Minireview

Structure and Molecular Assembly of the Dermal-Epidermal Attachment Complex in Skin

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Abstract: The basement membrane between the epidermis and the dermis contains unique structures that maintains the attachment of the epithelium. The components of the attachment complex provide links to the intracellular intermediate filament network of basal keratinocytes and to the extracellular matrix of the papillary dermis. One of the key components of the anchoring complex is laminin 5. Laminin 5 is essential to epidermal attachment, as genetic mutations in the laminin 5 genes cause the severe blistering phenotype of Herlitz's junctional epidermolysis bullosa. It is clear that laminin 5 binds the hemidesmosomal integrin $\alpha 6\beta 4$, but the mechanism by which laminin 5 binds either components of the basement membrane or of the papillary dermis still remains incompletely understood. Recent research progress indicates the following results: (1) laminin 5 is uniformly distributed along the epidermal basement membrane while type VII collagen is concentrated under hemidesmosomes; (2) laminin 5 is extracted from tissues in two forms, as a monomer and as a covalent complex with laminin 6 or 7, and the laminin 5-6/7 complex can interact with other basement membrane components through an interaction mediated by nidogen; (3) laminin 5 binds the NH_2-terminal NC-1 domain of type VII collagen; (4) monomeric laminin 5 constitutes the anchoring filaments and bridges integrin $\alpha 6\beta 4$ with type VII collagen; (5) in $\alpha 3$ integrin null mice, a normal basement membrane appears beneath the hemidesmosomes, but it lacks the basement membrane in the interhemidesmosomal regions. From these observations, we propose that monomeric laminin 5 is the major species contributing to the epidermal-dermal stability of attachment, while the laminin 5-6/7 complex contributes to the assembly and/or stability of the basement membrane between hemidesmosomes.

Key words: anchoring fibril, anchoring filament, basement membrane, dermal-epidermal junction, hemidesmosome, laminin 5

Introduction

The attachment of external epithelia to the underlying stroma is mediated by a unique unit of ultrastructural entities within the basement membrane zone, called the attachment complex[1]. This complex includes hemidesmosomes on the basolateral surface of the epithelium, anchoring filaments that bridge the hemidesmosomes with lamina densa, and anchoring fibrils that form an extended network surrounding stromal fibrous elements and insert into the basement membrane[2]. This complex represents a continuum of intermolecular interactions that originates within the epithelial cells, spans the plasma membrane via hemidesmosomal transmembrane proteins, extends into the underlying extracellular matrix, and functions to enhance cell-matrix attachment. The attachment helps maintain the integrity of tissues that are subjected to externally applied force, such as skin, oral and vaginal mucosa, esophagus, cornea, and amnion[2]. Indirect evidence in skin that the attachment complex plays an important role in epidermal-dermal integrity comes from studies of the diseases bullous pemphigoid and epidermolysis bullosa[3].

In this mini-review, we concentrate on one of the well-studied attachment complex-containing tissues, the dermal-epidermal junction of skin. In skin, the keratinocytes overlie and are attached to the basement membrane that consists of at least two morphological layers, the lamina lucida and the electron-dense lamina densa, as shown in Fig. 1. The attachment sites within the plasma membrane of the keratinocytes are numerous electron-dense plates, called hemidesmosomes, which are connected to the cytoskeletal keratin filaments[2]. On their extracellular side, the hemidesmosomes are connected to the lamina densa through microfilamentous structures (anchoring filaments) localized within the lamina lucida[2,4]. The basal lamina is attached to the papillary dermis via thicker centrosymmetric microfilamentous structures known as anchoring fibrils[5-7]. Recent advances in our understanding of the composition of the anchoring complex indicates that it derives from multiple molecules. Therefore, in this review we attempt to summarize the characterization of the molecules within the anchoring complex and the interactions of these molecules which provide the functions ascribed to this complex.

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Hemidesmosomes

Ultrastructurally, hemidesmosomes appear as electron-dense plaques found along the basolateral surface of keratinocytes.

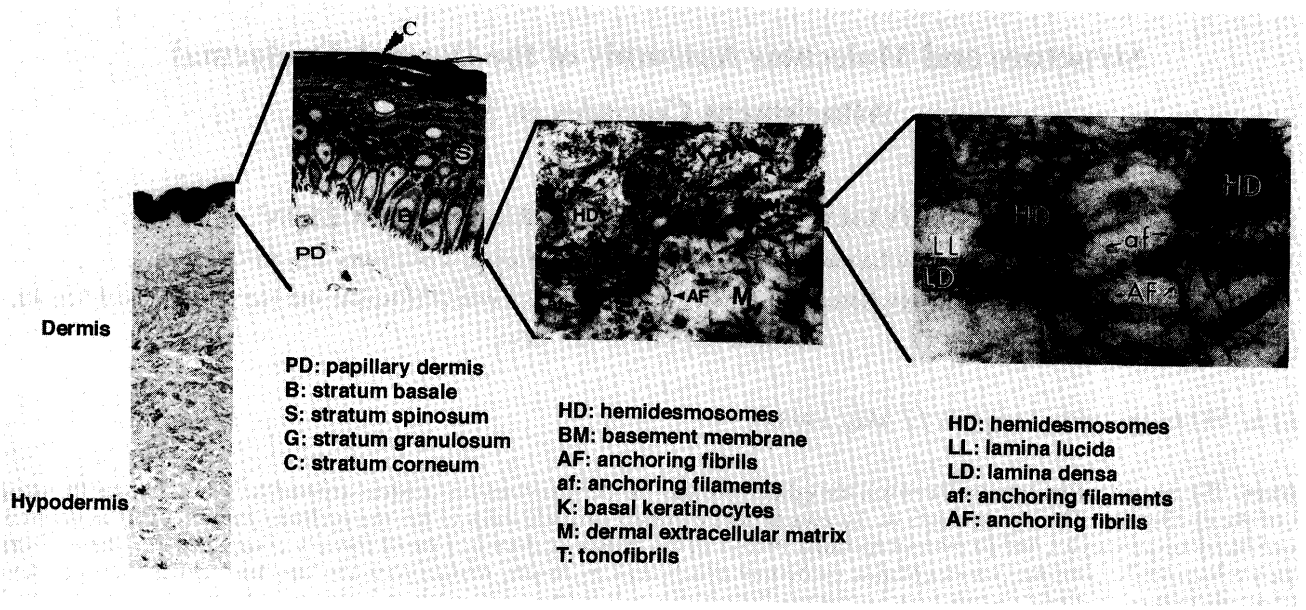

Fig. 1. Light micrograph and transmission electron micrograph of the dermal-epidermal junction of human skin (from whole skin to basement membrane zone).

A striking feature of hemidesmosomal plaques is the insertion of fine electron-dense intracellular filaments into the plaques from both the inside of the cell and from the basement membrane as seen in Fig. 1. The intracellular filaments, or tonofilaments, are composed of keratin intermediate filaments. The tonofilaments look very much like the fibrillar elements of the cytoskeleton that converge into desmosomes. The extracellular filaments, or anchoring filaments, extend into the basal lamina and appear to be continuous with anchoring fibrils, which entrap collagen fibrils in the papillary dermis[2].

Molecular characterization of the hemidesmosomes indicates that they contain at least two transmembrane proteins, the integrin $\alpha 6\beta 4$[2,8,9], and BPAG-2 (bullous pemphigoid antigen 2)[10,11]. The intracellular domains of these two proteins interact with BPAG-1 (bullous pemphigoid antigen 1) and with the molecule plectin (HD-1)[12-14]. Plectin and BPAG-1 bind the keratin filaments, thus bridging the cytokeratin network with the transmembrane hemidesmosomal proteins. The exodomain of BPAG-2 contains amino acid sequences that predict a triple-helical structure, so that BPAG-2 has also been termed type XVII collagen[11].

Anchoring filaments

The anchoring filaments span the lamina lucida and insert into the lamina densa. At high resolution, the anchoring filaments appear to bridge the hemidesmosome with the centrosymmetrically banded anchoring fibrils, as shown in Fig. 1. The subbasal dense plate parallels the plasma membrane at the extracellular side and appears to serve as an attachment site for the anchoring filaments[15]. The molecular assembly of the dense plate is still obscure.

The molecules contained in the anchoring filaments have

Table 1. Composition and localization of laminins

Laminin	Components	Localization
laminin 1	$\alpha 1\beta 1\gamma 1$	central nervous system, kidney
laminin 2	$\alpha 2\beta 1\gamma 1$	peripheric nerve, striated muscle. pancreas, placenta
laminin 3	$\alpha 1\beta 2\gamma 1$	synaptic junctions
laminin 4	$\alpha 2\beta 2\gamma 1$	muscle, placenta
laminin 5	$\alpha 3\beta 3\gamma 2$	epithelium: skin, amnion
laminin 6	$\alpha 3\beta 1\gamma 1$	epithelium: skin, amnion
laminin 7	$\alpha 3\beta 2\gamma 1$	epithelium: skin, amnion
laminin 8	$\alpha 4\beta 1\gamma 1$	heart, lung, placenta
laminin 9	$\alpha 4\beta 2\gamma 1$	heart, lung, placenta
laminin 10	$\alpha 5\beta 1\gamma 1$	skin, heart, lung, placenta, kidney
laminin 11	$\alpha 5\beta 2\gamma 1$	skin, heart, lung, placenta, kidney

been partly identified and characterized. These include the exodomains of integrin α6β4 and BPAG-2, and a molecule now known to be a laminin. As described in Table 1, the laminins are a growing rapidly family of molecules, and there are presently 11 published laminin subtypes[16-18]. Of these, laminin 5 localizes to the anchoring filaments[4]. Laminin 5 contains three unique subunits, α3, β3, and γ2[19-21]. In skin laminin 5 is proteolytically processed after secretion[22]. The 200-kD α3 chain is cleaved to 165-kD and finally to 145-kD. The β3 chain remains intact, while the γ2 chain is processed from 155-kD to 105-kD by Bone Morphogenic Protein-1 (BMP-1) [23], previously known as type I procollagen C-proteinase. This processing may be required for the assembly of the epidermal basement membrane of skin[23]. The γ2 chain lacks the nidogen binding consensus sequence and thus cannot bind nidogen under physiological conditions[24], therefore laminin 5 alone cannot associate with the collagen IV networks in the basement membrane. Laminin 5 also lacks the short arm domains believed to be required to promote assembly into laminin networks[25]. The mechanism of the interaction of laminin 5 with the lamina densa and with the anchoring fibrils has only recently been elucidated.

It is known that monomeric laminin 5 binds integrin α6β4 at the α3 COOH-terminal G domain[26]. In a recent study, Rousselle et al.[27] have demonstrated that monomeric laminin 5 can bind the NH_2-terminal NC-1 domain of type VII collagen. The relative binding of type VII collagen NC-1 to type IV collagen is moderate, but the binding to other basement membrane components is minimal as compared with the strong binding to laminin 5. The binding is dependent upon the native conformation of both laminin 5 and type VII collagen NC-1.

Anchoring fibrils

The anchoring fibrils originate within the lamina densa and project into the upper regions of the papillary dermis. About 800 nm in length, the anchoring fibrils either loop back to reinsert into the lamina densa, or insert into structures termed "anchoring plaques" that appear as junctions between two or more anchoring fibrils[7]. Additional anchoring fibrils originate in the plaques and extend further into papillary dermis. The resulting network engulfs banded collagen fibers and other fibrous elements (elastic microfibrils, beaded microfilaments, and so on) of the papillary dermis, thus assuring the strength of the union of the basement membrane and the upper dermis.

Anchoring fibrils are disulfide bond-stabilized dimers of type VII collagen[6]. The biosynthetic product is a monomer containing a large NH_2-terminal globular domain, NC-1, an unusually long and interrupted triple-helical domain, and a relatively small COOH-terminal globular domain, NC-2, that is proteolytically removed during maturation of the anchoring fibrils, and is probably involved in the dimerization process[28-31]. Immunoelectron microscopic studies of the localization of well-characterized epitopes within type VII collagen indicate that the NC-1 domain is contained within the lamina densa[7]. The ultrastructure of the anchoring complex also suggests a direct interaction of the anchoring filaments with the anchoring fibrils within the lamina densa[5]. Both observations are consistent with the reported direct binding of laminin 5 to type VII collagen. Therefore, a model of the interactions that stabilize the epidermal-dermal attachment is now proposed as shown in Fig. 2 that postulates keratinocyte binding to the G domains of laminin 5 α3 chain via integrin α6β4 (and perhaps through BPAG-2 as well), strong binding of the laminin 5 to the type VII collagen NC-1 domain, and moderate binding of the type VII collagen NC-1 domain to the basement membrane through interactions of NC-1 with type IV collagen[17,27,32].

The epithelial basement membrane contains at least two additional epithelial-specific laminins, laminins 6 (α3β1γ1) and 7 (α3β2γ1). Laminin 6 has no detectable affinity for type VII

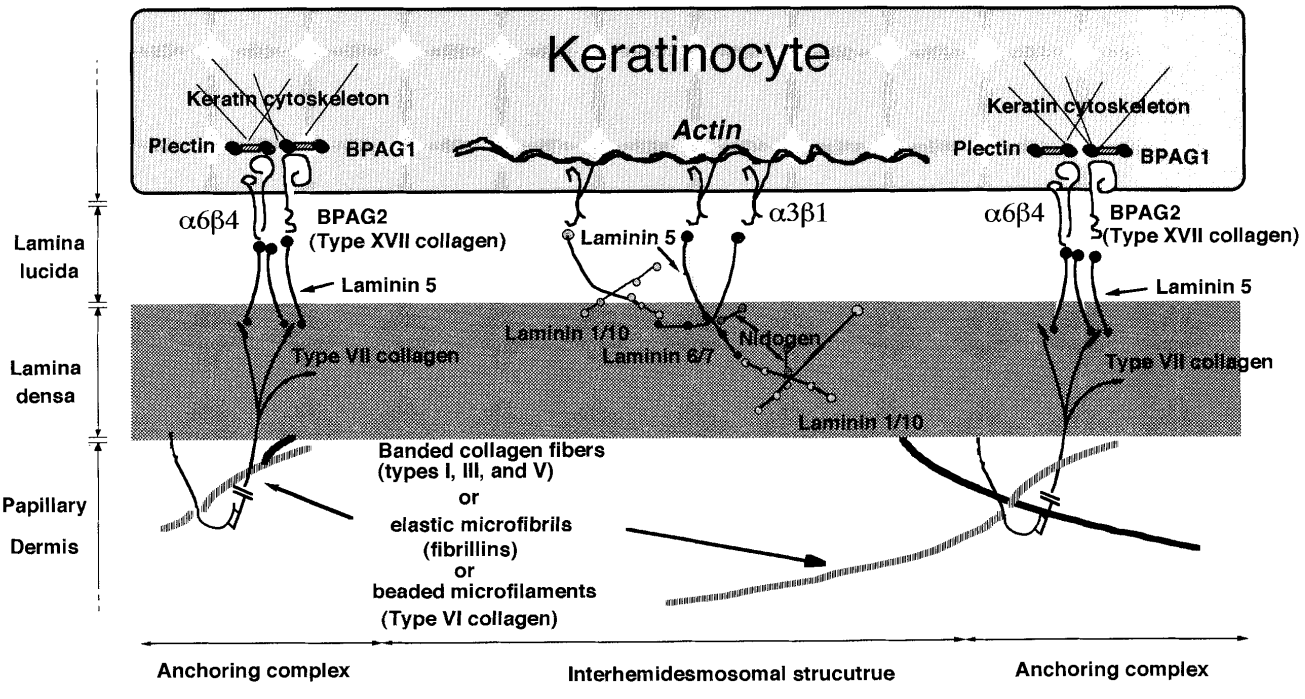

Fig. 2. Model for the presence of monomeric laminin 5 and complexed laminin 5 within a dermal-epidermal junction.

collagen NC-1, indicating that the binding is mediated by the β3 and/or γ2 chains of laminin 5. Thus, neither laminin 6 nor laminin 7 are predicted to substitute for laminin 5 within the hemidesmosome. The genetic analyses of mutations in the laminin 5 chains in patients with junctional epidermolysis bullosa are consistent with this prediction.

Interhemidesmosomal structures

Ultrastructurally, laminin 5 is uniformly distributed along the epithelial basement membrane[4], while anchoring fibrils and type VII collagen are concentrated under hemidesmosomes and show insertion of the ends of the fibrils into the lamina densa at hemidesmosomes[5,6]. The observations summarized above support a model predicting that monomeric laminin 5 constitutes the anchoring filaments and is the primary bridge between hemidesmosomal integrin α6β4 and type VII collagen as shown in Fig. 2. However, the precise roles of laminins 6 and 7 are less clear.

Laminin 5 can be incorporated into the basement membrane through a unique mechanism involving cross-linking of laminin 5 with laminin 6 or laminin 7[17]. Approximately one-half of the laminin 5 solubilized from human amnion or skin is covalently complexed with laminin 6 or laminin 7, the other half being monomeric[17]. The adduction occurs between the NH_2 terminus of laminin 5 and the branch point of the short arms of laminin 6 or laminin 7. The results are consistent with the presumed orientation of laminin 5, having the COOH-terminal G domain apposed to the hemidesmosomal integrin and the NH_2-terminal domains within the lamina densa. The presence of the laminin γ1 chain within laminins 6 or 7 within this complex allows strong binding of the complex to nidogen, and therefore also to type IV collagen[24].

The conclusion that the characteristics of monomeric laminin 5 best fit its direct role in the anchoring complex as described above, and the observation that laminin 5 is uniformly distributed along the epidermal basement membrane[4], suggests that the laminin 5-6/7 complex is likely to be present within the basement membrane between hemidesmosomes. It is capable of associations with the basolateral plasma membrane through integrin α3β1, since α3β1 integrin can bind to the G domains of laminin α3 chain[33,34]. The predicted presence of the laminin 5-6/7 complex within this space further suggests that the complex initiates basement membrane assembly at these sites or stabilizes the basement membrane once assembled. This model is illustrated in figure 2[27].

The recent studies of a mouse engineered as a genetic ablation of the gene encoding the α3 integrin subunit supports this model of interhemidesmosomal assembly[35]. The newborn mouse suffers blistering at the dermal-epidermal junction, although the phenotype is less severe than in integrin α6-null or integrin β4-null mice[34, 36, 37]. Ultrastructural analysis of the skin of this mouse indicates that a normal appearing basement membrane is present beneath the hemidesmosomes, but the basement membrane in the interhemidesmosomal region is lacking.

Conclusions

Recent studies suggest a model for the molecular composition and arrangement of the dermal-epidermal attachment complex in skin as described in Fig. 2. Monomeric laminin 5 visualized as the anchoring filaments, bridges the hemidesmosomal transmembrane proteins, integrin α6β4 and type XVII collagen, with type VII collagen, the anchoring fibrils. The tight binding of laminin 5 to α6β4 and to type VII collagen provides the primary resistance to frictional forces. The cytoskeletal proteins, plectin and BPAG-1, bridge the hemidesmosomal transmembrane proteins with the cytokeratins. The laminin 5-6/7 complexes are present within the interhemidesmosomal spaces where they bind at least integrin α3β1, and where they may mediate basement membrane assembly or stability, but they contribute less significantly to epidermal friction resistance.

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