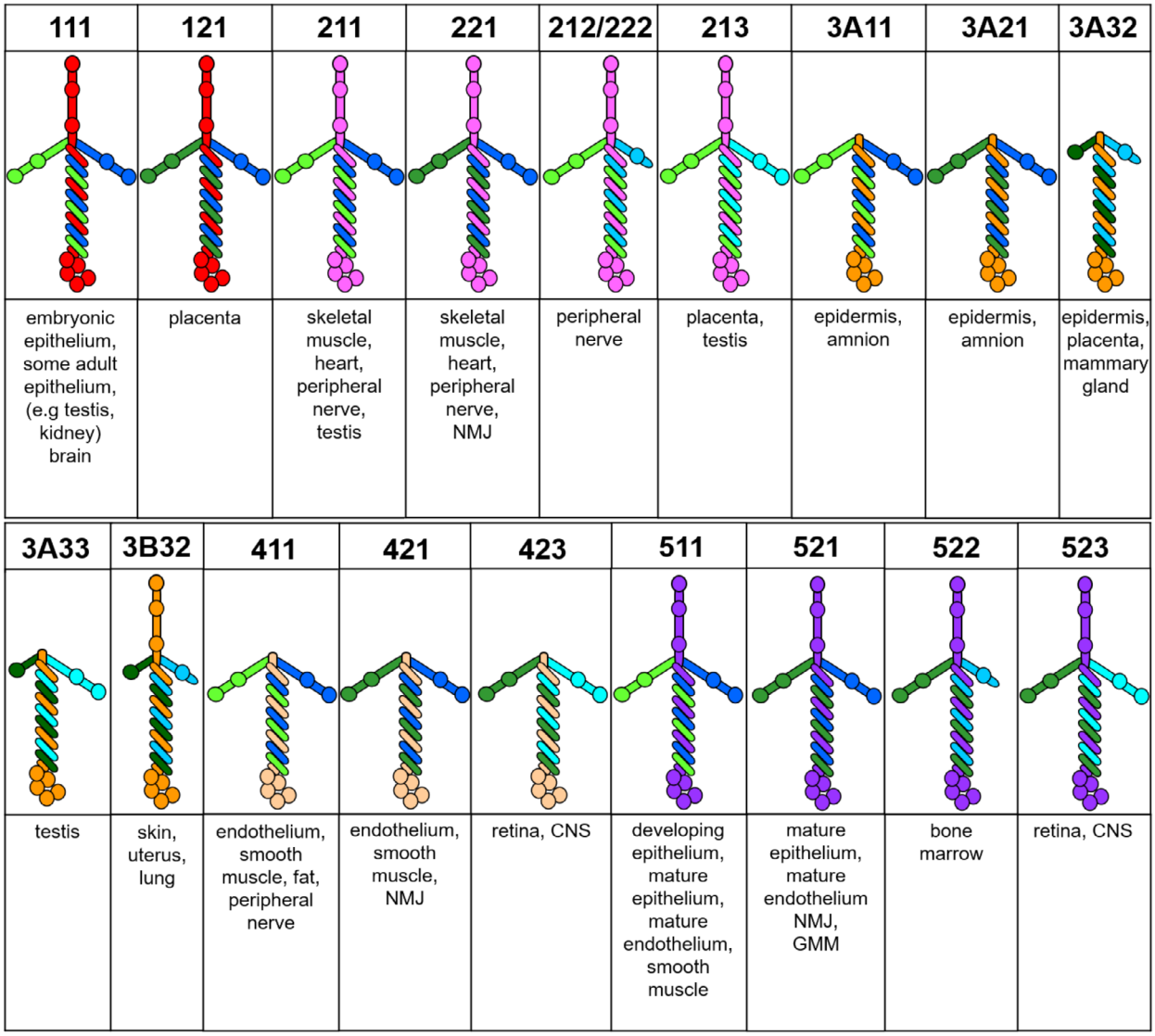

Fig. 6. Schematic representation of laminin trimers and their main sites of expression. Laminins are composed of three polypeptide chains; α, β and γ. Five α (α1–α5), three β (β1–β3) and three γ (γ1–γ3) can assemble to form different trimeric molecules named according to their chain composition. Laminin-111, for example, is composed of α1 (red), β1 (light green) and γ1 (blue) chains. A majority of the depicted laminins have been biochemically isolated but the existence of some is based on co-immunoprecipitation and immunohistochemical analyses. Laminins form cross-shaped, T- (or Y-) shaped or rod-shaped structures with N-terminal globular domains, a coiled-coil region through which the three chains are assembled and C-terminal globular domains. Laminins self-polymerize through interactions with their N-terminal globular domains and bind to cell surface receptors via their C-terminal globular domains. NMJ, neuromuscular junction; GBM, glomerular basement membrane. This figure has been reproduced from Ref. 495.

chains, ending with the C-terminal globular domain (LG domains) of the α chain. The laminins are attached to the cell surface via binding of the LG domains to various cell surface receptors, such as integrins and α-dystroglycan to mention a few, and the structures of the integrin- and dystroglycan-binding fragments of laminins have been elucidated at the atomic level. Laminins interact with a wide variety of other BM proteins (e.g., perlecan, nidogen, and agrin). Hence, laminins form supramolecular networks that are vital for embryogenesis and multiple adult organs and systems [55,145,493–496,498].

The generation and characterization of loss-of-function mouse models have greatly increased our understanding of laminin functions. All genes encoding laminin chains have been constitutively knocked out in mice and several also in specific tissues. In addition, some double knock-outs have been produced as well

as various knock-ins [55]. Mice devoid of laminin α1 chain die very early due to defects in the Reichert's membrane, an extraembryonic BM that is only present in rodents. Absence of laminin α1 in the embryo does not affect embryonic development but leads to retinal defects, behavioral abnormalities and aberrant cerebellum formation. Deprivation of laminin α2 chain causes a very severe form of muscular dystrophy and postnatal death and deficiency of laminin α3 chain (as well as β3 and γ2) leads to progressive blistering in the skin after birth and mice die within a few days. Mice devoid of laminin α4 chain display defects in various postnatal organs whereas laminin α5 chain is required for organogenesis. Tissue-specific deletion of laminin α5 chain has also revealed crucial roles in for example lung, kidney, neuromuscular junction and micro- and macro-vessel endothelial function. Mice lacking laminin β1 and γ1, respectively, die very early during embryogenesis due to defects in extraembryonic and embryonic BMs. Inactivation of laminin γ1 chain expression in certain cells have furthermore revealed a large variety of important functions of this laminin polypeptide. Laminin β2-null mice develop postnatal kidney defects and a neuromuscular junction phenotype. In contrast to all these laminin mutant mice, which either die during embryogenesis or display postnatal severe phenotypes, mice devoid of laminin γ3 chain only show minor abnormalities [55,494,495].

Finally, all laminin subunits have now been associated with human diseases. Mutations in *LAMA2, LAMA3, LAMB3, LAMC2,* and *LAMB2* cause rare but severe congenital disorders; congenital muscular dystrophy (a skeletal muscle disease), junctional epidermolysis bullosa (a skin blistering disease) and Pierson syndrome (a kidney disease with ocular abnormalities) [499–502]. Notably, the corresponding mouse models mirror the human conditions very well and are thus excellent animal models for studying disease mechanisms and treatment strategies. Strikingly, an overlying spectrum of brain phenotypes are features of mutations in *LAMA1*, *LAMA2*, *LAMB1*, *LAMC1* and *LAMC3* [503–507]. Mutations in *LAMA4* lead to dilated cardiomyopathy [508] and *LAMA5* mutations have recently been implicated in a wide range of disorders [509–511] (Table 3). In summary, laminins are of uttermost importance for BM integrity, early embryonic development, organogenesis and for the maintenance and survival of many tissues. During the last decade it has also become evident that recombinantly expressed laminins are significant tools in the generation of xenogeneic-free and defined cell differentiation protocols [512].

Integrins: The adhesion and signaling mediators between ECM and cells

Integrins structure and the ECM ligands

Integrins are a superfamily of TM cell adhesion proteins and their role is to link the ECM with cell

Table 3. Human laminin genes, chromosome locations, corresponding polypeptides, genetic disorders and OMIM phenotype entries. EMI, emilin, FNIII, fibronectin III, NC, noncollagenous, TSP, thrombospondin.

Gene	Cytogenetic location	Protein	Disease	OMIM
LAMA1	18p11.31	α1	Poretti-Boltshauser syndrome	615960
LAMA2	6q22.33	α2	Congenital muscular dystrophy (partial or complete absence)	607855, 618138
LAMA3	18q11.2	α3	Epidermolysis bullosa, junctional, Herlitz type	226700
			Epidermolysis bullosa, junctional, non-Herlitz type	226650
			Laryngoonychocutaneous syndrome	245660
LAMA4	6q21	α4	Cardiomyopathy, dilated, 1JJ	615235
LAMA5	20q13.33	α5	Connective tissue abnormalities	na
			Presynaptic myasthenic syndrome	na
			Bone dysplasia	na
LAMB1	7q31.1	β1	Lissencephaly 5	615191
LAMB2	3p21.31	β2	Pierson syndrome	609049
			Nephrotic syndrome, type 5, with or without ocular abnormalities	614199
LAMB3	1q32.2	β3	Epidermolysis bullosa, junctional, Herlitz type	226700
			Epidermolysis bullosa, junctional, non-Herlitz type	226650
			Amelogenesis imperfecta, type IA	104530
LAMC1	1q25.3	γ1	Dandy-Walker malformation	na
LAMC2	1q25.3	γ2	Epidermolysis bullosa, junctional, Herlitz type	226700
			Epidermolysis bullosa, junctional, non-Herlitz type	226650
LAMC3	9q34.12	γ3	Cortical malformations, occipital	614115

cytoskeleton. Integrins have important role in transduction of intracellular signaling pathways, as well as for the interactions with ECM molecules. Integrins form heterodimers between the α-subunits and β-subunits, with at least twenty-four unique combinations. Integrins α-subunits are consisted of eighteen different types and β-subunits of eight different types [513]. Integrin α- and β-subunits are type I TMEMs composed of a large extracellular domain, a single TM domain and a short cytoplasmic domain [513]. Among the different subunits some of them are abundant in more heterodimers, such as β1 in twelve different heterodimers and αv in five different heterodimers [514]. The extracellular domain, especially the αI domain, provides ligand specificity with several different ECM macromolecules or counter receptors on adjacent cell surfaces. There are four broadly grouped categories: (a) The arginine-glycine-aspartic acid, or RGD motif, (b) Laminin receptors, (c) Leukocyte-specific receptors, (d) Collagen receptors (Table 4). The binding of ECM ligands either in both subunits or on a specific domain of the α-subunit as well as the different combinations between various α-subunits and β2 integrins in the case of hematopoietic cells, provide ligand specificity to integrins heterodimers. Regarding RGD-binding integrins, the RGD ligand binds to an interface between the α and β subunits, the R residue fitting into a cleft in a β-propeller module in the α subunit, and the D coordinating a cation bound in a vWF A-domain in the β subunit [515]. Another acidic motif, called LDV, is functionally related to RGD, and even though there is no structural information, it is highly possible that binds in a similar way as RGD at the junction between the α and β subunits [516]. Fibronectin, VCAM-1, and MAdCAM-1 are molecules that contain LDV motif and bind to α4β1, α4β7, and α9β1, as well as to β2 subfamily and αEβ7 integrins [516].

Osteopontin is also a binder to α4β1, α4β7, and α9β1 via the SVVYGLR peptide motif [517,518]. An inserted A-domain in the α subunit provides ligand binding specificity to many β-subunit families, such as β1, β2, and β7 subunits. In the case of β2 family specific ligand sites, they possess structural similarities to the LDV motif and the major difference between β1/β7 ligands is that β2 utilizes glutamate instead of aspartate residue for cation coordination (Table 5) [519]. A-domain from α1, α2, α10 and α11 subunits forms heterodimers with β1 and create laminin- and collagen-binding families [516]. Specifically, α2 A domain interacts via GFOGER motif with triple helix of collagen [520]. On the other hand, non-αA domain containing integrins, such as α3β1, α6β1, α7β1, and α6β4, have highly selectivity for laminin ligands.

Table 4. Main categories of integrins based on ligand substrate.

Integrin ligand	Integrin heterodimer
RGD receptors	α5β1, αVβ3, αVβ1, αVβ5, αVβ6, αVβ8, and αIIbβ3
Laminin receptors	α1β1, α2β1, α3β1, α6β1, α7β1, and α6β4
Leukocyte-specific receptors	αLβ2, αMβ2, αXβ2, and αDβ2
Collagen receptors	α1β1, α2β1, α3β1, α10β1, and α11β1

Table 5. Proteins binding to different integrin heterodimers.

Binding protein	Integrin heterodimer
Collagen	α1β1, α5β1, α10β1, α11β1, αXβ2
Laminin	α3β1, α6β1, α6β4, α7β1, α1β1, α2β1, α10β1
Thrombospondin	α3β1, α2β1, α4β1, αvβ3, α3β1, αIIbβ3
Fibronectin	αvβ3, αvβ6, αIIbβ3, αvβ1, α5β1, α8β1, α4β1, α4β7
OPN	α4β7, α4β1, α9β1, α8β1, α5β1, αvβ1, αvβ6, αvβ3, αvβ75
Bone sialoprotein	αvβ3, αvβ5
Developmental endothelial locus-1	αvβ3, αvβ5
Vitronectin	αvβ3, αvβ5, α8β1, αIIbβ3
vWF	αvβ3, αIIbβ3
TN	αvβ3, α8β1, α9β1
Platelet endothelial cell adhesion molecule 1	αvβ3
Mucosal addressin cell adhesion molecule 1	α4β7, α4β1
Intercellular cell adhesion molecule	αXβ2, αMβ2, αLβ2, αDβ2
Latency associated peptide transforming GF	αvβ1, αvβ8, αvβ6, αvβ3
Fibrillin	αvβ3
Fibrinogen	αvβ3, αIIbβ3, αXβ2, αMβ2
Factor X	αMβ2
Inactivated complement component C3b	αXβ2, αMβ2
E-cadherin	αEβ7

Integrin activation and roles in physiology and disease

The regulation of integrin activity was first discovered in blood cells [521]. Platelet and leukocyte integrins are the most well-studied systems, but integrins are widespread in many different cell types with significant role in angiogenesis, cell migration and ECM remodeling. Integrin activation involves talin binding to cytoplasmic tail of β1 subunit [522]. Binding of talin leads to conformational change of both subunits by separation of cytoplasmic region and extension of

extracellular region providing higher affinity with the ligands. After initial binding of talin to cytoplasmic domain, other effectors are binding to the cytoplasmic domain and support the activation, as well as the clustering with the different adhesive complexes (Fig. 7) [523]. Kindlin, is one of the major effectors of inside-out integrin signaling and is involved in ECM interactions and cell spreading via either activation of β- subunit cytoplasmic tail or recruiting of focal adhesion molecules as paxillin, which activates RHO GTPase RAC1 and directly polymerize actin by Arp2/3 complex leading cell spreading [524]. During adhesion maturation talin-induced integrin activation is maintained by binding of tensin-1 and tensin-3 to β1- subunit. Even though the exact integrin-tensin mechanism is not elucidated, there is a switch from talin binding to tensin binding during adhesion maturation, because of the overlapping of their binding sites on β1 subunit [525]. Integrins-mediated cell-ECM interactions trigger the formation of complexes and regulate downstream signaling pathways, such as activation of FAK, SRC, AKT, and ERK pathways and small GTPases of the RHO family [526,527]. These pathways are crucial for integrin-mediated cell behavior, such as cell death or survival, regulation of cytoskeleton dynamics, cell migration via controlling cell polarity, and tissue integrity [528].

Extracellular matrix molecules regulate the structure and the biochemical signals, which affect a wide range of processes during embryonic development and cancer

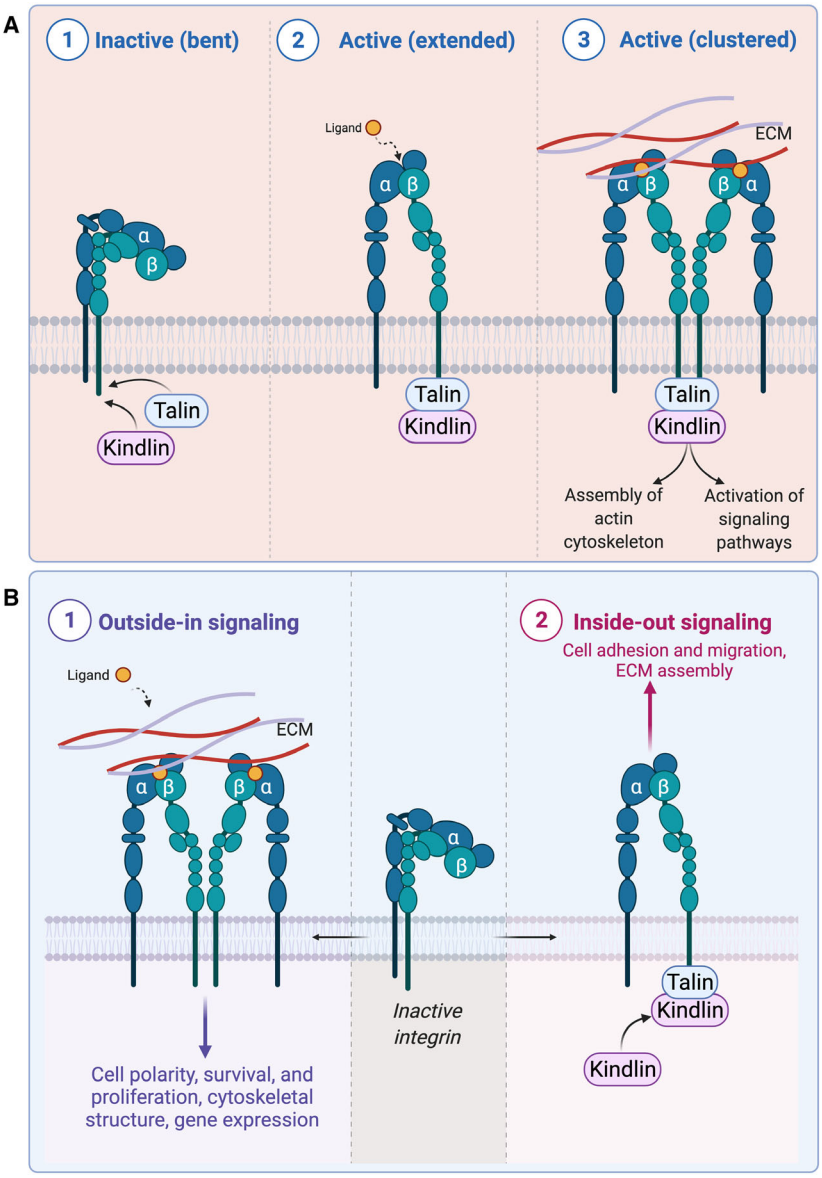

Fig. 7. Schematic representation of integrins activation process. (A) Integrins as inactive molecules characterized by bent extracellular domain. After binding of talin and kindlin integrins are activated and the extended extracellular domain binds to ligands, which trigger the activation of downstream signaling pathways and regulate actin cytoskeleton assembly. (B) Integrins activation can lead to either outside-in signaling, after binding of ECM molecules on their extracellular domain leading to cell polarity differences, cell survival and proliferation and cytoskeleton rearrangement, or to inside-out signaling, after binding of talin and kindlin to cytoplasmic domain, which affect the cell adhesion and migration. Adapted from 'Outside-in and Inside-out Integrin Signaling Pathways & Integrin Structure and Activation', by BioRender.com (2020). Retrieved from https://app.biorender.com/biorender-templates.

progression [529]. In this ECM network, integrins have outstanding role on regulation of cell migration by controlling the substrate-bound ligands in the ECM, diffusible ligands, or ECM rigidity. Integrin-mediated mechanisms of cell migration control the speed of migratory cells in response to ECM rigidity [530]. As the most abundant ECM proteins are fibronectin and collagen, during normal development, the presence of both proteins is crucial for the formation of ECM assembly, since deposition of fibrillar collagen presupposes the fibronectin network [531]. Fibronectin-binding integrins are required for the formation of fibronectin network, since the integrin heterodimers such as α5β1 transmit forces to fibronectin and expose cryptic binding sites necessary for polymerization [532]. During malignant condition the CAFs deposit excessive amounts of ECM components, especially collagen [533]. The increased deposition of collagen is caused by the activation of integrin signaling pathways, such as FAK and YAP/TAZ, which drive the disease progression [534]. In addition to collagen-related cancer progression, recent evidence highlights that deposition of fibronectin leads to cancer invasion in prostate cancer, in which the remodeled fibronectin, via actomyosin-contractility-driven traction forces and α5β1 integrin, generate aligned fibers that promote cell migration [535]. In breast cancer, β1 subunit is increased by TWIST1 and contributes to cancer invasion. TWIST1 is an EMT transcription factor which is related to metastatic tumors with poor prognosis [536]. Integrins are also involved in complexes with GF receptors and syndecans [537]. In MCF-7 breast cancer cell line inhibition of IGFR signaling lead to significant reduction of the adhesive capacity in fibronectin and laminin caused by lower cell surface expression levels of α5β1, αvβ3, αvβ5 and αvβ6 integrins and endocytosis of syndecan-4 [538,539].

The dynamic character of ECM—critical enzymes involved in remodeling

Tissue development, repair, regeneration, and homeostasis require a dynamic character for ECM, called remodeling [5,14]. Any alterations to this well-balanced procedure can lead to pathogenesis [8,129,133]. HPSE is an endoglycosidase that cleaves the side chains of Hep and HS of PGs, a process named shedding. Regardless of its presence in late endosomes and perinuclear lysosomes, under proper stimuli HPSE can be found in the ECM [540]. HPSE together with HYALs, which degrade HA, can modify GAGs in ECM altering their structure and functions. The activity of HPSE defines the fate of Hep and HS binding partners such as GFs, cytokines and enzymes, participating in the remodeling of ECM. Moreover, HPSE via its interaction with TMEMs can activate signaling cascades such as Akt, ERK, p38 and Src. By this way, HPSE can manipulate cell motility, angiogenesis, inflammation, exosome production, and autophagy [541].

Proteinases are a large family of enzymes responsible for the rearrangement of ECM ingredients and the manipulation of cellular function. The metzincin superfamily includes the MMPs and the adamalysin group, with a disintegrin and metalloproteinases (ADAMs) and ADAMTSs to be the main members [3,542]. MT-MMPs and most ADAMs are TMEMs, thus they manipulate the local microenvironment of BMs. ADAMs are mainly sheddases interacting with cytokines, GFs and their receptors and regulate cell migration, adhesion and fate determination [543].

MMP, ADAMTS, and ADAM metalloproteinases

Matrix metalloproteinases (matrixins) and the related ADAMs and ADAMTSs are subfamilies of proteolytic enzymes belonging to the metzincin clan. They have important roles in ECM homeostasis and regulation of autocrine and paracrine signaling. MMPs and ADAMTSs are secreted enzymes, although some MMPs and several ADAMTSs are localized on the cell surface or in the pericellular environment [544]. ADAMs, on the other hand, are TM metalloproteinases that act primarily on membrane-localized substrates.

Matrix metalloproteinases, ADAMTSs, and ADAMs are modular enzymes, containing a conserved catalytic domain and various ancillary domains that regulate substrate selectivity and enzyme localization. As the name suggests, the proteolytic activity of metzincins is dependent on an active site Zn^{2+} ion, which is coordinated by 3 histidine residues in the conserved HEXXHXXGXX(H/D) consensus motif [545]. The glutamic acid residue in this motif activates a Zn^{2+}-bound H_2O molecule to generate the nucleophile that cleaves substrate peptide bonds. Metzincins also have a conserved methionine residue downstream of the catalytic motif, forming the Met-turn that dictates the architecture of the active site.

Matrix metalloproteinases

Physiological and pathological functions

Matrix metalloproteinases were named for the substrates and functions first ascribed to them when they

were discovered, namely degradation of ECM molecules such as collagens in the context of tissue turnover and remodeling. MMPs can act to a wide range of ECM substrates and based on that and their homology, they can be categorized to six groups; collagenases, stromelysins, matrilysis, gelatinases, furin-activated, and other MMPs. Their action is monitored in transcriptional level, in proteolytic activation of the synthesized inactive zymogens and the interaction with endogenous tissue inhibitors such as tissue inhibitors of MMPs (TIMPs). MMPs are crucial during development, tissue remodeling, cellular migration, and apoptosis [14,546]. For example, MMP-1 seems to contribute to bone tissue regeneration through the activation of JNK and ERK signaling molecules, inducing the osteogenic differentiation of bone mesenchymal stem cells [547].

The prototypic MMP, MMP-1, was discovered as the enzyme responsible for degrading type I collagen during resorption of the tadpole tail during metamorphosis. Along with the cysteine protease cathepsin K, the MMP 'collagenases' (i.e., MMP-1, MMP-2, MMP-8, MMP-13, MMP-14) are the only mammalian enzymes capable of cleaving native triple-helical collagen [548]. Subsequently, other members of the MMP family were found to have similarly important roles in matrix turnover, cleaving substrates such as fibronectin, laminin, and elastin during physiological processes such as wound healing and angiogenesis [549].

Subsequently, it has been appreciated that these enzymes also play more subtle roles in protein processing, with their proteolytic activity leading to activation or deactivation of bioactive substrates such as GFs, cytokines, cell surface receptors, and adhesion molecules. As such, MMPs are important regulators of biological processes including inflammation, immunity, tissue repair, and differentiation.

Pathological roles have also been ascribed to MMPs in diseases including cancer, cardiovascular disease, osteoarthritis, and emphysema [550]. MMPs are thought to be particularly important for cancer invasion and metastasis, as a result of their ability to degrade BMs and other ECM barriers. They can additionally promote tumor angiogenesis, growth and survival [551]. Clinical trials of MMP inhibitors in cancer were unsuccessful, largely due to low inhibitor specificity, which led to off-target inhibition of physiologically important metalloproteinases and hence undesirable side effects [552]. Advances in understanding of MMP biology are allowing design of more selective and targeted inhibitors [553] that may be more clinically effective.

Domain architecture

The 23 MMPs in the human genome are synthesized as pre-pro-enzymes, with a signal peptide followed by a pro-domain that maintains latency, the catalytic domain, a linker or hinge region, and, in all but MMP-7 and MMP-26, a C-terminal hemopexin domain that participates in substrate recognition (Fig. 8). For example, the hemopexin domain of collagenolytic MMPs contains an exosite that is essential for collagen cleavage [554]. The membrane-type MMPs contain either a TM domain and short cytoplasmic region or a GPI anchor that tethers them to the cell surface. The 'gelatinases' MMP-2 and MMP-9 contain three fibronectin type II repeats in their catalytic domains that promote interaction with gelatin.

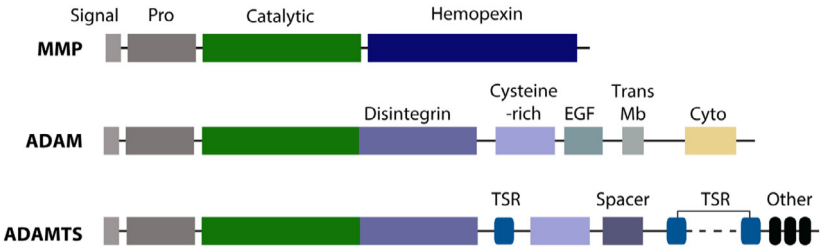

Fig. 8. Domain architecture of metalloproteinases. All metalloproteinases contain a signal peptide, a latency-maintaining pro-peptide that is removed during maturation, and a conserved metalloproteinase catalytic domain containing the HEXXHXXGXX(H/D) consensus motif. These regions are followed by C-terminal ancillary domains that control substrate selectivity and enzyme localization. Most MMPs have C-terminal hemopexin domains, and some (the membrane-type MMPs, MT-MMPs) have additional TM and cytoplasmic domains (not shown). ADAM metalloproteinases contain disintegrin domains adjacent to the catalytic domain, followed by cysteine-rich and EGF-like domains, a TM region and cytoplasmic domains of varying size. ADAMTS metalloproteinases also have disintegrin domains adjacent to their catalytic domains, followed by a thrombospondin repeat (TSR), cysteine-rich and spacer domains, and varying numbers of C-terminal TSR and other domains.

Regulation of activity

In addition to transcriptional and post-transcriptional control of expression, MMPs are synthesized as latent zymogens that are activated either in the extracellular environment by removal of the pro-domain (e.g., by serine proteases such as plasmin) or intracellularly by proprotein convertases such as furin. Activation of proMMP-2 occurs at the cell surface, through interaction with a trimolecular complex of TIMP-2 bound to a dimer of MT1-MMP [555].

Matrix metalloproteinase activity is further controlled by mechanisms such as localization (on the cell surface, pericellularly, or in the ECM) [544] and endocytic clearance (e.g., by low-density lipoprotein receptor-related protein 1, LRP1) [556]. Their activity is additionally inhibited by interaction with their endogenous inhibitors, the tissue inhibitors of metalloproteinases. Post-translational modifications such as glycosylation can also modulate MMP activity, localization, and interaction with substrates and other proteins [557].

ADAMTSs

Physiological and pathological functions

The 19 mammalian ADAMTSs are important for ECM assembly and turnover in adult and embryonic tissues [558]. Prototypic substrates have been identified for many of the ADAMTSs, but there is still much to learn about the *in vivo* functions and substrate repertoires of this structurally complex group of enzymes. These enzymes have narrow substrate specificity and roles the collagen maturation, the cleavage of PGs such as aggrecan, versican and brevican, the homeostasis of blood coagulation and the inhibition of angiogenesis [559,560].

The procollagen N-proteinases ADAMTS-2, ADAMTS-3, and ADAMTS-14 cleave the aminopropeptide of fibrillar procollagens, promoting collagen maturation and ECM assembly [561]. Mutations in *ADAMTS2* cause the rare dermatosparaxis type of Ehlers-Danlos syndrome, characterized by weakening of connective tissue and skin fragility. Additional roles in processes such as TGFβ signaling are emerging [562].

The proteoglycanases ADAMTS-1, ADAMTS-4, ADAMTS-5, ADAMTS-8, ADAMTS-9, ADAMTS-15, and ADAMTS-20 cleave CS-rich PGs such as versican, brevican, and aggrecan [563]. Versican cleavage by ADAMTSs is crucial for morphogenetic events such as cardiac development and interdigital web regression [564]. ADAMTS-4 and ADAMTS-5 are thought to drive aggrecan loss in osteoarthritis, making them potential drug targets [565]. Versican, brevican and aggrecan also have roles in perineuronal net formation, so these ADAMTSs are of interest in synaptic plasticity and neuronal disorders.

ADAMTS-7 and -12 were first characterized for their ability to cleave COMP, contributing to matrix degradation in arthritis [566]. Additional cardiovascular substrates of ADAMTS-7 were recently identified, along with preferential inhibition by TIMP-4, which is selectively expressed in cardiovascular tissues [567].

ADAMTS-13 cleaves vWF multimers into smaller fragments, controlling its interaction with platelets and hence modulating hemostasis. Reduced activity of ADAMTS-13, through genetic mutation or more commonly due to formation of inhibitory autoantibodies, gives rise to the clotting disorder thrombotic thrombocytopenic purpura [568]. Cleavage is regulated by shear stress-induced conformational changes in both vWF and ADAMTS-13 [569].

Domain architecture

As with the MMPs, ADAMTSs are secreted metalloproteinases consisting of a catalytic domain and various combinations of C-terminal ancillary domains. The disintegrin domain forms an extension of the catalytic domain, and is more similar to a cysteine-rich fold than to prototypic disintegrin domains [570]. The C-terminal domains modulate substrate recognition and enzyme localization, with the disintegrin, cysteine-rich, and spacer domains of ADAMTS-1, ADAMTS-4, and ADAMTS-5, for example, greatly enhancing cleavage of aggrecan and versican [571,572]. ADAMTSs also contain variable numbers of thrombospondin motifs and other ancillary domains in some cases.

Regulation of activity

ADAMTSs activity is regulated at multiple levels, ranging from transcriptional control, to intracellular activation by proprotein convertases, and inhibition by TIMPs [563]. The ADAMTSs are preferentially inhibited by TIMP-3, with generally lower affinity for TIMP-1, TIMP-2, and TIMP-4. Endocytic clearance via LRP1 is also an important mechanism regulating activity of ADAMTS-4, ADAMTS-5, and ADAMTS-9 [556,573] and potentially other family members.

ADAMs

Physiological and pathological functions

ADAMs are TM metalloproteinases with critical roles in development, cell fate determination, cell migration,

adhesions, inflammation, and immunity [574]. There are 20 ADAMs in the human genome, and 12 of these (ADAM-8, ADAM-9, ADAM-10, ADAM-12, ADAM-15, ADAM-17, ADAM-19, ADAM-20, ADAM-21, ADAM-28, ADAM-30, and ADAM-33) are predicted to be catalytically active metalloproteinases, with the remaining 8 (ADAM-2, ADAM-7, ADAM-11, ADAM-18, ADAM-22, ADAM-23, ADAM-29, and ADAM-32) lacking the HEXXHXXGXX(H/D) consensus motif. The catalytically active ADAMs 'shed' or release the ectodomain of bioactive TM substrates such as cytokines, GFs, adhesion molecules, and receptors.

The best studied of the ADAMs is ADAM17, which has a broad range of substrates and critical roles in development and in regulation of the immune system [575]. ADAM17 is also known as TNFa-converting enzyme due to its ability to cleave the TM form of TNF and so release a soluble form of this cytokine that has systemic effects on the immune system [576]. ADAM17 further regulates the inflammatory response through its shedding of the IL-6 receptor (IL-6R), allowing IL-6 trans-signaling via gp130 [577]. ADAM17 also has a central role in EGFR signaling through its shedding of proteins such as EGF, HB-EGF, TGFα, epiregulin, amphiregulin, and betacellulin [578]. Dysregulation of ADAM17 activity is associated with pathologies such as chronic inflammation and cancer [577,579].

ADAM10 has critical roles in development (e.g., in Notch signaling) and regulation of cell-cell contacts (e.g., Eph/ephrin signaling) [579,580]. ADAM10 is indispensable for early embryonic development in its role as an alpha-secretase for regulated intramembrane proteolysis (RIP) of Notch [580]. ADAM10 also participates in RIP of amyloid-precursor-protein and thus has roles in Alzheimer's Disease [579,581].

Domain architecture

ADAMs have the same N-terminal architecture as MMPs and ADAMTSs, consisting of a signal peptide, a pro-domain and a conserved metalloproteinase catalytic domain. C-terminal to this are the disintegrin, cysteine-rich and EGF ancillary domains, followed by TM and cytoplasmic domains [570]. Crystallographic information on ADAMs is limited, but available data indicate that the metalloproteinase, disintegrin, and cysteine-rich domains form a dynamic C-shaped arrangement, with potential conformational flexibility upon binding of substrates [570]. Phylogenetic analysis shows that the ADAM10 and ADAM17 are more similar to each other than to other ADAMs.

Regulation of activity

Following on from transcriptional and post-transcriptional regulation, the maturation of ADAMs is controlled by proprotein convertases that remove the pro-domain in the Golgi apparatus. Intracellular trafficking and maturation of ADAM17 is further dependent on iRhom chaperone proteins, which remain associated with the enzyme on the cell surface and influence substrate selection and enzyme stability [582].

The activity of ADAM10 and ADAM17 can be rapidly activated on the cell surface, pointing to conformational regulation. Additional factors such as enzyme and substrate localization and phosphorylation, dimerization and disulfide bond isomerization have also been shown to regulate ADAM17 activity [575,583]. Tetraspanins are implicated in regulation of ADAM10 [583].

The catalytic activity of ADAMs can be inhibited by TIMPs, most commonly TIMP-3, although some ADAMs are also inhibited by TIMP-1 (e.g., ADAM10) or show reduced sensitivity to TIMPs (e.g., ADAM8, 9). In some cases, sensitivity to TIMPs is reduced by association of the ADAM with the substrate prior to hydrolysis.

Tissue inhibitors of MMPs

In plasma and fluid environments (e.g., synovial fluid), the activity of MMPs, ADAMs, and ADAMTSs can be inhibited by α2-macroglobulin, a broad-spectrum inhibitor of multiple proteinase classes. In tissues, these enzymes can be inhibited by the four mammalian TIMPs [584,585]. Through their ability to post-translationally inhibit metalloproteinase activity, TIMPs can have powerful effects on tissue homeostasis and cell behavior. TIMP-1, 2 and TIMP-4 inhibit the activity of most MMPs and a few ADAMs, while TIMP-3 is distinctive in having the broadest inhibitory profile, and inhibiting MMPs, most ADAMs and ADAMTSs [585]. TIMP-3 is also distinctive in its ability to bind to ECM sulfated GAGs, which protects it from endocytic uptake via LRP1 and increases affinity for some target enzymes [586].

Tissue inhibitor of metalloproteinases interact reversibly with target metalloproteinases in 1 : 1 stoichiometric complexes of low nanomolar affinity, blocking substrate access to the catalytic site [587]. TIMPs are composed of 2 domains, with the N-terminal domain containing the necessary elements for metalloproteinase inhibition, and the smaller C-terminal domain contributing to molecular interactions, such as

interaction of TIMP-2 with proMMP-2 and interaction of TIMP-3 with ECM PGs.

Tissue inhibitor of metalloproteinases are widely expressed, with the exception of TIMP-4, which has found primarily in the brain, heart and adipocytes. TIMP expression is dysregulated in pathologies such as cancer, osteoarthritis and fibrosis [585].

Heparanases

Heparanase is an endoglycosidase that catalyzes the cleaving of the side chains of HSPGs. In 2000, the homologous *HPSE-2* was identified and it shares ~ 40% similarity with HPSE-1 and has no glycosidase activity but appears to act as an inhibitor for HPSE-1 by interacting with HS chains [588]. The cleavage site of HPSE-1 is the β (1,4) glycosidic link between glucuronic acid and *N*-sulfonylated glucosamine present in HS chains. Only a limited number of these bonds are affected by the activity of HPSE-1 and the specificity is due to the recognition of a trisaccharide substrate with a defined HS sulfation pattern (GlcNX-GlcA-GlcNS, where X = S or Ac) thus generating 5–10 kD HS fragments [589].

Heparanase-1 structure and activity

The transcription and translation of the *HPSE-1* gene generates a protein of 65 kDa in the form of a proenzyme (pro-HPSE-1) which, after a post-translational cut, gives rise to two subunits of 50 and 8 kDa not covalently linked and which constitute the active form of the enzyme [590]. This maturation process begins in the endoplasmic reticulum where the precursor is synthesized, to continue, after the removal of the signal peptide, in the Golgi apparatus from where it is finally secreted into the extracellular environment. The conversion of the inactive form of the enzyme into the active one requires its re-absorption by endocytosis and transport to the lysosomes where cathepsin L catalyzes the cut that will give rise to the two subunits that form the mature enzyme.

Several membrane-bound molecules have been shown to mediate the binding and internalization of pro-HPSE-1. In particular, the receptors for mannose 6-phosphate and for low-density lipoproteins are to be considered as high-affinity receptors for HPSE-1. It has been reported that plasma membrane-bound syndecan-1 mediates HPSE-1 internalization [591].

In 2015, the crystal structure of human HPSE-1 was revealed, highlighting that this enzyme comprises a TIM-barrel domain containing the catalytic site and a C-terminal domain necessary for secretion and for regulation of its enzymatic and nonenzymatic activity. Regarding the catalytic site, it includes a putative proton donor at Glu 225 and a nucleophile at Glu 343, as well as having two binding domains for Hep / HSe (HBD1 and HBD2), which are located near the micropocket of the active site [592].

Heparanase-1 as a multitasking protein

HPSE-1 has been posited and defined by some authors as a multitasking protein with enzymatic and nonenzymatic activities. Thanks to its activity as endoglycosidase, it catalyzes the cutting of the side chains of the HSPGs, contributing decisively to the remodeling of the ECM and the BMs. Furthermore, since HSPGs through the sulfated disaccharide domains act as a 'sponge' providing numerous docking sites for bioactive molecules such as cytokines, GFs, enzymes and/or inhibitors, the enzymatic activity of HPSE-1 also favors the release of these various biomolecules linked to HS. In this way, HPSE-1 facilitates cell motility and proliferation, angiogenesis and inflammation [541].

The functions performed by HPSE-1 are not limited only to enzymatic activity toward HS chains but also include nonenzymatic and/or 'signaling' activities. By interacting with a possible not-yet-identified membrane receptor, both pro-HPSE-1 and mature HPSE-1 have been shown to be able to activate certain signaling pathways and to regulate gene expression. Clustering of syndecans by means of HPSE facilitates cell adhesion and spreading by PKC, Rac and Src activation. HPSE-1 can enhance the phosphorylation of protein kinases such as Akt, p38-MAPK, STAT, Src, Erk which in turn increase the transcription of several genes involved in tumorigenesis. In turn, Src activation phosphorylates EGFR, thus increasing cell proliferation and tumorigenesis [593].

Cellular localization studies have also shown that HPSE-1 can be found at the nuclear level where, contributing to the loss of nuclear syndecan-1, it can regulate the transcription of various genes involved in neoangiogenesis (VEGF-A and VEGF-C) and in ECM turnover (MMP-9) in tumor cells. The regulation of gene expression by HPSE-1 has been ascribed to the promotion of histone acetyltransferase activity in this further expanding of the repertoire of functions and modes of action of HPSE [594].

HPSE in physiology and disease

In healthy tissues, the cellular expression of HPSE-1 is strictly regulated in order to prevent the uncontrolled degradation of HSPG both at the membrane and

ECM level. In most tissues its expression is constitutively inhibited by epigenetic regulation of the promoter of the *HPSE-1* gene [595] and by the activity of the wild-type transcription factor p53 [596]. Consequently, the expression of HPSE is limited only to keratinocytes, to the placental trophoblast, to platelets and to some immune-defense cells such as mast cells and leukocytes [597].

In mice, during the early stages of gestation, HPSE-1 increases in the tissues of the uterus and contributes to tissue remodeling and the release of GFs necessary for implantation of the blastocyst and the development of the embryo [598]. The growth of the hair follicle in humans is regulated by HPSE-1 whose expression is localized in the inner root sheath thus controlling its differentiation [599]. In wound healing, HPSE-1 is required for tissue repair as it stimulates angiogenesis and keratinocyte migration [600]. In addition, HPSE-1 released by de-granulated platelets and immune cells facilitates the interaction of leukocytes with subendothelial BM and their extravasation as well as blood clotting [597].

Thanks to its degradative function toward HSPG and the signaling mechanisms, HPSE-1 has been shown to be strongly involved in various pathological conditions. The overexpression of HPSE-1 has in fact been shown to have a role in tumors, inflammatory and degenerative diseases (Table 6). In these pathological conditions, various factors are responsible for the overexpression of HPSE-1 including mutated variants of p53, estrogen, ROS, hypoxia, inflammatory cytokines, hyperglycemia and albuminuria.

Table 6. Heparanase expression and function in diseases.

Pathological condition	Reference
Cancer	
Sustaining proliferative signaling such as FGF2, TGFβ, HGF, VEGF and EGF	[638–642]
HPSE inhibits apoptosis via HS-mediated signaling	[643]
Autophagy regulation	[644,645]
Inducing angiogenesis	[540,646,647]
Activating invasion, metastasis, EMT and ECM degradation	[608,609,623,648]
Involving in exosome formation	[615,617]
Inflammation	[611,613,614,618,619,624]
Diabetes	[620,621]
Coagulation dysfunctions	[622,625,626]
Amyloid disease	[627–630]
Kidney disease	[631–634]
Organ fibrosis	[600,635,636,649,650]
Pancreatitis	[651]
Viral Infection	[652–654]

HPSE-1 is upregulated in almost all human cancer [601] and many studies have shown that it participates in tumor initiation, angiogenesis, growth, metastasis and chemoresistance [602,603]. Basal HPSE-1 expression is regulated by Sp1 transcription factor [604], whereas inducible HPSE expression seems to be under Egr1 control [605]. HPSE-1 expression is also controlled at epigenetic level: hypermethylation of promoter region reduces HPSE expression [595]. It has been identified that HPSE-1 is regulated at post-transcriptional levels by the presence of AU-rich elements in the 3′ untranslated region which favor mRNA degradation, and the loss of this region contributes to HPSE production [606]. HPSE-1 is also regulated by hormones, tumor suppressors, oncogenes, and miRNAs [607].

It has been recently established that estrogen receptor-beta, ERβ, suppression in the aggressive MDA-MB-231 breast cancer cells strongly inhibits their invasive phenotype by affecting their morphological characteristics and functional properties, leading to a partial MET and critically affecting matrix reorganization. Among other major ECM components HPSE is strongly downregulated following ERβ suppression [608] and seems to be correlated with several functional miRNAs, including miR-10b, miR-200b and miR-145 that modulate breast cancer cell properties in ER-dependent manner [609,610].

Moreover, HPSE-1 is deeply involved in inflammation since it regulates the migration of dendritic cells, monocytes, eosinophils and neutrophils [611], but also participates in the activation and polarization of macrophages toward a pro-inflammatory/pro-tumorigenic phenotype mainly by regulating TLRs signaling [612–614].

Over time, published scientific evidence has proved the involvement of HPSE in multiple other pathological conditions such as diabetes [615,616], dysfunction of the coagulation system [617,618], amyloid disease [619–622], renal disease [615–628], fibrosis [629–632], pancreatitis and viral infections [633–636] (Table 6).

Concluding remarks and future perspectives

In this article, we present a detailed description of the composition and functions of the ECMs. The last decade shed plenty of light with the significant advances in research related to ECM macromolecules as well as the ECM interactions networks. ECMs are responsible

for tissue integrity and play regulatory roles in cell signaling, gene expression, and cell functional properties in physiological and pathological conditions. Despite the accumulated and in depth gained knowledge, we believe is essential a guide to describe and summarize the entire field of ECM in terms of its main macromolecular components and their biological roles. Although it is not possible to cover all different types of ECM effectors, we presented the most important and abundant macromolecules of the ECM networks. Among them are the family of collagens and its receptors, elastin, laminins, the various PGs and hyaluronan with its well-studied receptor CD44. The matrix-degrading proteolytic and glycolytic enzymes were also described as their affect tissue remodeling in health and disease.

Despite the strong correlation between several matrix macromolecules with disease development and progression, the area of ECM, although an emerging field for intense research to understand the mechanisms underlie the basis of several diseases, has been underestimated in terms of designing novel strategies for disease treatment. We believe that the ECM community should aim toward the understanding of cellular mechanisms governing the matrix-based disease development and progression as well as to develop comprehensive strategies that will drive future pharmacological targeting, disease diagnosis, prognosis and treatment.

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Conflicts of interest

The authors declare no conflict of interest.

Author contributions

All authors contributed to writing, reviewing and edited the manuscript. NKK organized, reviewed and submitted the manuscript.

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