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Extracellular matrix in skin diseases: The road to new therapies

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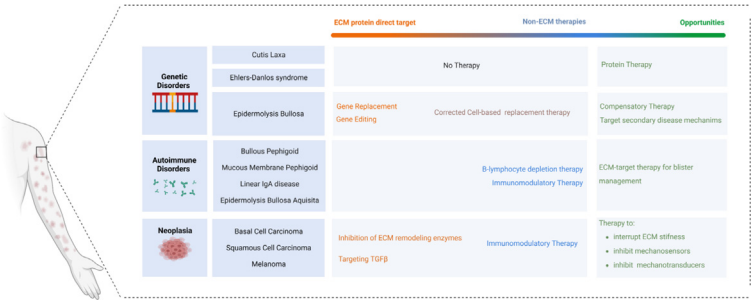
HIGHLIGHTS

- ECM-target therapy for skin diseases has been overlooked and many opportunities remain unexplored.
- Numerous breakthroughs have been made in EB, but not for other ECM-related genetic skin disorders.
- Targeting ECM stiffness seems promising, but countless options await to be explored in skin tumors.
- Several questions regarding autoimmune bullous dermatoses pathomechanisms remain unanswered.
- Multidisciplinary approaches might help overcome potential constraints of ECM-target therapies.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: The extracellular matrix (ECM) is a vital structure with a dynamic and complex organization that plays an essential role in tissue homeostasis. In the skin, the ECM is arranged into two types of compartments: interstitial dermal matrix and basement membrane (BM). All evidence in the literature supports the notion that direct dysregulation of the composition, abundance or structure of one of these types of ECM, or indirect modifications in proteins that interact with them is linked to a wide range of human skin pathologies, including hereditary, autoimmune, and neoplastic diseases. Even though the ECM's key role in these pathologies has been widely documented, its potential as a therapeutic target has been overlooked.

Aim of review: This review discusses the molecular mechanisms involved in three groups of skin ECM-related diseases - genetic, autoimmune, and neoplastic – and the recent therapeutic progress and opportunities targeting ECM.

Key scientific concepts of review: This article describes the implications of alterations in ECM components and in BM-associated molecules that are determinant for guaranteeing its function in different skin disorders. Also, ongoing clinical trials on ECM-targeted therapies are discussed together with future opportunities that may open new avenues for treating ECM-associated skin diseases.

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Background

In skin tissue, there are two distinct forms of ECM that can be identified based on their location and composition. The interstitial dermal matrix, which is mainly composed of collagens and elastic fibers, cushions the skin from stress and strain while the basement membrane (BM) separates the keratinocytes from the dermal stroma [1,2]. Although the implication of both forms of skin ECM in cutaneous diseases, ranging from hereditary or autoimmune disorders to neoplasia, has been widely documented, its promise as a therapeutic target is still a long way off.

Heritable genetic diseases affecting dermal ECM proteins are grouped as Ehlers-Danlos syndrome (EDS) or Cutis Laxa (CL) [3], both translated into significant alterations in skin tissue biomechanics. In EDS, skin hyperextensibility and fragility are owed to deviations along the course of collagen fiber formation, from transcription to fiber assembly [4]. In turn, CL wrinkled, redundant, inelastic, and sagging skin results from defective elastogenesis, particularly in the synthesis and assembly of elastic fibers [5]. Epidermolysis bullosa (EB) forms another group of genetic disorders yet characterized by skin blistering. Blisters result from dermal-epidermal separation after minor trauma due to mutations in proteins composing the or associated with BM [6]. Up to now, EB has been linked to mutations in 18 distinct genes and classified into four major types according to the ultrastructural level of skin blistering: above (epidermolysis bullosa simplex- EBS), at (junctional EB - JEB), below (dystrophic EB - DEB), or at multiple sites of (Kindler EB) the BM [6]. The disruption of the BM and the formation of subepidermal blisters are also manifestations of autoimmune skin diseases, which are caused by autoantibodies against proteins of the dermal-epidermal adhesion complex [7]. Despite their mechanistic similarities, these diseases are classified into different groups based on the associated clinical manifestations and the type of autoantibodies. Bullous Pemphigoid (BP) and Mucous Membrane Pemphigoid (MMP) are mainly characterized by IgG antibodies production, but BP predominantly affects skin while MMP has mucosal and skin involvement [8]. Linear IgA disease (LABD) shares many BP features but, as the name implies, is linked to IgA autoantibodies [9]. On the other hand, the Epidermolysis bullosa acquisita (EBA) although clinically resembling hereditary EB, is considered an autoimmune disease caused by IgG autoantibodies against collagen VII [10].

The ECM biochemical and biophysical properties have been also increasingly associated to the aggressiveness and the metastatic ability of the three most common types of skin cancer: basal cell carcinoma (BCC), cutaneous squamous cell carcinoma (cSCC), and melanoma [11]. Degradation of BM and remodeling of dermal ECM constituents are linked with cancer progression and the ability of cancer cells to outgrow in the epidermis, penetrate the dermis, and access to lymphatic or blood vessels [12,13]. In fact, the most metastatic skin cancer (metastasizes in 40.5 % of the patients) – melanoma – is characterized by an overexpression of ECM remodeling enzymes, as compared to non-melanoma ones, with a metastatic rate of 0.003 % - BCC - and 2–6 % - cSCC [14,15]. Likewise, this different metastatic ability between cSCC and BCC appears to be associated with the level of BM degradative enzymes.

Therefore, this review focuses on the different ECM modifications associated with skin disorders. We detail the mutations that cause EDS and CL and how the pathways of dermal collagen or elastic fiber formation are respectively altered in those diseases. Additionally, we describe the mutations that result in EB and the involvement of the BM proteins and associated structures in intraepidermal adhesion and dermo-epidermal anchoring, which are disrupted in these hereditary bullous diseases. We further clarify the molecular mechanisms of autoimmune bullous diseases, fundamentally different from the hereditary ones, emphasizing the distinctions between the existing groups - BP, MMP, LABD and EBA. Although the ECM is not directly involved in the initiation of carcinogenesis, it is critical for tumor cell invasiveness. Thus, using BCC, cSCC, and melanoma as examples, we debate the correlation between their metastatic potential and the expression of ECM remodeling enzymes, highlighting what is known regarding suppression of tumor progression if those enzymes are knockdown. Finally, we review current treatments for each of the disorders in focus and discuss potential options for future ECM-targeted therapies.

Changes in dermal extracellular matrix and its implications

Collagen fibers alterations in Ehlers-Danlos syndrome

Mutations in the genes encoding for collagen I (*COL1A1* or *COL1A2*), III (*COL3A1*) and V (*COL5A1* or *COL5A2*) directly affect the primary structure of the collagen fibrils and represent the major group of mutations implicated in EDS [4] - Fig. 1A. *COL1A1* and *COL1A2* mutations influence type I collagen *N*-propeptide removal from the fibril [16], restricting its lateral growth by steric hindrance. This results in reduced diameter and irregular shape of the fibril cross-sections and diminished strength [17]. In turn, *COL3A1* mutations cause the synthesis of abnormal type III procollagen, which is retained intracellularly [18]. Therefore, the reduced amount of collagen III in the ECM leads to an abnormal collagen III: I ratio and severely malformed collagen fibrils [19]. Moreover, as type V collagen is an initiator of fibril formation and regulates the self-assembly of types I and III collagen fibrils [20], the reduction in type V collagen observed in EDS favors lateral expansion of

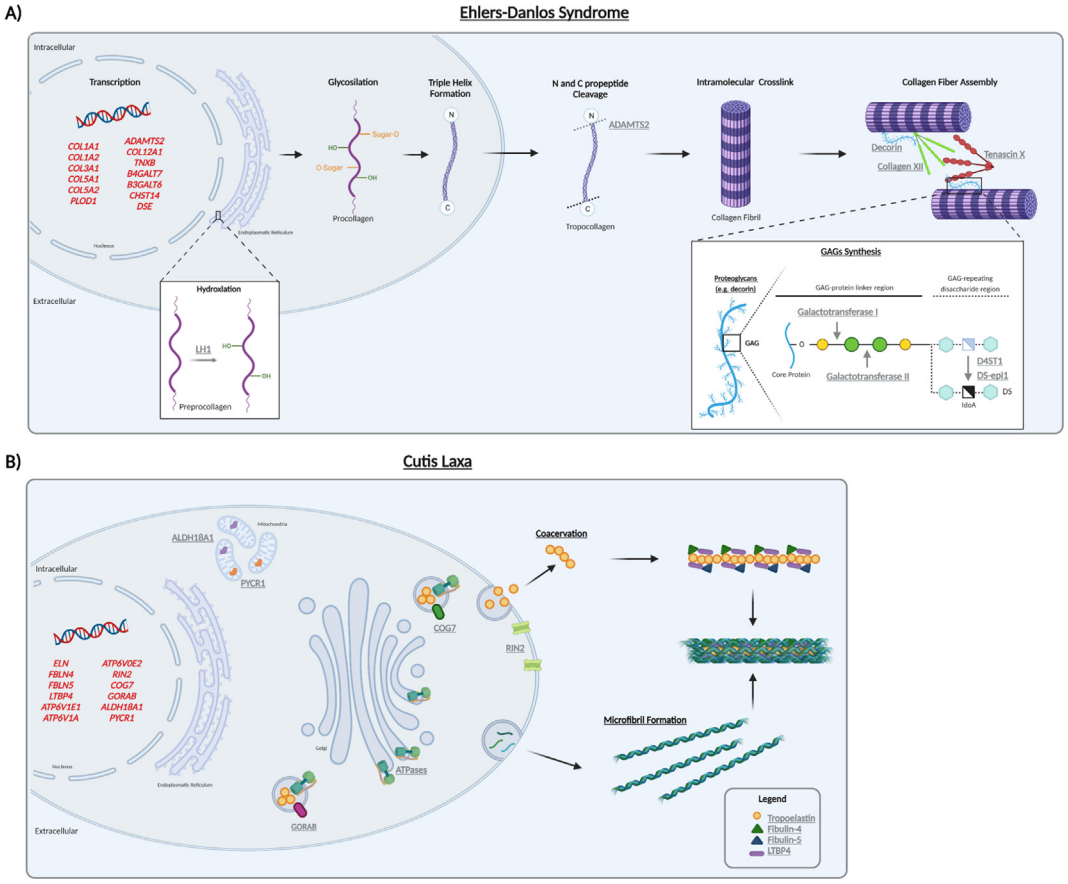


Fig. 1. Heritable genetic diseases Ehlers-Danlos syndrome (EDS) or Cutis Laxa (CL) are owed to mutations that affect the normal course of collagen and elastic fibers formation. A) In EDS, those mutations occur in genes encoding for various collagens, collagen processing enzymes, and enzymes involved in GAG synthesis. B) Cutis Laxa is associated with mutations in genes involved in the biogenesis of elastic fibers, either directly (elastic fiber components mutations) or indirectly (mutations in enzymes involved in cellular metabolism and secretory pathways). Mutations are indicated in red within the nucleus, while the corresponding affected proteins are underlined in grey. Figure created with BioRender.com. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

existing fibrils over new fibril formation contributing also to the lower number and density [21].

Mutations that introduce defects in collagen-processing enzymes such as lysyl hydroxylase 1 (LH1) and procollagen *N*-proteinase ADAMTS2 - Fig. 1A - are also implicated in EDS pathology [4]. Reduced LH1 activity caused by *PLOD1* gene mutations hinders lysine post-translational hydroxylation resulting in lower hydroxylysine content in collagen fibrils [22]. Therefore, intramolecular collagen crosslink through this amino acid is compromised leading to inferior tensile strength of the fibers and to skin mechanical instability [23]. In turn, the deficient activity of ADAMTS2 impairs *N*-propeptide cleavage restricting collagen fibrils lateral growth [24], similarly to what happens with *COL1A1* and *COL1A2* mutations.

EDS is also linked to impaired assembly of collagen fibers due to mutations in *COL12A1* or *TNXB* genes, encoding for collagen XII and tenascin X, respectively [4] - Fig. 1A. Since these proteins are responsible for forming flexible bridges between collagen fibrils, the qualitative and/or quantitative alterations caused by those mutations, lead to more loosely packed collagen fibers composed of disorganized fibrils with increased interfibrillar spacing [25,26].

New EDS-associated mutations identified in the last decade broadened insights into the role of abnormal glycosaminoglycans (GAGs) in the collagen fibers assembly and stability [27] - Fig. 1A. Mutations in *B4GALT7* and *B3GALT6* genes, encoding for galactosyltransferase I and II, prevent the elongation of the proteoglycans glycan chain from the first or second galactose, respectively [28]. This severe deficiency in GAGs synthesis particularly impacts decorin, involved in the interfibrillar collagen bridging directly or indirectly through the interaction with collagen XII and tenascin X [27,28]. Further downstream in the biosynthetic pathway of GAGs, mutations in *CHST14*, which encodes dermatan 4-O-sulfotransferase 1 (D4ST1), or in *DSE* encoding for DS-epimerase 1 (DS-epi1)), also cause EDS. These two enzymes are crucial for the introduction of L-Iduronic acid (IdoA) residues, which are responsible for providing flexibility to dermatan sulfate (DS) chains [29]. As about 60 % of the decorin chain consists of DS [29], impaired function of D4ST1 or DS-epi1 leads to reduced flexibility of those chains, which are unable to normally surround collagen fibrils and to tightly bundle them forming collagen fibers. Thus, in EDS, decorin chains extend perpendicular to collagen fibrils spreading across interfibrillar spaces inhibiting normal supramolecular collagen fibril assembly [27,30].

Altered elastic fibers in Cutis Laxa

Mutations in the last 5 exons of the elastin gene (*ELN*) result in the substitution of the C-terminus of tropoelastin - elastin precursor - by a missense peptide sequence [31] - Fig. 1B. The mutant tropoelastin exhibits increased self-association at the first step of elastic fiber assembly – coacervation – resulting in increased globule formation [31]. Nevertheless, the conformational changes in the mutant tropoelastin interfere with its binding to the microfibrils resulting in lower amount mature elastic fibers [31,32].

Fibulin-4 (*FBLN4*), fibulin-5 (*FBLN5*) and latent transforming growth factor beta-binding protein 4 (*LTBP4*), essential proteins for proper elastogenesis, are all implicated in CL [33–35] - Fig. 1B. The fibulin-4 mutant protein is less capable of binding to tropoelastin and the crosslinking enzyme lysyl oxidase, affecting this step of elastogenesis [36]. Also, decreased amount and/or structural changes of fibulin-5 alters its interaction with both tropoelastin and fibrillin-1, interfering with the association of tropoelastin aggregates with microfibrils [37–39]. Premature-termination mutations in *LTPB4* lead to a severe reduction in the mRNA amount [40], and consequently, in protein abundance. Because LTBP4 directly binds to tropoelastin, fibulin-4, and fibulin-5, mutations in that gene also affect tropoelastin binding to microfibrils [35,41].

A growing body of evidence has been allowing identifying metabolic abnormalities also as a cause of CL. Mutations in ATPase H + Transporting V1 Subunit E1 (*ATP6V1E1*), V1 Subunit A (*ATP6V1A*) and V0 Subunit A2 (*ATP6V0A2*) cause intracellular tropoelastin accumulation due increased pH and coacervation, which normally occurs upon released to the extracellular space [42,43] - Fig. 1B. Mutations in *RIN2*, *COG7* or *GORAB* which encode Golgi enzymes involved in intracellular trafficking were also identified in CL [44,45] - Fig. 1B. Although their causality to impaired elastogenesis is still unknown, it was already shown that CL patients with *RIN2* mutations have a decreased expression of fibulin-5 in the dermis [45]. Furthermore, mitochondrial enzymes involved in the biosynthesis of proline – one of the major amino acids composing tropoelastin - encoded by *ALDH18A1* and *PYCR1* genes, have been identified in CL - Fig. 1B - but the mechanisms behind this phenotype of the disease remain unknown [46,47].

Basement membrane alterations and the paradigm of blistering disorders

Inherited Epidermolysis Bullosa

The simplex form of EB (EBS) represents the mildest and most common form of EB, mainly characterized by non-scarring blisters in the hands and feet. Mutations in *KRT5* and *KRT14* genes, encoding for keratin 5(K5) and 14(K14), respectively - the main constituents of the intermediate filament (IF) - are its prime cause [48] - Fig. 2. Alterations in any of these proteins result in abnormalities in the keratin IF network, compromising the structure and function of the basal layer keratinocytes, which become prone to cytolysis, weakening the dermal-epidermal bond [49]. More rarely, mutations in genes encoding for the hemidesmosomes proteins plectin (*PLEC1*) [50], which directly crosslinks with IF, and α6β4 integrin (*ITGA6, ITGB4*), which anchor basal keratinocytes to lamina lucida, can also cause EBS [51]- Fig. 2.

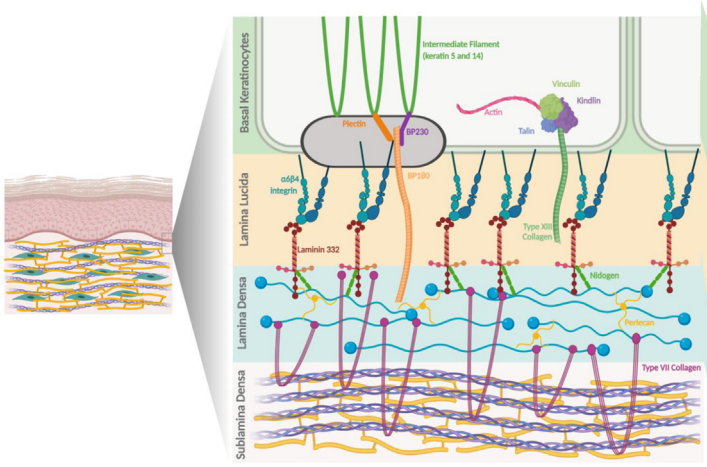
Mutations in the *LAMA3*, *LAMB3* and *LAMC2* genes which encode respectively α3, β3 and γ2 chains of laminin 332 – main constituent of anchoring filaments - originate JEB, a more severe and less common type of EB characterized by a plane of cleavage through the lamina lucida [52,53] - Fig. 2. In the complete absence of laminin 332 occurs a mucocutaneous blistering at birth that, with time, involves most of the body surface leading to patients' death within the first year of life [54]. More sporadically, JEB arises from mutations in genes encoding for BP180 (*COL17A1*) [55] or integrin α6β4 (*ITGA6*, *ITGB4*) [54,56] - Fig. 2 - both of which are located in basal keratinocyte hemidesmosomes and bind to laminin 332 in the lamina lucida.

Over 700 mutations in *COL7A1* gene, encoding for collagen VII, have been identified so far in DEB - Fig. 2 - that can be inherited in a dominant (DDEB) or recessive (RDEB) manner [57,58]. Partial or total loss of type VII collagen - the major constituent of anchoring fibrils - results in sublamina densa fragility with loss of adhesion between the dermis and the basement membrane [57]. Although with a considerable phenotypic overlap, generally RDEB is a more severe variant with widespread blistering and erosions that result in extensive scarring, contrasting to the localized sores that characterize the milder variant (DDEB) [53].

The Kindler EB is extremely rare, with only 250 known cases worldwide and is caused by mutations in the *FERMT1* gene – encoding for the kindlin-1, an intracellular protein of focal adhesions [53,59] - Fig. 2. It seems that *FERM1* loss-of-function mutations disrupt the attachment of the actin cytoskeleton of basal keratinocytes to the BM, hence weakening the dermal-epidermal bond [60]. However, the mechanisms that led to the variable degree of skin cleavage found in these very few documented patients are still unknown [53].

Autoimmune bullous dermatoses

Bullous pemphigoid (BP) is the most diagnosed form of pemphigoid blistering diseases, being characterized by the production of autoantibodies against the epidermal hemidesmosome proteins BP180 and/or BP230 - Fig. 2. Autoantibodies against BP180 appear to be the major trigger of BP as they are found in nearly 90 % of the



Affected Protein	Skin Disease	
	Autoimmune	Genetic
Kindlin-1	-	Kindler EB
Keratin 5	-	EB Simplex
Keratin 14	-	EB Simplex
Plectin	-	EB Simplex
α6β4 integrin	Mucous membrane pemphigoid	EB Simplex Junctional EB
Laminin 332	Mucous membrane pemphigoid	Junctional EB
BP180	Bullous Pemphigoid Mucous membrane pemphigoid Linear IgA bullous disease	Junctional EB
BP230	Bullous Pemphigoid Mucous membrane pemphigoid	-
Type VII Collagen	Epidermolysis Bullosa Acquisita	Dystrophic EB

Fig. 2. Blistering genetic and autoimmune disorders result from the disruption of the dermal-epidermal adhesion complex which can occur at different levels -basal keratinocytes, lamina lucida, lamina densa or sublamina densa - depending on the affected protein. Those proteins are either mutated - different Epidermolysis Bullosa types – or attacked by autoantibodies – different types of Autoimmune Bullous Dermatoses. Figure created with BioRender.com.

patients [61,62]. The role of the BP230-autoantibodies in BP pathogenesis is controversial despite some evidences suggesting that they might cause the disease [63,64]. The pathological mechanism of BP has been associated to both complement-dependent and complement-independent pathways. The complement-dependent pathway occurs in nearly 80 % of the BP cases due to the high ability of IgG autoantibodies to activate complement cascade when they bind to their targets [65]. Once the cascade is activated, the complement factors C3a and C5a induce the chemotaxis of neutrophils and eosinophils, as well as the degranulation of mast cells [65]. Activated eosinophils accumulate in the BM and secrete matrix metalloproteinase (MMP)9 that specifically degrades BP180 resulting in dermal-epidermal detachment and blister formation [66]. Also, IgG autoantibodies are able to prompt the formation of blisters via direct interaction with BP180, in a complement-independent manner [67]. Cross-linking of IgG autoantibodies with BP180 triggers the macropinocytosis of this complex, resulting in the depletion of BP180 from the hemidesmosome weakening dermal-epidermal adhesion [68]. It is also known that the autoantibodies promote the secretion of proinflammatory cytokines, such as IL-8 and IL-6 by keratinocytes [69,70], but their direct implication in the progression of the disease is yet to be understood.

Mucous membrane pemphigoid (MMP), is also predominantly characterized by IgG autoantibodies directed against BP180 - Fig. 2 - however, majorly affecting the mucous membranes, and only in around 25 % of the cases the skin [71,72]. Interestingly, while patients with MMP and BP both have IgG autoantibodies against BP180, the recognized epitopes in this protein are specific to each one of the dermatoses [8]. Although more rarely, other MMP subtypes are also linked to autoantibodies against BP230 (typically associated with reactivity to BP180) [73], laminin 332 [74] or $\alpha 6\beta 4$ integrins [73] - Fig. 2. So far, it is known that complement activation is also involved in these MMP subtypes, all of which associated to disruption of the lamina lucida causing blister formation.

Linear IgA bullous disease (LABD) is also characterized by the presence of autoantibodies against BP180 - Fig. 2 - being driven by both complement-dependent and -independent pathways. In contrast to BP, LABD is defined by the production of IgA, which is a weaker activator of the complement-dependent pathway than IgG. Thus, this signaling occurs in less than 30 % of LABD cases [9,75] and alternatively, IgA promotes complement-independent granulocyte activation and migration after direct crosslinking with its receptor (FcαRI) in those cells [76].

Antibodies against type VII collagen due to abnormal activation of the immune system can also originate epidermolysis bullosa acquisita (EBA) - Fig. 2, an autoimmune bullous dermatosis characterized by sublamina densa destruction. The produced IgG antibodies mediate Fcγ-dependent neutrophil activation in the complement-independent cascade [10] and upon interaction with Col VII induce complement activation, solely through the complement factor C5a [77].

Epidermal Neoplasias: Extracellular matrix driving the metastatic route

In the early stages of malignancy, skin cancer cells are confined in the epidermis limited by the BM that once breached, encourage invasion of the near and potentially distant tissues towards metastization [78]. Proteolytic enzyme overexpression has been a hallmark of tumor cells invasion capacity due to their ability to degrade the majority of BM and ECM components. The level of those enzymes has been therefore correlated to the metastatic potential of cutaneous tumors [79] - Fig. 3. Heparanase (HPA), that cleaves heparan sulfate proteoglycans promoting BM disruption by disturbing the link of perlecan with laminin and collagen IV [80], is expressed in significantly greater amounts in cSCC compared to BCC and normal skin tissue [81]. Higher expression of HPA has been also correlated with melanoma patients' poor survival [82]. Importantly, *in vivo* studies showed that knockdown of HPA is sufficient to suppress cSCC and melanoma invasion [83,84]. Similarly, urokinase plasminogen activator (uPA) expression seems to be positively correlated with skin tumors invasive capacity [85,86]. This enzyme converts plasminogen to plasmin, which directly degrades BM laminin and collagen IV [87] and is detected at the edge of invasively growing strands of cSCC [85] as well as in advanced primary melanoma [86]. On the other hand, neither benign lesions nor BCC express uPA [85,86]. Additionally, uPA indirectly promotes BM disruption by the activation of other proteinases like MMP3 and 9 that degrade laminin and collagen IV [88]. These enzymes are involved in the early stages of melanoma progression by cleaving E-cadherin, the prime adhesion mediator between melanocytes and keratinocytes in the epidermis [89]. Its loss weakens cell–cell adhesion, favoring the melanoma cells motility and initial local invasion in the epidermis [90]. Interestingly, these two MMPs are not detectable in BCC and are expressed at a low level in cSCC [91], suggesting that the lower metastatic behavior of these tumors compared to melanoma might be also linked to the preservation of E-cadherin [92]. Additionally, these different invasion patterns are possibly associated to the abundance of MMPs 2/9 and MMP11 shown to be higher in cSCC stroma than in BCC [93–95].

Dermal invasion is a crucial step – particularly for melanoma and to a lesser extent for cSCC - for tumor cells progression - Fig. 3. In cSCC, MMPs 2/9, besides the BM, also degrade dermal ECM proteins like collagens, elastin, fibronectin and fibrillin enabling invasion [96]. On the other hand, during melanoma invasion towards the dermis, MMP3 and MMP7 breaks down aggrecan and fibronectin, together with dermal collagens in the case of MMP7 [97,98]. Also, MMP1 is highly expressed during melanoma vertical growth phase degrading type I and III collagens and activating PAR-1 (protease activator receptor 1), which in turn induces the expression of cancer specific genes with known functions in tumor growth, invasion and metastasis [99]. In fact, PAR-1 blockage has been shown to reduce tumor growth and metastasis in both *in vitro* and *in vivo* melanoma models [100,101].

Proteolytic enzymes, besides directly remodeling dermal ECM, can indirectly promote tumor invasion through the activation of molecules involved in the metastatic cascade - Fig. 3. uPA, by converting plasminogen in plasmin, mediates the activation of certain growth factors, such as fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF), further increasing the invasion potential of melanoma. FGF2 was shown to promote melanoma cell migration via syndecan-4 dependent mechanism [102]. VEGF stimulates autocrine tumor growth, protects melanoma cells from death by activating anti-apoptotic proteins, and enhances endothelial cell proliferation and sprouting promoting angiogenesis [103,104]. Angiogenesis is also promoted by MMPs 2/9-induced release of VEGF via an integrin $\alpha v5$/phosphoinositide-3-kinase-dependent pathway[105,106]. In turn, MMP-1 promotes the release of FGF, VEGF, TGF-β via PAR-1 establishing a feedback loop that enhances ECM remodeling [99]. Additionally, both FGF and VEGF growth factors stimulate the production of the motility factor thrombospondin-1 (TSP-1), a chemotactic and haptotactic attractant with a direct pro-invasive effect on melanoma cells' motility within the dermal ECM [107].

Translational Challenges: From Disease Awareness To Treatment

Despite the knowledge that has been generated regarding the potential of ECM components as therapeutic targets in various skin

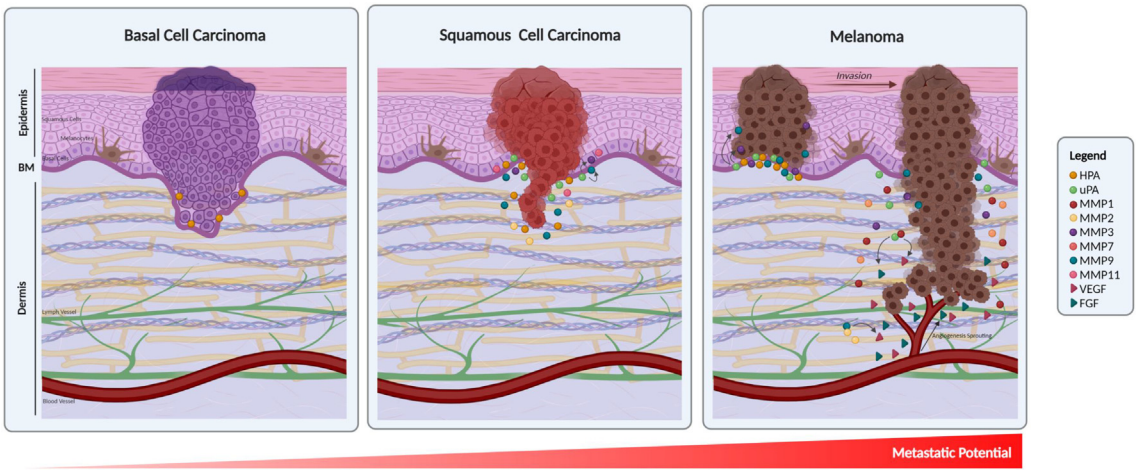


Fig. 3. The progression of epidermal neoplasias is driven by the disruption of skin basement membrane and dermal extracellular matrix (ECM) remodeling.The expression of ECM degradative is linked with cancer progression and the ability of cancer cells to outgrow in the epidermis, penetrate the dermis, and access to lymphatic or blood vessels, being therefore correlated with skin cancer metastatic potential. The number of dots indicates the relative amount of each enzyme in comparison between the three different skin tumors illustrated. Figure created with BioRender.com.

disorders, so far directed therapies are a reality only for epidermolysis bullosa. Additionally, ongoing clinical trials for other skin disorders are mainly focused on immunomodulatory approaches or management of associated comorbidities - Table 1.

The full restoration of BM strength was achieved using autologous genetically modified cultured epidermal stem cells in JEB patients harboring mutations in *LAMB3 gene* [108,109]. Spurred by this favorable outcome, similar trials (NCT03490331, NCT02984085) are currently ongoing in RDEB and JEB patients to evaluate safety and efficacy of autologous epidermal grafts expressing *COL7A1* and *COL17A1* transgenes, respectively. A phase I/II study investigating the long-term efficacy of autologous gene-corrected keratinocyte sheets in chronic RDEB wounds showed that 70 % of treated sites have more than 50 % of the wound closed at year five [110]. Additionally, exploratory phase I/II trial data (NCT03536143) in RDEB patients regarding the use of a *COL7A1*-carrying viral vector delivered in a topic gel showed complete healing of 90 % of wounds and confirmed the production of functional collagen type VII deposited along the BM [111]. As such, a phase III trial (NCT04491604) is currently ongoing. Another topical gene delivery therapeutic for RDEB under investigation uses a highly branched polymer-based vector to deliver the full-length *COL7A1* [112]. So far, preclinical results in a murine model confirmed type VII collagen expression after 10 weeks following three topical applications in RDEB-associated wounds [113]. Also, intradermal injection of *COL7A1*-modified autologous fibroblasts in RDEB patients led to C7 expression restoring 12 months after treatment [114].

The great advantage of gene replacement therapy is the possibility to target a high number of mutations in the same gene with a single therapeutic strategy, extending a single approach to patients with different mutations. Hence its attractiveness for monogenic disorders - such as DEB – or genetic diseases caused predominantly by mutations in one gene - *LAMB3* in JEB. Dominant ECM-associated skin disorders, in which disease results from one altered copy of the gene, are not amenable to correction through the addition of the defective gene. Instead, CRISPR/Cas9 based gene editing, although still early, has demonstrated repair of dominant mutations within *KRT14* [115] and *KRT5* [116,117] genes in *in vitro* keratinocytes from EBS patients. Also, the same approach has allowed the restoration of *COL7A1* [118,119] expression in keratinocytes and fibroblasts from DEB patients and *COL17A1* [120,121] expression in keratinocytes from JEB patients. For the treatment of EDS and CL, little progress has been made so far. Likewise, therapies for autoimmune skin diseases have not advanced significantly, despite the resemblances in clinical manifestations and common involved ECM players with genetic blistering disorders. In fact, the clinical investigation is solely focused on B lymphocyte-depleting therapies to reduce levels of deleterious autoantibodies, known to be responsible for their onset.

Dynamic matrix remodeling that occurs during tumorigenesis is becoming an appealing target for therapeutic intervention. For example, overexpressed ECM remodeling enzymes promote the digestion of BM components creating breaches in this physical barrier and allowing cancer cells invasion. Thus, inhibition of MMP2/MMP9 [122] and uPA [123] was shown respectively to reduce lung melanoma metastasis and abrogate the invasiveness of melanoma cells *in vitro*. Nonetheless, clinical trials with MMP inhibitors have failed due to off-target effects, and novel strategies to increase therapeutic specificity are currently being investigated [124]. Another example is the increasing ECM rigidity because of excessive collagen production/deposition, which has been also associated with cancer cell invasion [125]. Due to the high degree of cell confinement, the stiffness of the ECM promotes the formation of tumor clusters and drives collective migration via a protease-dependent process, hence increasing tumor cells migration speed [126]. In skin carcinogenesis, the most studied strategy for reducing ECM stiffness has been the inhibition of TGF-β signaling by blocking its binding to serine/threonine kinase receptor (TGFβR1) and the subsequent activation of SMAD pathway, impairing collagen gene expression [127]. In melanoma, anti-TGFβR1 was effective in diminishing tumor growth and invasion [128,129] and reducing bone metastases [130] in murine models. Furthermore, two phase I clinical trials using anti-TGFβ monoclonal antibodies have shown preliminary evidence of antitumor activity in patients with advanced melanoma [131,132]. The importance of ECM stiffness in creating a permissive tumor microenvironment is also well illustrated in aggressive cSCC that commonly arise in previously injured skin sites in RDEB patients. A preclinical study targeting TGF-β signaling cascade in a murine model of RDEB demonstrated reduced fibrosis of chronically injured forepaws that delayed the development of mitten deformities and prevented cSCC [133].

Table 1
Clinical Trials for skin ECM-related disorders.

	Disease	Number Clinical Trials	Objective	Treatment	Biological Target
Genetic	Ehlers-Danlos Syndrome	3	Symptoms Relief	Compression garments	–
	Cutis Laxa	3	Symptoms Relief	Dietary Supplement	–
				Devices to improve skin laxity	–
	Epidermolysis Bullosa Simplex	3	Symptoms Relief	Cannabinol Cream	–
			Pain Management	Botulinic Toxin	–
	Junctional Epidermolysis Bullosa	5	Symptoms Relief	Antibiotic; Cannabinol Cream	–
			Wound Healing	Thymosin beta-4 Cream	–
			Treat the disease cause	Genetically Modified Epidermal Stem Cells	LAMB3; COL17A1
	Dystrophic Epidermolysis Bullosa	20	Pain Management	Analgesic	–
			Symptoms Relief	Antibiotic; Cannabinol Cream	–
			Wound Healing	Thymosin beta-4 Cream; Skin Graft	–
			Treat the disease cause	Mesenchymal stem cells	–
				Gene Correction	COL7A1
	Kindler Epidermolysis Bullosa	1	Symptoms Relief	Cannabinol Cream	–
Autoimmune	Bullous Pemphigoid	11	Immune System Modulation	Monoclonal Antibodies	CD20 CD125 IgE C1S C5aR IL12 IL4 IL23 PD-1/PDL-1
	Mucous Membrane Pemphigoid	3	Immune System Modulation	Monoclonal Antibody	CD20
			Pain Management	Corticosteroids (Dexamethasone)	–
	Linear IgA Bullous Disease	–	–	–	–
	Epidermolysis Bullosa Acquisita	–	–	–	–
Neoplasia	Basal Cell Carcinoma	43	Target Therapy	Small-molecules	Hg pathway CK2 JAK/SYK HDAC
			Immune System Modulation	Monoclonal Antibody	PD-1
	Squamous Cell Carcinoma	93	Immune System Modulation	Monoclonal Antibodies	PD-1 EGFR
				Oncolytic virus	*T*-VEC (target GM-CSF)
				Small-molecules	mTOR pathway TLR9 Multiple kinase EGFR
				Monoclonal Antibodies	IL15 IL7 CD47/CD40
	Melanoma	188	Immune System Modulation	Monoclonal Antibodies	CTLA-4 PD-1
				Oncolytic virus	*T*-VEC (target GM-CSF)
				Adoptive cell therapy	Natural killer cells Lymphokine-activated killer cells Cytotoxic T cells Dendritic cells
			Target Therapy	Monoclonal Antibodies	BRAF (BRAF-mutant melanoma) MEK (MEK -mutant melanoma)

The table reports registered clinical trials on https://clinicaltrials.gov focused on the different diseases in study. Access date: 15 November 2022. Filters: Recruitment: "Recruiting", "Not yet recruiting" and "Active, not recruiting"; Study Type: "Interventional (Clinical Trial); Intervention/treatment: "Drug" and "Biological."

Concluding remarks and future perspectives

A growing body of research demonstrates that the ECM plays a critical role in the pathophysiology of cutaneous disorders. Over the last two decades, numerous breakthroughs have been made by successfully applying that knowledge to the development of innovative therapeutic approaches for EB. The promising prospects could theoretically be extended to EDS and CL. Yet, little progress has been made in that line. Possibly because these disorders involve various distinct ECM-related genes each affecting a relatively small number of people, which makes this area of research not as attractive for the pharmaceutical industry. In what concerns therapies for cutaneous tumors, inhibitory strategies towards ECM remodeling enzymes or to reduce ECM stiffness are still the realms of basic and early translational research. However, countless options await to be explored as increasing evidence indicates that ECM dramatically influences cancer progression. Regarding autoimmune bullous dermatoses, numerous unanswered ques-

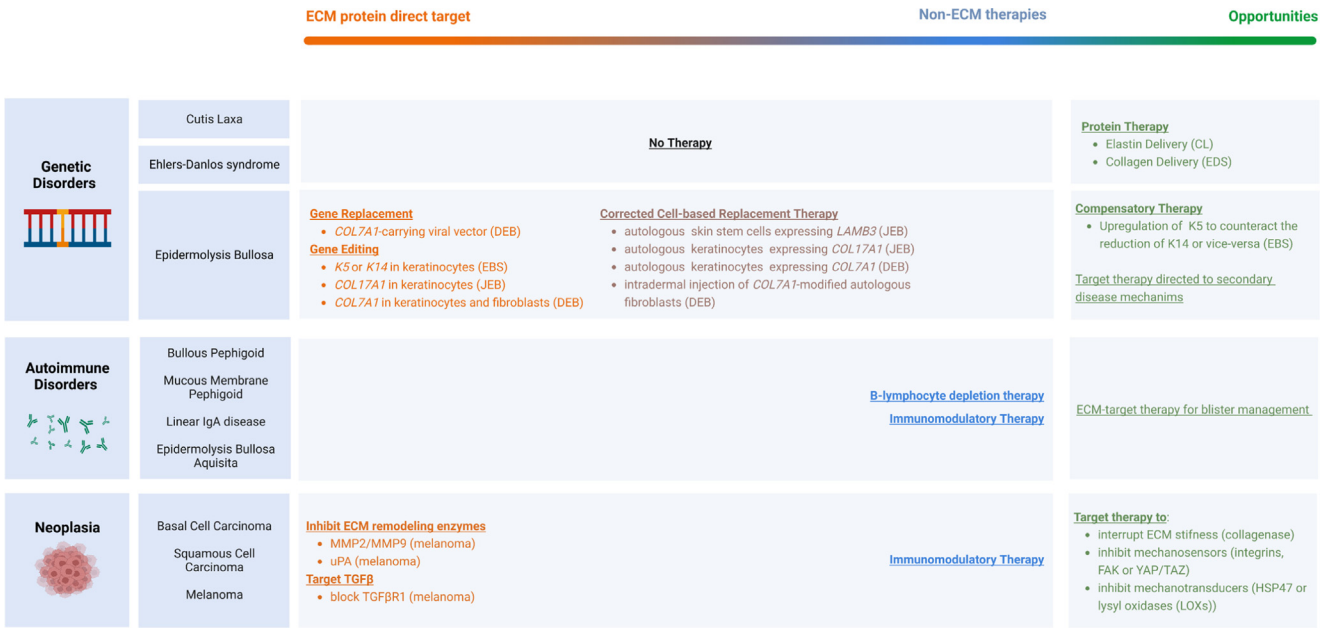


Fig. 4. Current Treatments and Opportunities to treat ECM-associated disorders. Current treatments either target directly ECM proteins or do not consider ECM. Figure created with BioRender.com.

tions remain, and it is now critical to understand what triggers the production of autoantibodies against specific ECM components. This will provide new insights into the pathomechanisms of these diseases leading in the future the development of new treatment options.

Targeting ECM as a strategy to interfere with specific skin diseases has yet to attain its full therapeutic potential and several opportunities await to be explored - Fig. 4.

In dominant genetic disorders - one altered copy of the gene causes the disease - research has not yet proven to be fruitful. Thus, it might be useful to target unaltered genes to trigger compensatory mechanisms. This could be applicable in EB simplex, in which an upregulation of K5 might counteract the reduced K14, or vice-versa, leading the assembly of healthy intermediate filaments. Other disorders that are in urgent need of therapeutical options are CL and EDS. Because they are associated with mutations in varied genes involved in different stages of protein synthesis, approaches that deliver the final molecule, such as elastin and collagen, to the skin might be considered. In contrast, while the repertoire of potentially curative EB therapies is expanding, it appears highly improbable that a "one-size-fits-all" treatment will ever exist. Rather than that, the choice between the use of gene-, protein- or cell-based therapies would have to be better supported by knowledge of mechanisms secondary to the disease. Moreover, lessons from these can also be employed, not in curative therapies, but as alternative or adjuvant treatments for blisters' management in autoimmune disorders. In the case of skin tumors, approaches to interrupt ECM stiffening, such as for example the use of recombinant collagenase [134] might have significant advances in the short term. Also, inhibition of ECM stiffness-regulators - HSP47 and lysyl oxidases (LOXs) - or mechanosensors - integrins, FAK and YAP/TAZ- explored in other tumors [135] could be used. Yet, the selection of the optimal target, from the multitude of options, balancing efficacy and fewest adverse effects remains the main challenge.

Taken together the complex dynamics of ECM-related disorders, it seems probable that combinations of different therapeutic principles will bring the best results. For example, interval therapies using combining or alternating ECM- and non-ECM-targeted therapies can be used to combat the causes or the secondary disease manifestations. Nonetheless, the translation of new findings to clinical practice must overcome critical issues - direct treatment of skin manifestations cannot boost extracutaneous symptoms or induce immunologic consequences. Technological advancements and emerging multidisciplinary approaches, such as the combination of cell biology, material science, and nanotechnology tools, might help overcoming such potential constraints to ultimately improve the quality of life of patients suffering from skin pathologies.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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