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The extracellular matrix of the dermis: flexible structures with dynamic functions

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Abstract: The current understanding of the role of extracellular matrix proteins is mainly based on their structural properties and their assembly into complex networks. The multiplicity of interactions between cells, cytokines and growth factors within the networks determines functional units dictating the biophysical properties of tissues. This review focuses on the understanding how alterations in the genes, modifying enzymes or biological functions of extracellular matrix molecules, lead to inborn or acquired skin disorders. Analysis of the disease mechanisms provides the basis for the emerging concept that not solely structural defects of single extracellular matrix proteins are at fault, but rather that the functional unit as a whole is not working properly, causing similar clinical symptoms although the causative genes are entirely different. The understanding of these disease-causing pathways has already led to surprising new therapeutic developments applied to rare inborn disorders. They now permit to design new concepts for the treatment of more common diseases associated with the accumulation of connective tissue and alterations of the biomechanical properties of the extracellular matrix.

Key words: collagen – Ehlers-Danlos syndromes – fibrillin – fibrillogenesis – integrins – laminin – Marfan syndrome – skin blistering diseases – TGF-β

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Introduction

Most extracellular matrix proteins are macromolecules consisting of either a single polypeptide chain, like nidogens, fibrillins, fibulins or several, together associated, polypeptide chains, for instance collagens or laminins (Table 1). At the structural level, extracellular matrix proteins are constructed from a relatively small number of well-defined modules, such as the collagen triple helix, the fibrillin- and laminin-type epidermal growth factor–like module, the von Willebrand factor A domain, follistatin-like and EF-hand Ca^{2+}-binding motif or coiled-coil oligomerization domains (1,2). An important characteristic of extracellular matrix proteins is that they assemble into complex networks, whose composition and architecture determine the biophysical properties of tissues such as stiffness, compliance and resilience (3). Deposition and assembly of extracellular matrix proteins into insoluble and complex polymers are regulated processes adapted to development, tissue remodelling, repair, ageing and wound healing. Conversely, stiffness and compliance of tissues are important factors regulating the functions of the cells embedded into or apposed to the matrices (4). This regulation proceeds either directly because proteins within the networks interact with cell surface receptors to initiate specific signalling pathways or indirectly because activity and availability of compounds such as cytokines and growth factors are controlled through transient sequestration within the networks (5,6).

There are heritable disorders as well as many acquired diseases, for instance fibrosis and inflammatory pathologies, associated with dysfunction in extracellular matrix protein expression, function and metabolism (7–10). The molecular mechanisms responsible for the diseases are complex and often involve multiple pathways. In this overview, a brief summary of collagen biosynthesis and genetic diversity, as well as of the supramolecular organization of extracellular matrix proteins into functional units, will be presented to address the emerging heterogeneity of pathways leading to extracellular matrix diseases with skin manifestations.

Collagen biosynthesis is complex and proceeds through multiple steps

Collagens form the most abundant and largest family of genetically, structurally and functionally diverse molecules (11). Collagen genes are first transcribed into messenger RNAs, leading to the synthesis of precursor polypeptides, the α chains (7), followed by multiple biosynthetic steps, which have been best detailed for the major interstitial collagens. In particular, the precursor polypeptides of interstitial collagens undergo a series of post-translational modifications in the rough endoplasmic reticulum and the Golgi, including hydroxylation of certain prolyl and lysyl residues and glycosylation, i.e. attachment of galactose or glucosylgalactose onto certain hydroxylysyl residues (12–14). Prolyl hydroxylation is a prerequisite for folding of three α chains along their entire length or only part of it into a triple helix, so that the monomer contains at least one stretch with a triple-helical structure, which defines the so-called collagen domain. Folding of three α chains into a triple-helical conformation is permitted by the occurrence of glycine (Gly) in about one-third of the amino acids, such as the polypeptide consists of repeating Gly-X-Y triplets, with the X and Y positions often occupied by proline and hydroxyproline, respectively (7). The protein quality control taking place in the endoplasmic reticulum permits that properly folded collagen precursors only will be exported out of the cell (8,15). Lysyl hydroxylation is needed for cross-link formation to stabilize collagen networks in

Table 1. Most frequent extracellular matrix proteins

Family name	Number of genes and name of the proteins
Collagens (COL)	44 genes, 28 proteins (COL I to XXVIII)
Elastin	1 gene
Fibronectin	1 gene, 20 splice variants
Fibulins	7 genes, 7 proteins (fibulin-1 to 7)
Fibrillins	3 genes, 3 proteins (fibrillin-1 to 3)
Latent TGF-β–binding proteins (LTBPs)	4 genes, 4 proteins (LTBP-1 to 4)
Laminins (LMs)	11 genes, 16 proteins (LM-111, LM-332, LM-511,...)
Matrilins	4 genes, 4 proteins (matrilin-1 to 4)
Nidogens	2 genes, 2 proteins (nidogen 1 and 2)
Tenascins	4 genes, 4 proteins (tenascin-C, -X, -R and-W)
Thrombospondins (TSPs)	5 genes, 5 proteins (TSP-1 to 5)
Vitronectin	1 gene, 1 protein
Proteoglycans	>40 different species

Most extracellular proteins form families. For each protein family, the family name, the number of genes and the denomination of the family members are indicated.

the extracellular space (13). After secretion into the extracellular space, those collagen precursors with amino- and/or carboxyl-terminal extensions (procollagens) are converted to collagens by the action of specific amino- and carboxyl-terminal proteinases removing the amino- and carboxyl-terminal propeptides, respectively, followed by the spontaneous alignment of collagen molecules into fibres (11,16). Fibrils are stabilized by intramolecular and intermolecular covalent bonds, or cross-links, following the oxidative deamination of certain lysyl and hydroxylysyl residues by lysyl oxidase. Interactions of the collagen oligomers and polymers with other proteins of the extracellular matrix, many growth factors and cytokines lead to specific structural networks with biological activity and biophysical properties important for the skin. These networks will be eventually remodelled by enzymes from the matrix metalloproteinase (MMP) family (17). In particular, MMP-1 and MMP-13 initiate the degradation of collagens I and III, the most abundant collagens present in skin. For instance, MMP-1 catalyses the cleavage of the α1(I) chain at a specific site, giving the characteristic three-quarter/one-quarter collagen fragments. At physiological temperature, the fragments are unstable and denature into gelatin, which is then susceptible to attack by other proteinases.

Variety in collagen genes and supramolecular assemblies with other extracellular matrix components in the dermis

Forty-two different collagen genes in the human genome give rise to twenty-eight different homo- or heterotrimers, collagens I–XXVIII, at least half of them being present in the skin (11). Collagens contain at least one triple helical domain, and some members of the family contain one or several non-collagenous modules. Based on the length and number of triple helical collagen domains and non-collagenous modules, as well as on the architecture of their assembly in tissues, the genetically distinct collagens are subdivided into several classes: fibril-forming, microfibrillar, network-forming, FACIT (fibril–associated collagen with interrupted triple helix) and transmembrane collagens. All of these classes are represented in the skin, with the fibril-forming collagens I, III and V being the most abundant. These collagens assemble together into the large and parallel fibril bundles with the typical cross-striation seen in the dermis by transmission electron microscopy. Collagens I and III represent close to 90% and 10%, respectively, in the composition of dermal collagen fibrils, and collagen V is present as a minor fraction of about 2% (18). The fibrils provide a scaffold for anchoring other proteins (Fig. 1a), in particular for collagens XII and XIV belonging to the FACITs group (19). By associating with collagen fibres, these collagens as well as small proteoglycans, such as decorin, fibromodulin or lumican, are thought to regulate fibril formation, diameter and spacing (2,20). Decorin, an ubiquitous component of connective tissues, is particularly abundant in the dermis (21), where it is thought to contribute to collagen fibrillogenesis by mediating or

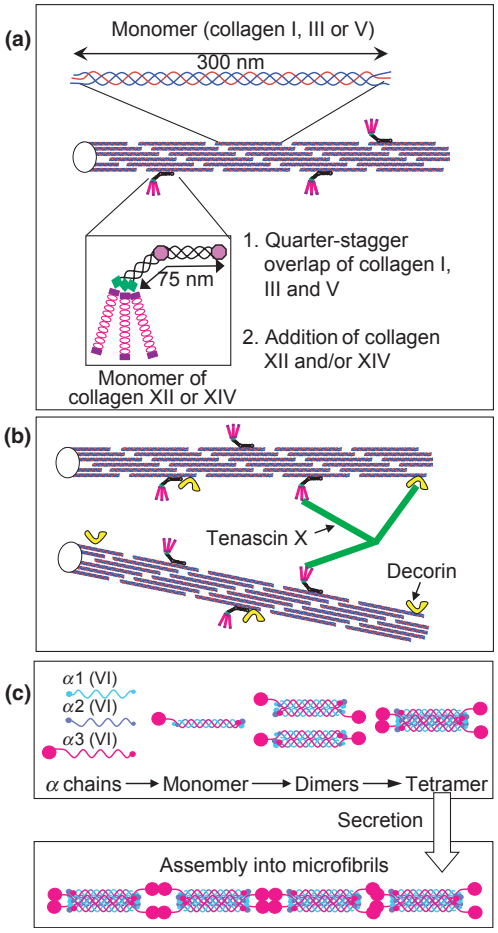

Figure 1. Supramolecular assembly and interactions of collagens in the dermis. (a) Monomers of fibril-forming collagens I, III and V align head-to-tail and in parallel to form large fibrils. Collagen XII and XIV from the FACIT family are homotrimers with a short triple helical domain, and large non-collagenous domains, and they associate with the fibril-forming collagens. The drawing illustrates the modular structure of collagen XII, with two short triple helical collagenous domains (black), two von Willebrand factor A domain (blue), one thrombospondin N-terminal domain (green) and multiple fibronectin type III repeats (pink). (b) Non-collagenous molecules regulate fibrillogenesis by direct (decorin, yellow) or indirect (tenascin-X, green) binding to collagen fibrils. (c) Collagen VI form its own microfibrillar network of collagen VI. Collagen VI monomers arise from the assembly of three different α chains. Intracellularly, the monomers form antiparallel dimers, which in turn associate into tetramers. The tetramers are secreted in the extracellular space and align end-to-end in a unique microfibrillar pattern of thin and long aggregates in the dermis.

stabilizing interactions between collagen I, FACITs and tenascin-X, a member of the tenascin family of extracellular matrix proteins (Fig. 1b). Altogether, the fibrils with their associated proteins confer tensile strength to the skin and are pivotal for the general organization and stability of the dermal extracellular matrix. Another relatively abundant dermal collagen is the microfibril-forming collagen VI. In contrast to the fibril-forming collagens whose assembly is initiated in the extracellular space after conversion of the procollagens into collagens, collagen VI assembly starts inside the cells (22,23). Before secretion, collagen VI monomers form antiparallel dimers, which in turn associate into tetramers (Fig. 1c). After secretion in the extracellular space, the tetramers align to form thin microfibrils, which are thought to bridge different supramolecular assemblies, such as collagen fibres, and protein networks of the basement membrane at the dermal–epidermal junction.

Concurrent fibrillar networks in the dermis: the elastic fibres

Besides collagen-based networks, other supramolecular assemblies are highly relevant to skin biology and pathology. This is the case of the elastic fibre network endowing tissues with elasticity and resilience (10,24–26). The network consists of elastin and microfibrils composed by several proteins (Table 1) such as fibrillins, latent transforming growth factor (TGF)-β–binding proteins (LTBPs), fibulins and microfibril-associated glycoproteins (MAGPs). The primary sequence of fibrillins, latent transforming growth factor (TGF)-β–binding proteins (LTBPs) and fibulins are dominated by multiple calcium-binding, epidermal growth factor (EGF)-like motifs (Fig. 2a). These proteins are of considerable interest because they modulate TGF-β bioavailability (27,28). Cells secrete TGF-βs as a small latent complex, in which the latency-associated propeptide prevents binding of the growth factor to its receptors, or as a large latent complex with LTBP, which mediates interactions with fibrillin-1 and probably fibulin 4/5 and thus anchorage to microfibrils for storage (Fig. 2b). Binding of MAGPs to microfibrils is thought to displace the small latent complex and release free TGF-β. TGF-β is probably the most powerful growth factor–controlling expression, deposition and turnover of collagens and other extracellular matrix proteins in the skin. It elicits paracrine and autocrine signalling pathways acting on the transcriptional and/or translational levels, and it also induces the expression of other growth factors, in particular connective tissue growth factor.

Polymers and networks associated with the basal lamina

The classical and ubiquitous components of basement membrane are collagen IV, laminins, nidogens and perlecan (9,20). Among the six different α(IV) chains known to exist, four are expressed in human skin, the α1(IV), α2(IV), α5(IV) and α6(IV), with $[\alpha1(IV)]_2\alpha2(IV)$ being the predominant heterotrimer (29). Imperfect Gly-X-Y triplets in the collagen IV α chains confer flexibility to the triple helical molecules. The supramolecular organization of collagen IV into roughly hexagonal networks proceeds by the formation of dimers, through the assembly of two amino-terminal non-collagenous globular domains, and tetramers, via lateral association of the short carboxyl-terminal stretches of four monomers (30). Nidogens and perlecan connect the collagen IV network to laminin scaffolds. At least two different laminins, laminin 332 and laminin 511 (31), are present in the basement membrane of the dermal–epidermal junction. Laminin 511 is widely expressed in the organism, whereas laminin 332 is of a strictly epithelial origin and specific to the basement membranes underlying stratified epithelia such as the epidermis of skin and mucosa (32,33). Laminin 332 is particularly important for the functional properties of the dermal–epidermal junction because it is central to the structure anchoring the epidermis to the dermis (Fig. 3). First, the carboxyl-terminal portion of laminin 332 directly interact with cell surface receptors expressed by basal keratinocytes, in particular the $\alpha3\beta1$ and $\alpha6\beta4$ integrins (9,34). Second, the amino-terminal domains of laminin 332 associate with two collagens specific to the epithelial–mesenchymal interface, collagens VII and XVII, present in the anchoring fibrils and anchoring filaments, respectively (9). Collagen VII has a 450-nm-long triple-helical domain, and it forms antiparallel dimers via interaction of the carboxyl-terminal ends (35). The dimers loop around collagen fibrils of the upper dermis, while the amino-terminal domains interact with collagen IV and laminin 332 (Fig. 3). Collagen XVII is a transmembrane protein anchored in the plasma membrane of basal keratinocytes (36,37). Its extracellular domain, or ectodomain, interacts with laminin 332, and together they form the anchoring filaments reaching the lamina densa (38). Finally, there are other collagens found in basement membranes (11), but whether and how they integrate within specific supramolecular assemblies to contribute to skin physiology is presently not known.

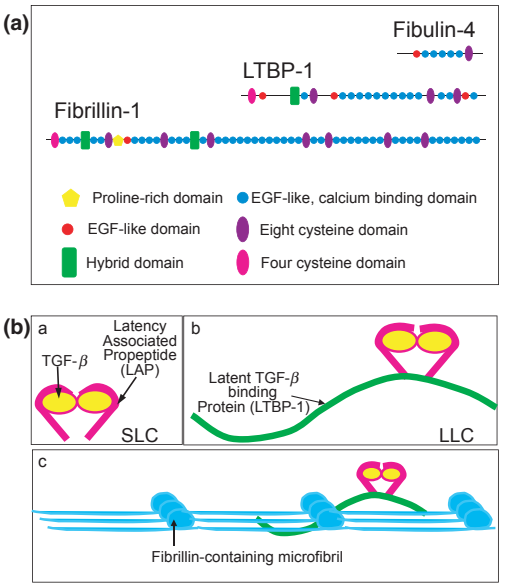

Figure 2. Structure and assembly of proteins forming elastic fibrils. (a) Domain organization of microfibrillar proteins fibulin-4, latent transforming growth factor-β–binding protein-1 (LTBP-1) and fibrillin-1. Each protein belongs to a different gene family (Table 1). Family members thought to be important for the regulation of TFG-β storage and bioavailability are represented. These proteins share common motifs, the most abundant being EGF-like domain with (blue) and without (red) calcium affinity. Other motifs are proline-rich domain (yellow), hybrid domain (green) and motifs with eighth (purple) and fourth (pink) cysteine residues. (b) Regulation of TGF-β storage and activity by association with elastic microfibrils. Cells secrete TGF-β either as a small latent complex (SLC, a) associated with the latency-associated propeptide (LAP) preventing TGF- β binding to its receptor or as a large latent complex (LLC, b) associated with the latent TGF-β binding protein (LTBP). The latter mediates binding to fibrillin-containing microfibrils for storage (c). Dissociation of the protein complex leads to activation of TGF-β and a rapid availability depending on the biological requirements.

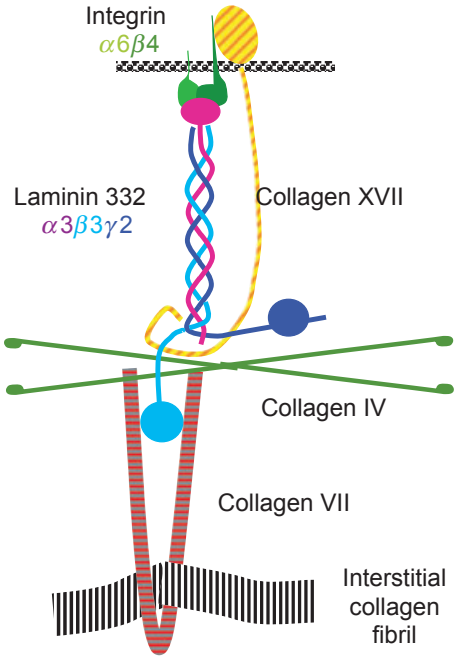

Figure 3. The extracellular architecture maintaining epidermal anchorage to the dermis. Laminin 332, composed of the $\alpha 3$, $\beta 3$ and $\gamma 2$ chains, is a central element of the basement membrane network of the dermal–epidermal junction. Its C-terminus attaches to $\alpha 6\beta 4$ integrins anchored in the plasma membrane of basal keratinocytes, while its N-terminus interacts with collagens VII and XVII, which in turn are involved in associations with collagens IV and other proteins of the sublamina densa and the upper dermis.

Extracellular matrix dysfunction and cutaneous diseases

There are many inherited diseases with skin involvement that are caused by mutations in the genes coding for extracellular matrix proteins (Table 2), enzymes responsible for their post-translational modifications and processing, or proteins and small molecules participating in the supramolecular assembly of extracellular matrix proteins into networks with biophysical activity. Previously, it was assumed that the structural alteration in a protein caused by a genetic mutation was directly explaining the clinical phenotype. Today, we know that the situation is far more complex and many disease-causing pathways involving non-structural alterations have been disclosed. Understanding these new disease-causing mechanisms has several important implications for treating not only rare inborn diseases but also more common acquired diseases involving the extracellular matrix. In the following, we have chosen three types of disorders, the Ehlers–Danlos syndromes, the Marfan syndrome and related pathologies, and skin blistering disorders to discuss various disease-causing mechanisms and how multiple and different gene defects lead to related disorders.

Disorders of collagen fibrils and their consequences: the Ehlers–Danlos syndromes as a model

The Ehlers–Danlos syndromes (EDS) are a group of phenotypically related disorders, with skin hyperextensibility, velvety and easy bruising. The genetic defects are, however, very heterogenous, and mutations occur in the genes coding for collagens I, III or V, lysyl hydroxylase involved in post-translational modifications of fibril-forming collagens, perhaps a Zinc transporter, procollagen amino-peptidase responsible for processing procollagen to collagen, or tenascin-X, a non-collageneous, fibril-associated protein (Fig. 4).

Table 2. Disorders with skin manifestations caused by mutations in the genes encoding extracellular matrix proteins

Collagen I	Achondroplasia EDS (EDS VIIa and VIIb)
Collagen III	Vascular EDS (EDS IV)
Collagen IV	Familiar porencephaly, hereditary angiopathy
	Alport syndrome, leiomyomatosis
Collagen V	Classical EDS (EDS I and II)
Collagen VI	Bethlem myopathy
	Ulrich muscular dystrophy
Collagen VII	Dystrophic epidermolysis bullosa
Collagen XVII	Epidermolysis bullosa junctionalis
Elastin	Cutis laxa
Fibrillin 1	Marfan's syndrome
	Weill-Marchesani syndrome
Fibrillin 2	Contractural arachnodactyly
Fibulin-4	Cutis laxa
Fibulin-5	Cutis laxa
Laminin 332	Epidermolysis bullosa junctionalis
Tenascin-X	Hypermobility EDS (EDS III)

For the Ehlers–Danlos syndromes (EDS), both the new and old (between parenthesis) nomenclatures are used.

Mutations in the *COL5A1* or *COL5A2* genes cause EDS types I and II, or classical EDS (39–41). Several of the mutations induce mRNA instability, followed by nonsense-mediated decay and, consequently, haploinsufficiency. Under these conditions, there are no abnormal molecules, but the amount of collagen V is reduced to a level likely to be insufficient for healthy collagen fibrils to form. Other mutations are supposed to perturb the folding into a triple helix of two $\alpha 1$(V) and one $\alpha 2$(V) chains. For instance, mutations inducing glycine substitution by a bulky amino acid probably hinder the formation of a properly folded triple helix. In this case, either the protein will be recognized as misfolded and targeted for degradation, causing reduced amount of collagen V, or alternatively, abnormal collagen V molecules will be produced, secreted

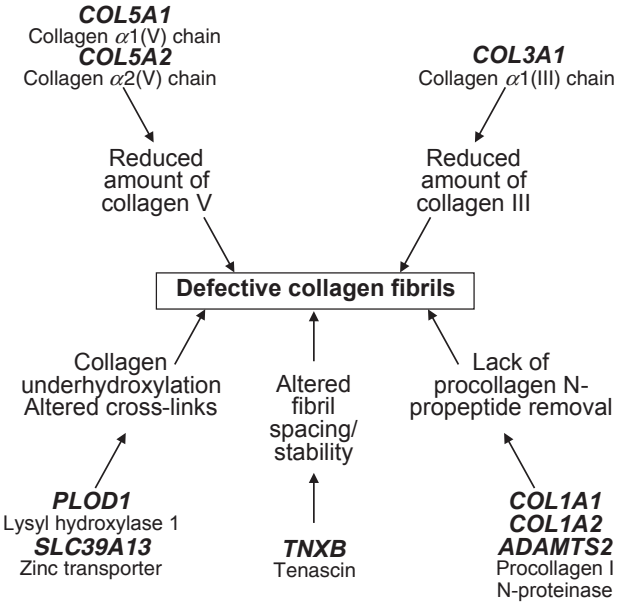

Figure 4. The diversity of genetic mutations in Ehlers–Danlos syndromes leading to defective collagen fibrils. Gene symbols (bold) and names of the corresponding proteins are indicated in italics.

and incorporated into fibrils, thereby creating defective collagen fibrils (Fig. 4). The EDS type IV, or vascular EDS, is caused by mutations in the *COL3A1* gene. Here, again some mutations (mostly splice site mutations) are likely to cause mRNA instability and nonsense-mediated decay, while others (glycine substitutions) presumably hinder the folding of three collagen α1(III) chains into a triple helix (42,43). Interestingly, a recent study showed that both splice site and glycine substitution mutations resulted in a drastic reduction in the amount of collagen III at the protein level (44), supporting the notion that misfolded collagen trimers fail to pass the quality control in the endoplasmic reticulum and consequently are targeted for degradation (8,15). The outcome of both scenarios is that reduced amounts of an otherwise structurally normal collagen III are insufficient to form functionally correct collagen fibrils (Fig. 4). The EDS type VI, or kyphoscoliotic EDS, has been attributed to a deficiency in lysyl hydroxylase in some of the patients (45), causing paucity in hydroxylysyl residues, reduction of stabilizing cross-links and altered physical properties of the collagen fibrils. Interestingly, lysyl hydroxylase displays a normal activity in another group of patients with EDS type VI. For these patients, mutation in a zinc transporter gene (*SLC39A13*) presumably causes accumulation of zinc ions in the endoplasmic reticulum, which compete with ferrous ions, normally required as cofactor for lysyl and prolyl hydroxylase activity (46).

Mutations in at least three different genes lead to EDS type VII, a syndrome in which the amino-terminal propeptide of procollagen I is not removed as it should be. Here, again the fibrils are physically abnormal because the propeptide is very likely a steric hindrance for fibril packaging. EDS type VII was first recognized in dermatosparaxis, a cattle disease, quoted EDS VIIc in human. The disorder is caused by mutations in the *ADAMTS2* gene coding for procollagen N-proteinase, causing abnormal retention of the procollagen N-propeptide (47). In two other phenotypically identical disorders, EDS VIIa and VIIb, a mutation in either the *COL1A1* or *COL1A2* genes modifies the cleavage site of the procollagen N-proteinase in the procollagen α1(I) or α2(I) chains, respectively, preventing the removal of the N-propeptide (48). As a consequence, the procollagen N-propeptides are supposed to hinder packaging and alignment of collagen monomers into fibrils in all three forms of EDS VII. Finally, mutations in the gene coding for tenascin-X have been identified in patients with EDS type III or hypermobility type (49). As a result of its bridging function of collagen fibres (Fig. 1b), tenascin-X has been proposed to be responsible for the stiffness of collagenous networks (50). At last, it should be remembered that although the list of genes whose mutations cause EDS is already long, there are patients who are negative for the mutations described above, and abnormalities in other genes await to be demonstrated. For instance, no inherited disorder involving decorin has been described, although mice with a deletion of the decorin gene display abnormal collagen fibrils and skin fragility reminiscent of EDS (51), supporting the notion that decorin is important for correct collagen fibril formation or architecture. Along these lines, mutation in the gene coding for carbohydrate sulphotransferase 14 (*CHST14*) has been shown to be associated with alterations in the glycosaminoglycan pattern of decorin in a disorder resembling EDS (52).

To summarize, EDS are disorders of collagen fibrils, whether caused by mutations in the collagen genes, deficits in collagen-modifying enzymes or proteins involved in the architecture and biomechanical function of collagen fibrils (Fig. 4). These disorders are a vibrant illustration that integrity of dermal collagen fibrils is crucial for skin biophysical properties.

The elastic fibre network, Marfan's syndrome and growth factor–regulating function of the extracellular matrix

Evidence is accumulating that many extracellular matrix proteins participate in regulatory functions which can be disturbed and lead to disease processes. This is, for instance, the case for Marfans's syndrome. The disease is characterized by thin skin and abnormalities affecting the ocular, skeletal and cardiovascular systems. Mutations that affect the structure or lead to a reduced synthesis of fibrillins are the cause of Marfan's syndrome (28). Genetic, biochemical and functional analysis of cells and patients affected with the Marfan syndrome, as well as mouse models, revealed that mutations in the fibrillin gene are associated with increased TGF-β signalling (24,27). The critical role of TGF-β signalling in the pathogenesis of Marfan syndrome was underscored by the fact that treatment of fibrillin mutant mice with TGF-β neutralizing antibodies or losartan (an angiotensin II type 1 receptor blocker) ameliorated the symptoms, in particular mitral valve prolapse (53). Similarly, treating patients with Marfan's syndrome with angiotensin II inhibitors slowed the development of aortic root dilatation (54). Thus, more than being the consequence of solely a structural mutation in fibrillin, the disease is caused by exacerbated TGF-β signalling. It is also now clear that a dysfunction in the pathways under the control of TGF-β leads to abnormal and uncontrolled accumulation of collagens, a characteristic hallmark of progressive systemic sclerosis and other fibrotic conditions (55–57). The detailed understanding of TGF-β bioavailability and activation gained from studying a rare disease, as well as disclosing how TGF-β dysfunction leads to accumulation of connective tissues and fibrosis, has major implications for the design of novel therapeutic approaches (58).

The basement membrane architecture and skin blistering diseases

Several acquired and inherited skin blistering disorders originate from autoantibodies targeting proteins involved in the anchorage of basal keratinocytes or from mutations in the genes encoding the components of the anchoring complexes. For instance, the most common autoimmune blistering disorders are bullous pemphigoid, associated with autoantibodies against the ectodomain of collagen XVII (37,59), epidermolysis bullosa acquisita, characterized by autoantibodies are against collagen VII (60,61), and mucous membrane pemphigoid, triggered by autoantibodies against several of the components of the anchoring complexes (62). In these diseases, the dysfunction is thought to be caused by accumulating deposition of the autoantibodies within the structure, thus impairing interactions between components, very likely by steric hindrance. Finally, a whole range of severe inherited skin blistering disorders characterized by trauma-induced epidermal detachment from the dermis are caused by mutations in the genes coding for laminin 332, its $\alpha 6\beta 4$ integrin receptor, collagen VII or collagen XVII (9,35,37,61–64). Many of the mutations are associated with the lack of one of the protein in the molecular chain linking the epidermis to the dermis (Fig. 3). Mutations leading to abnormalities in the expression, structure, and interactions of the various components of the dermal–epidermal junction have also

been reported. The exquisitely different molecular defects causing these disorders illustrate the notion that extracellular matrix proteins are organized into specific suprastructures working as functional units and that altering a single building block leads to the construction falling apart.

Emerging disease-causing mechanisms and outlook

As outlined in the preceding paragraphs, mutations in the genes coding for key extracellular matrix proteins often lead to structural and functional consequences associated with severe clinical symptoms. Although gene mutations certainly explain a number of symptoms, not all clinical abnormalities are directly and solely due to defects in protein structure or to the complete lack of a protein. There is mounting evidence that additional disease mechanisms originate from decisions taking place in the endoplasmic reticulum (8,15). The accumulation of misfolded mutant or unfolded polypeptides in the endoplasmic reticulum induces detrimental processes of diverse severity, ranging from increased protein targeting to the proteasome for destruction, macroautophagy, general reduction in protein synthesis including that of the abnormal protein, to complete cellular dysfunction with apoptosis of the cells (Fig. 5). When an extracellular matrix protein is formed by two or more genetically different chains, for instance collagen I or laminin 332, it should be considered whether accumulation of the unfolded, otherwise non-mutated chains, has deleterious consequences for the cell or whether dysfunction results solely because, owing to the mutation, one chain is missing or abnormal.

Finally, an important function of the extracellular matrix is to control cell behaviour, including migration, survival, differentiation, contraction, transmission of forces and expression of specific genes. To this end, extracellular matrix proteins interact with cell surface receptors to activate specific signalling pathways (Fig. 6). The best-known receptors are syndecans and glypicans (65,66), the discoidin domain receptors (67) and integrins (68). These molecules span or are anchored in the plasma membrane, thereby connecting extracellular matrix networks to intracellular scaffolds such as the actin cytoskeleton or keratin intermediate filaments depending on the cell context (66,69). Because integrins are devoid of enzymatic activity, they recruit a myriad of intracellular proteins into platforms at the cell surface to activate signalling pathways (Fig. 6). In addition, integrins allow cells to sense the rigidity of the environment and accordingly they transmit forces to their interior via connections to the cytoskeleton (70,71). It can easily be anticipated that defects in one of the many components constituting the platforms, as well as in the receptor's extracellular ligands, might eventually result in a diversity of dysfunctions. Also, because integrins adjust to specific physiological and pathological changes in the microenvironment, they will adapt the cellular response to the biomechanical changes in the extracellular matrix taking place during wound healing, inflammation, fibrosis and cancer (72,73), and very likely to the metabolic changes associated with skin ageing (74–76). During these processes, many of the extracellular matrix macromolecules deposited in tissues undergo remodelling by various proteases, which specifically cleave defined domains of the macromolecules (17). This remodelling generates fragments, many of them either retaining the original biological activity or displaying a previously cryptic biological property, as is the case for matricryptines originating from collagen IV (tumstatin), collagen XV (restin), collagen XVIII (endostatin) or perlecan (endorepellin). Once released from the tissue, the matricryptines develop their own autocrine or paracrine signals through direct or indirect interactions with integrins. At the same time or independently, tissue remodelling leads to the release or activation of growth factors acting in concert with integrins (72). For example, growth factor activation by αv integrins has been suggested to occur in the development of lung fibrosis and angiogenesis. Eventually, accumulation of extracellular matrix proteins contribute to modify the mechanical properties of their networks resulting in a local increase in tissue rigidity, as it is the case in the microenvironment of tumors, contributing to enhance integrin signalling (73).

Obviously, mechanical tension directly transmitted via integrins to the cytoskeleton has an important regulatory function for many cellular activities. It is involved not only in repair processes and fibrosis but also in the tissue response to tumor formation and invasion. Modulation of cellular interactions with the surrounding extracellular matrix either by interfering with integrin binding or by regulating expression and activity of critical intracellular integrin-binding partners could therefore be a promising approach to

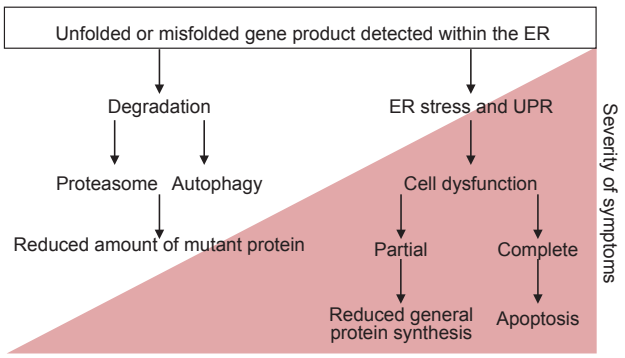

Figure 5. Quality control in the endoplasmic reticulum. Misfolded extracellular matrix polypeptide chains harbouring mutations, or excess of unfolded polypeptides, are either targeted for degradation or their accumulation within the endoplasmic reticulum induces the unfolded protein response, leading to partial or complete cell dysfunction and apoptosis.

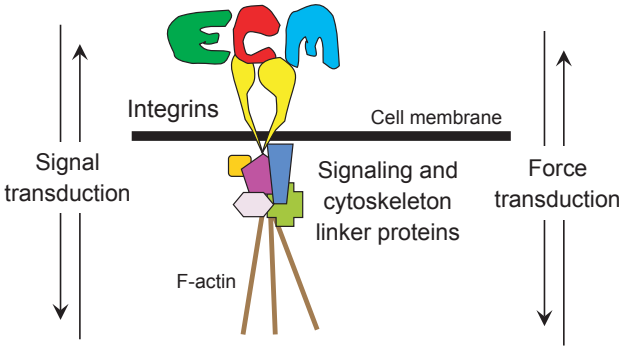

Figure 6. Cellular interactions with the extracellular matrix. The main cellular receptors for extracellular matrix constituents are integrins. They consist of two non-covalently associated subunits (α and β). The large extracellular domains of both subunits provide a binding site for extracellular ligands. The short intracellular tails of the subunits recruit cytoskeleton-associating and adaptor proteins required for signal transduction and transmission of forces, both outside-in and inside-out.

influence cellular activities in tissue remodelling and associated diseases.

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Conflict of interest

The authors state no conflict of interest.

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