REVIEW

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Modulation of the immune response by extracellular matrix proteins

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Abstract

Inflammation entrains a focused and coordinated response from many different elements. Soluble factors such as chemokines and cytokines direct the recruitment, differentiation, and fate of leukocytes. Cells and pathogens are killed and consumed, yet where the response is effective, inflammation will melt away, leaving a healthy functioning tissue. All this commonly takes place in an environment known as the extracellular matrix (ECM). The ECM is not a passive partner in the process and recent work demonstrates the important role that proteins found in this environment play in connecting different parts of the immune response together. In this review we will focus on these connections and the proteins that make them. One emerging trend that we will highlight is the ability of endogenous molecules to interact with receptors that are better known as sensors of the molecular fingerprints of infection. We propose that this may be particularly relevant in the context of autoimmunity, since the provision of such signals may be crucial in breaking tolerance.

Key words: extracellular matrix, inflammation, matricellular protein.

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INTRODUCTION

The extracellular matrix (ECM) is a mixture of secreted complex carbohydrates and proteins that serves as a mechanical scaffolding within which tissues and organs can be assembled and as a lattice through which cells can move. The major structural components of the ECM include collagens, hyaluronan (HA), fibronectin, and proteoglycans; receptors for these components of the ECM serve to anchor cells within the matrix. In addition to molecules that have a major structural role, the ECM contains a host of other constitutive components and also, in response to specific developmental or pathological events, a number of components that appear transiently. Some of these minor ECM proteins have been grouped together as "matricellular proteins", whose function is achieved by binding both matrix and cell surface receptors. Members of this family, which includes the thrombospondins, osteopontin (OPN), and osteonectin, have functions that are primarily regulatory rather than structural [7]. Other components such as mindin and biglycan (BGN) are constitutive, but play an important role in modulating the response to infection. Fragments of larger ECM proteins such as HA and fibronectin that are released during the process of tissue damage and inflammation may also elicit their own cellular responses. And newly recognized interactions, for example between the β-galactose-binding protein galectin-9 and Tim-3-expressing T cells, continue to expand the realm of these important observations. The following review considers some of the functions of these ECM components in the context of an inflammatory immune response.

PROMOTION OF INFLAMMATION BY ECM COMPONENTS

The successful inflammatory response is a multi--faceted process involving the sequential release of soluble mediators and the recruitment and activation of inflammatory cells by various inflammatory stimuli [35]. This is followed by the elimination of the eliciting insult, the remodeling of tissue, and the restoration of normal or near normal function. Inflammation may be more dangerous in some locations than in others, such as the eye or brain, where additional specialized mechanisms of control are observed. We may divide the inflammato-

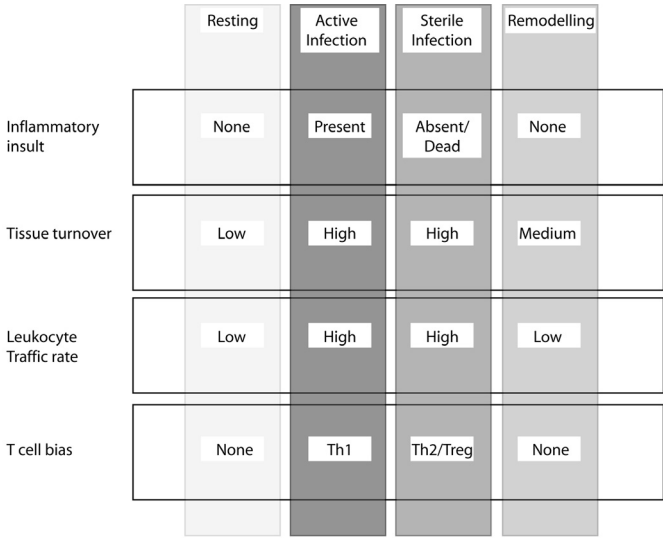

Fig. 1. Overview of features of acute inflammation influenced by the ECM.

ry response into four phases, with different characteristics in terms of the eliciting insult, tissue turnover, cell trafficking, and cell function (Fig. 1), but all these characteristics interlock. Different resting tissues may vary greatly in terms of tissue turnover, leukocyte traffic rate, and the presence of T cells, but whatever the baseline, there are relative changes in response to inflammation.

ECM PROTEINS INTERACT WITH PATTERN-RECOGNITION RECEPTORS

The ECM can serve as a binding site for microbial colonization. Pathogens bind to ECM molecules via adhesins and can then use the ECM as a site for propagation and spreading, causing a breakdown of the ECM and release of integral components. ECM breakdown also occurs as a result of the release of matrix metalloproteinases during inflammation. Fragments of specific ECM proteins have pro-inflammatory functions, which can augment the immune response both by activating the innate immune response and by acting as regulators of inflammatory cell apoptosis [24].

The ability to recognize and respond rapidly to pathogenic components is essential for the successful clearance of invading microorganisms. Innate immune responses are critical in this early recognition process. One early mechanism of pathogen recognition is provided by specific receptors called pathogen-recognition receptors (PRRs). This group of receptors, different members of which are found intra-and extra-cellularly, recognize conserved motifs found in microbes, termed pathogen-associated molecular patterns (PAMPs) [17]. Well-classified examples of PAMPs include bacterial cell wall components such as lipopolysaccharide (LPS) and lipoteichoic acid, as well as internal structures such as sequences of bacterial DNA (CpGs) [30]. The receptors that interact with these molecules have become well known in recent years, and one of the most studied is Toll-like receptor (TLR)4. In a molecular complex involving CD14 and myeloid differentiation protein-2, TLR4 mediates the cellular response to LPS [32]. This interaction leads to the early release of pro-inflammatory cytokines including interleukin (IL)-6 and tumor necrosis factor (TNF)-α, a response that can be rapidly fatal when large doses of LPS are given systemically [10]. Strikingly, two different mutants, both with deletions of constitutive ECM proteins, show decreased sensitivity to LPS-induced shock.

Mindin is a member of the F-spondin family of ECM proteins and belongs to a group of proteins termed the thrombospondin type-1 (TSP-1) repeat superfamily [3, 20]. Mindin mRNA is detected in a number of tissues, including those of the immune system and the lung. When mindin knockout mice are challenged with LPS, they secrete less IL-6 and TNF-α than wild-type controls. These mindin-deficient animals show increased resistance to infections that cause septic shock, but are less able to clear bacteria from the lung. Mindin binds directly to bacteria expressing LPS and agglutinates them. Overall, mindin appears to focus and enhance the macrophage-driven LPS response, and might therefore serve to increase the early tempo of the innate response to bacterial colonization of the ECM [19]. However, mindin expression is not itself upregulated by LPS treatment.

In contrast, another ubiquitous ECM protein, BGN, is upregulated by active inflammation. BGN, along with decorin, is a member of the family of small leucine-rich proteoglycans (SLRPs) [22]. Although BGN is abundant in many tissues, it is further induced in macrophages by activation with LPS, IL-1β, and IL-6. BGN-deficient mice are also resistant to LPS-induced septic shock, but as compared with mindin-deficient mice, production of IL-6 by macrophages is less affected than production of TNF-α. BGN appears to interact directly with TLR2 and TLR4 and *in vitro* assays demonstrate that it can stimulate the production of pro-inflammatory cytokines from wild-type macrophages. The inflammatory response to BGN is abolished in TLR2- and TLR4-deficient macrophages [47]. BGN also demonstrates another interesting feature of ECM modulators of immune responses in that the activity of the soluble form of the molecule is different from that when the molecule is bound within the matrix [47].

In addition to the smaller ECM proteins, fragments of large ECM proteins have also been found to signal through TLR4, suggesting a role for these in initiation of the inflammatory response to bacterial invasion. Fibronectin within the ECM exists as an insoluble glycoprotein. In response to tissue injury, fragments of fibronectin that contain extra domain A are released. These fragments are able to bind to TLR4, and in so doing can induce responses in macrophages that are similar to those induced by LPS [38].

HA is usually present in the ECM as a high-molecu-

lar-weight glycosaminoglycan. However, under pathogenic conditions HA has been shown to be more polydisperse, with low-molecular-weight degradation products composing an increased fraction of the total amount [36]. Low-molecular-weight fragments of HA signal through both TLR4 and -2 and have been shown to induce IL-1β and TNF-α production by murine macrophages. This function was tracked to an activation of the transcriptional regulatory complex NF-κB/IκBα. This complex plays an essential role in transduction of signals leading to the expression of genes for proteins that are central to the inflammatory response [36]. High-molecular-weight HA may also play a role in the regulation of inflammation. In a bleomycin-induced model of lung injury, in which an increase of apoptosis is seen, high-molecular-weight HA is able to protect cells against pro-apoptotic stimuli by activation of NF-κB. This effect is abrogated in TLR2 and TLR4 knockout mice, indicating that the effect is TLR dependent [24].

In summary, many components of the ECM are implicated in interactions with PRRs. In circumstances where pathogens are thought to be absent, such as organ-specific autoimmune disease, it is easy to see how inflammation that releases such endogenous innate immune system stimulators might still trigger the innate immune response.

RECRUITMENT OF INFLAMMATORY CELLS IS INFLUENCED BY ECM PROTEINS

A critical function of the inflammatory response is the recruitment of leukocytes to the area of immunological insult. Leukocytes traffic through normal tissues at different rates, and normal trafficking may be disturbed by disrupting integrin- and selectin-mediated interactions [51]. In the event of immunological stimulation, leukocytes in the peripheral blood undergo a series of steps which result in large increases in the flux of cells across the endothelium [37, 55]. This multi-step process involves coordinated interaction of adhesion molecules with their receptors, both on cells and within the ECM. Changes in selectins and integrins on the endothelium are driven by many inflammatory mediators, such as cytokines and chemokines [50], but ECM proteins can also play a role in regulating this process. Mindin-deficient mice, which have normal neutrophil development, show impaired recruitment of inflammatory cells, particularly neutrophils and macrophages. Studies in knockout mice demonstrated that the absence of mindin could reduce the migration of activated neutrophils by up to 3-fold. This occurred whether the neutrophils came from wild-type or mindin-deficient mice, suggesting that the role of mindin was in adhesion rather than in cellular function. Use of selected anti-integrin antibodies (anti-α_4, -α_m, and -β_2) blocked the effect of mindin on neutrophil migration, indicating that the role of mindin in cell recruitment is via integrin binding [23].

TSP-1 is the canonical member of the TSP family, which consists of 5 different TSP proteins. TSP-1, which falls into subgroup A of the TSP family, is also a matricellular protein, defined as a group of matrix proteins that modulate cell function but do not appear to contribute directly to structural organization or physical properties [7, 8]. The TSP-1 protein is a homotrimer comprised of three identical chains, each of which is divided into different structural domains with distinct functions. Of particular interest in the context of the immune response are the type 1 repeats that are found in the thrombospondins and also in a variety of other proteins outside the TSP family, including mindin [19]. TSP-1 is expressed widely in the embryo, but during adult life its expression is limited. Under normal conditions it is found only in certain specialized tissues, such as the eye [31]; however, TSP-1 expression is upregulated during inflammation [2]. TSP-1 has been demonstrated as having distinct roles in various stages of T cell and monocyte recruitment, indicated by the fact that TSP-1-deficient mice have increased numbers of circulating lymphocytes [26]. In an antibody-driven model of glomerular nephritis in these animals, there is a reduction in the recruitment of interstitial $CD4^+$ and $CD8^+$ T cells, implying a role for TSP-1 in this process. Additionally, TSP-1 can play a role as a co-stimulatory molecule in the activation of $CD28^-$ $CD4^+$ T cells isolated from the synovium of patients with rheumatoid arthritis [54].

T cells are able to bind to TSP-1 *in vitro*, an interaction that is increased upon stimulation. This activation-dependent increase in TSP-1 binding can be inhibited by addition to the culture of antagonistic integrin anti-β_1, anti-α_4, and anti-α_5 monoclonal antibodies, indicating that changes in integrin expression and functional activity are responsible. As well as being incorporated into the ECM, TSP-1 may be found associated with the cell surface of human T cells. This TSP-1 is mobilized from intracellular storage vesicles to the cell surface where it is expressed in response to appropriate adhesive interactions, such as adhesion of the cell to ECM components. Further studies show that this cell surface TSP-1 has an involvement in the process of T cell motility, as pseudopodia formation and cellular flattening, two features that are prevalent amongst T cells migrating through the ECM, are absent in the presence of an antagonistic anti-TSP-1 antibody [28].

The expression of cellular adhesion molecules (CAMs) is upregulated in response to injury, allowing increased extravasation of leukocytes to areas of inflammation [6]. The addition of TSP-1 can lead to an increase in CAM expression on vascular endothelial cells. Treatment of endothelial cells with TSP-1 results in a 2.5-fold upregulation of vascular CAM-1 and a 3.8-fold upregulation of intercellular CAM-1 compared with unstimulated endothelial cells. This function of TSP-1 was tracked to the CD47-binding regions of the protein. Endothelial cells' responsiveness to both TSP-1 and TNF-α (another factor which results in upregulation of

CAMs) was abrogated in CD47 knockdowns, suggesting that TSP-1 plays a role in both TNF-α-dependent and TNF-α-independent upregulation of CAMs and as such is a factor in the recruitment of leukocytes to the ECM in response to injury [34]. In addition to differences in activation status, changes between a Th1 and Th2 phenotype alter T cell binding to TSP-1, a phenomenon which is also a result of differential integrin expression between these cell types [60].

REGULATION OF ACTIVATION AND T CELL POLARIZATION

Upon activation, T cells undergo polarization in response to the cytokine profile of the inflammatory milieu [1]. Cytokines such as IL-4 and IL-12 play a dominant role in T cell differentiation, but ECM proteins are also able to influence the process. This could serve to modify T cell responses within the target tissues. This modulation is not necessarily directly targeted to T cells. An example of an ECM protein that modulates antigen-presenting cell (APC) function and thus T cell responses is OPN. OPN is a secreted phosphoprotein whose transcription is upregulated in response to a number of pro-inflammatory factors, including TNF-α, IL-1, and platelet-derived growth factor [16]. A critical role for OPN in the development of Th1-mediated immunity has been demonstrated in a number of studies. Ashkar et al. [4] showed that OPN-integrin interaction in cultured mouse peritoneal macrophages results in the induction of IL-12 secretion which drives T cells towards a Th1 phenotype. In the same study, the ability of $OPN^{-/-}$ mice to develop a granulomatous response (a response whose development is a consequence of type-1 mediated immunity) to polyvinylpyrrolidone was shown to be impaired. Activated T cells have been shown to be a significant source of OPN, both *in vitro* and *in vivo*, and OPN also contributes to the development of a Th1 phenotype by acting on macrophages and dendritic cells (DCs), promoting IL-12 secretion by these cells [49].

In humans, OPN is associated with the development of Th1-mediated autoimmune responses. Serum OPN was significantly increased in patients suffering from Crohn's disease, and OPN was demonstrated in the plaques found in the central nervous system of patients with multiple sclerosis as well as in the reactive microglia from these patients [11, 45]. *In vitro* studies demonstrated that OPN is secreted by immature DCs, inducing terminal differentiation and secretion of TNF-α and IL-12 by these cells. This in turn results in the promotion of a Th1 phenotype and therefore interferon (IFN)-γ production by T cells [41]. Addition of OPN to T cells without the addition of a factor to provide a suitable second signal (e.g. CD40L ligation) does not influence T cell cytokine secretion [40, 41].

Osteonectin, or secreted protein acidic and rich in cysteine (SPARC), is a calcium-binding matricullular protein. It is able to bind to a number of different ECM proteins, including TSP-1 and collagen type IV, and its function in the ECM is primarily regulatory rather than structural [9]. SPARC knockout mice display a 50% reduction in collagen deposition in the dermal ECM [9]. This feature causes a reduction in the tightness of the ECM in these animals, allowing faster migration of DCs and hence results in an acceleration of T cell priming and a faster onset of delayed type hypersensitivity [44].

Decorin, a member of the SLRP family, is constitutively expressed in the ECM of a variety of tissues. Like IFN-γ, decorin is able to inhibit proliferation, promote cell survival, and enhance the activation of macrophages, as measured by upregulation of MHC II, nitric oxide synthase (NOS2), and pro-inflammatory cytokine expression. Interestingly, decorin also enhances the response by macrophages to LPS, which occurs via a pathway distinct from that of IFN-γ activation. Such effects of decorin on macrophage activation were shown to be due to the ability of decorin to bind and sequester transforming growth factor (TGF)-β secreted by the macrophages, thus reversing the inhibitory effects of TGF-β on macrophage activation and therefore enhancing the activation of these cells [13, 61]. Synthesis of decorin and BGN is stimulated by the presence of TGF-β, suggesting that decorin may serve as a negative-feedback regulator of TGF-β activity [39].

DOWNREGULATION OF INFLAMMATION

Excessive or prolonged inflammation can be deleterious, resulting in extensive tissue damage and distortion, granuloma formation, and in some cases promotion of neoplastic transformation by oxidative damage to DNA [35]. Hence, downregulation of inflammation is an important aspect of the response, and inflammation normally leads to the release of anti-inflammatory mediators [35]. Several ECM proteins have functions that contribute to the downregulation of inflammation. Decorin and BGN have been implicated in control of the complement cascade. Both proteins are able to bind C1q, a ligand-recognition molecule for components of both the classical and lectin pathways of the complement cascade. Binding of C1q to its ligands (principally IgG and IgM) results in activation of the complement cascade. By binding to C1q, both decorin and BGN are able to inhibit the classical pathway of complement activation. In addition to this, BGN is also able to inhibit the lectin pathway and to prevent C1q-induced production of MCP-1 and IL-8, so minimizing the pro-inflammatory cellular responses induced by C1q [18].

ECM proteins may also influence the cytokine-secreting profiles of macrophages [5, 11] and T cells [25, 45]. TSP-1 knockout mice display an inflammatory phenotype which, although less severe, shows similarity to that seen in TGF-β null animals [14]. TSP-1 had previously been shown to bind to and activate latent TGF-β *in vitro* [33, 48], and this study by Crawford et al. [14]

demonstrated that TSP-1 was a major activator of TGF-β *in vivo*. CD36 is an 88-kDa transmembrane protein that functions as a scavenger receptor for oxidized LDL and shed photoreceptor outer segments [52] as well as the target for cytoadherence by malaria-infected erythrocytes [63]. CD36 interaction with TSP-1 has been implicated in platelet-monocyte adhesion [56] and macrophage uptake of apoptotic cells [46]. Studies using anti-CD36 antibodies also suggest that the interaction of CD36 with TSP-1 is necessary for activation of latent TGF-β, as this interaction serves to tether latent TGF-β to the cell surface where is can be processed by plasmin [62].

TSP-1 may also influence the function of T cells by binding to CD47. CD47 is expressed by both APCs and T cells. One function of TSP-1 that has been suggested is to form a trimolecular bridge by binding to CD36 on APCs and CD47 on T cells, which could serve to stabilize interactions between APCs and T cells [54]. Binding CD47 on human monocytes has been shown to downregulate IL-12 production by these cells, which in turn would inhibit the development of Th1 cells. It is also thought that TSP-1-binding of CD47 on T cells could aid in deviating signal transduction away from the Th1 response following T cell receptor ligation by inhibiting responsiveness to exogenous IL-12 [5].

TSP-1 may also have an important role in the promotion of immune privilege and tolerance, particularly in the context of ocular immune privilege. The immune response to antigens encountered within the eye is highly specialized to prevent excess inflammation that would cause loss of visual acuity. Ocular APCs have been recognized as having the ability to present antigen in such a way that it leads to deviation of T cell activation away from a Th1 phenotype. This function can be conferred to APCs isolated from the peritoneal cavity by exposure to TGF-β [58]. Recently it has been demonstrated that exposure of APCs to TSP-1 results in an upregulation of the same genes that are upregulated in response to TGF-β. Moreover, TSP-1-treated APCs have the ability to promote immune deviation *in vivo* and, interestingly, treatment with TGF-β could not restore the tolerizing phenotype in TSP-1 knockout APCs [29].

Inflammation may also be downregulated by the elimination of infiltrating leukocytes. Mechanisms that are thought to control this process include the ligation of Fas ligand, which leads to apoptosis. Recently, a novel interaction between Tim-3 and galectin-9 has also been shown to be relevant. Galectin-9 has two homologous carbohydrate-recognition domains and binds sugars containing β-galactose. It is found both intracellularly and at the cell surface and its release is promoted by a number of different activation signals. Galectin-9 can bind Tim-3, a molecule expressed on Th1 T cells, and cause aggregation and death. Therefore, elevation in the local concentration of galectin-9 in the ECM would be expected to lead to downregulation of Th1 T cell responses [65].

DIVERSE FUNCTIONS OF ECM PROTEINS

Matricellular proteins can have bipartisan roles in the immune response, a feature resulting in considerable uncertainties when attempting to reconcile their overall function within an immune setting. Prime examples of these can be seen in the functions of BGN and OPN. In contrast to its ability to promote inflammation by binding TLR2 and TLR4, BGN also has roles in downregulation of the adaptive immune response. Recently, ligation of the mannose receptor by both monoclonal antibodies and natural ligands, including BGN, has been demonstrated to inhibit IL-12 production and promote IL-10 production by LPS-stimulated maturing DCs. Production of IL-10 by DCs promotes the development of Th2 cells, and so in this way BGN may also act as a regulator of adaptive immunity by skewing $CD4^+$ T cell maturation towards a Th2 type [12].

OPN, which has a major function in the promotion of a Th1 response, also has anti-inflammatory functions. *In vitro*, OPN downregulates the function of NOS2, thus inhibiting the production of NO, a factor released by activated macrophages that promotes tissue damage during an inflammatory response. NO itself promotes the expression of OPN, thus these two factors function together as an autoregulatory loop [42, 53].

CONCLUSION AND FUTURE DIRECTIONS

In conclusion, the literature reviewed in this article demonstrates that the ECM is a dynamic structure, not only serving as scaffolding for cells and organs, but also interacting with cells, providing signals for growth, differentiation, proliferation, and death [27]. In response to signals from an inflammatory environment, cells leave the circulatory system and migrate through the ECM to the site of insult. During this process, interactions with the matrix serve to regulate immune cell action in both a positive and negative fashion, tailoring cell function to one that is appropriate for the type and magnitude of insult. In addition to this, cells of the immune response may transiently secrete ECM proteins in response to specific signals, and these can then act in an autocrine or paracrine fashion. Larger ECM components that are relatively inert when bound within an uninjured matrix may be broken down within the inflammatory environment to smaller, biologically active fragments which can also influence infiltrating inflammatory cells. Some of the proteins secreted into the ECM may have longer half-lives than cytokines, whose presence is often short lived (Table 1). Inflammation may therefore cause a 'pro-inflammatory footprint', sensitizing trafficking cells through interactions with receptors of the innate immune system, thus maintaining a heightened level of surveillance as the tissue moves from infection back to sterility and remodeling.

Table 1. ECM proteins modulating immune function

Matrix protein [references]	Expression and half-life	Putative immunomodulatory ligands	Known functions within the immune response
Thrombospondin-1 [14, 15, 26]	Induced; constitutive only in certain adult organs $T_{½}$ 540 min in circulation	CD36, CD47, α3, α4 and α5 integrins	Inhibition of Th1 cell polarization Co-stimulation of inflammatory T cells TGF-β activation Macrophage function
Mindin [19, 23, 30]	Constitutive, $T_{½}$ not known	TLR4	PRR for multiple PAMPs Recruitment of leukocytes
Biglycan [18, 43, 47]	Constitutive, upregulated by inflammation $T_{½}$ 150 min (mRNA); 20 days (in tissue explant)	TLR4, C1q	PRR for multiple PAMPS Regulation of complement activation
Decorin [18, 39, 43, 64]	Constitutive $T_{½}$ 150 min to 20 days (in cell culture or in tissue explant)	C1q	Complement activation Th1 cell polarization
Osteopontin [16, 40, 57]	Constitutive, $T_{½}$ mRNA 300 min (cell lines)		Th1 cell polarization Co-stimulation
Galectin-9 [21, 65]	Constitutive, upregulated by inflammation $T_{½}$ not known	Tim-3	Elimination of Th1 T cells

Many of the roles of ECM proteins in inflammation have been demonstrated by the development of gene knockout mice, which enable study of these components both in health and disease [23, 26, 49, 59]. As such, the number of ECM components recognized as modulators of the immune response continues to grow, with new functions of already recognized proteins also awaiting discovery. Use of *in vitro* ECM models such as collagen gels may be used to address these questions in an appropriate setting. In addition, questions remain regarding the pathophysiological role of these proteins in human disease. As a more comprehensive understanding of the roles of these ECM proteins develops, many promising avenues open up before us with regard to their therapeutic potential for human disease.

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