The biomolecular and ultrastructural basis of epidermolysis bullosa

C. Solovan, M. Ciolan and L. Olariu

SUMMARY

Transmission electron microscopy, immunoelectron microscopy, immunofluorescence and antigenic mapping have improved our understanding of the dermo-epidermal junction. We have reviewed some ultrastructural and biomolecular aspects related to the dermo-epidermal junction. In part, they are implicated in the pathogenesis of a group of hereditary disorders characterized by skin fragility, collectively known as epidermolysis bullosa (EB). These disorders could benefit in the near future from a gene therapy approach but at present genetic counseling, prenatal diagnosis and conservative treatment measures offer little real benefit to patients.

KEY WORDS

epidermolysis bullosa, dermal-epidermal junction, ultrastructure, pathogenesis

Introduction

Epidermolysis bullosa (EB) comprises a group of genetically determined skin fragility disorders characterized by blistering of the skin and mucosa following mild mechanical trauma. The descriptive term "epidermolysis" is illogical since epidermal disruption is not the primary change in two of three main categories of EB (1 – 3). Classification of this complex and heterogeneous group is difficult and is based largely on the mode of inheritance, clinical, laboratory and epidemiological studies.

The most comprehensive classification available (4) is that proposed in 1991 by a Subcommittee of the National EB Registry (NEBR). However, it remains to be seen whether the 25 subtypes of EB included in this list should still be regarded as distinct entities. Recent advances in the molecular pathology of EB are already providing the basis for genotype-phenotype studies that should help to clarify and simplify the classification of these disorders. A suggested working classification is shown in Table 1.

Epidermolysis bullosa simplex

EB simplex (EBS) is the most frequent form of EB. The inheritance is generally autosomal dominant but rarely it can be transmitted as an autosomal recessive trait. In all forms of EBS, blister formation is intra-epidermal and generally begins with the sub-nuclear disrup-

Table 1. Classification of epidermolysis bullosa (EB).

Type of EB	***Inheritance***	***Protein***
1. EB Simplex (EBS) (intra-epidermal blisters)		
a) More common forms		
EBS of hands and feet (Weber - Cockayne)	AD	K5, K14
EBS herpetiformis (Dowling - Meara)	AD,	K5, K14
EBS Kobner	AD (AR rare)	K5, K14
b) Less common forms		
EBS associated with neuromuscular disease	AD	plectin
EBS with mottled pigmentation	AD	K5
EBS superficialis	AD	unknown
EBS Ogna	AD	K14, plectin
EBS with pyloric atresia	AD	plectin
2. Junctional EB (JEB) (lamina lucida blisters)		
a) More common forms		
JEB gravis (Herlitz)	AR	laminin 5
JEB mitis (non-Herlitz)	AR	laminin 5 Col XVII
b) Less common forms		
JEB with pyloric atresia	AR	$\alpha 6\beta 4$ integrin
Invers JEB	AR	laminin 5
Progressive JEB		unknown
Cicatricial JEB		unknown
3. Dystrophic EB (DEB) (sublamina densa blisters)		
a) more common forms		
Severe generalized DEB (Hallopeau - Siemens)	AR	Col VII
Mild, non-mutilating generalized/localized DEB	AR	Col VII
Classical DEB (Cockayne-Touraine; Pasini)	AD	Col VII
b) Less common forms		
Inverse DEB	AR	Col VII
Centripetalis DEB	AR	unknown
Pretibial DEB	AD	Col VII
EB pruriginosa	AD	Col VII
Bart's syndrome	AD	unknown
Transient bullous disease of childhood	AD	Col VII
Dystrophic epidermolysis bullosa	AD/AR (hetero)	Col VII
4. Disorders of uncertain cause, possibly not EB		
Mendes da Costa disease		
Kalin's syndrome		

tion of basal keratinocytes. The use of different approaches by a number of groups led to the discovery that mutations in the basal cell keratins (K5 and K14), could result in EBS. It was shown that expression of mutant keratins in cultured cells gave rise to dense keratin aggregates (5). Similar aggregates of keratin filament clumps were seen in the basal cells of the epidermis (6, 7) or cultured keratinocytes (8) of patients with the Dowling-Meara form of EBS. Immunoelectron microscopic studies showed that the abnormal filament clumps could be labelled with K5 and K14 antibodies (9). Further evidence came from studies in transgenic mice carrying a deletion in the K14 gene (10). The mice developed intra-epidermal blisters associated with abnormal keratin filament aggregates, similar to those seen in Dowling-Meara EBS. Heat-shock experiments have shown an altered sensitivity in cultured EBS cells on the application of heat (11). Mutations in K5 and K14 have subsequently been described in Weber-Cockayne EBS (12) where they generally occur outside the highly conserved boundary motifs, and chiefly in other parts of the rod domain or the L12 linker region

(13). However, there are exceptions where mutations in the 1A conserved boundary domain can give rise to a mild EBS-WC phenotype (Liovic et al, 2004: Exp Derm). In the Weber-Cockayne or Koebner variants of EBS and the form associated with mottled pigmentation (EBS-MP), keratin filaments in the basal cells are not consistently abnormal and do not show any major characteristic changes (14,15).

In a form of autosomal recessive EB associated with muscular dystrophy, electron microscopy showed that tissue separation first starts in the cytoplasm of basal cells just above the hemidesmosomes and adjacent basal plasma membrane (16-21). Various ultrastructural abnormalities of hemidesmosomes were seen including small inner plaques and a reduced association with keratin filaments (18-20).

Immunofluorescence microscopy of skin from affected individuals showed diminished or absent staining for plectin (18, 19), a 500 kDa protein, which is distributed among a variety of tissues, including stratified squamous epithelia, nerves and muscles (22). There is also a deficiency of HD1, a partly characterized hemidesmosome component with similarities to plectin (23). Homozygous mutation in the plectin gene, PLEC1 on chromosome 8q24 have been described in a few families with the diseases (24) and in two unrelated children with EB simplex associated with mucosal lesions in the respiratory tract but without evidence of myopathy (25).

Junctional epidermolysis bullosa

Junctional EB (JEB) encompasses a group of autosomal recessive disorders with a wide range of clinical severity. Conventionally, JEB is divided into two main categories, the Herlitz (or lethal) and non-Herlitz (non-lethal) forms. Electron microscopy shows that the level of tissue separation in all forms of JEB is through the lamina lucida of the basement-membrane zone immediately beneath the plasma membrane of epidermal basal cells. The hemidesmomes tend to be sparse and very small, especially in the more severe forms of the disease (26, 27). These hemidesmosome abnormalities are most prominent in the Herlitz form of JEB and in the subtype associated with pyloric atresia. Molecular analysis has subsequently showed that the Herlitz form

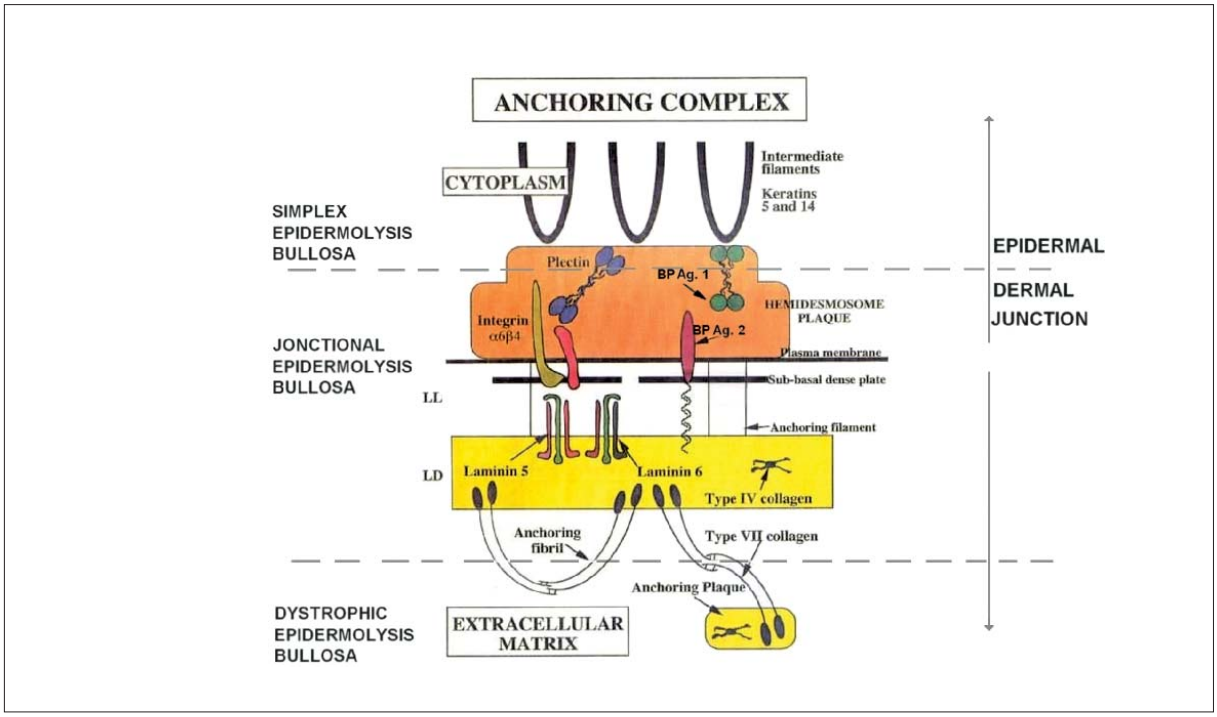

Figure 1. Principal components and their relative localization in the anchoring complex of the epidermal-dermal junction,and their correspondence to major types of epidermolysis bullosa. LL lamina lucida, LD lamina densa
(*modified after : Fine et al. J Am Acad Dermatol, 2000; 43: 135-7).

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of the disease may be caused by mutation in any of the laminin 5 genes (LAMA3, LAMB3 and LAMC2) encoding the α3, β3 and γ2 polypeptide chains respectively, which co-polymerize to form the laminin 5 protein. All mutations reported so far in Herlitz JEB have been homozygous or compound heterozygous premature termination codons (28-31).

Non-Herlitz EB may also result from a mutant laminin 5 gene (32-35). In a family with generalized atrophic benign EB there was a premature termination codon mutation in exon 3 of one LAMB3 allele and a missense mutation in the exon of the other allele. Exons 3 and 7 encode part of domain 6 on the short arm of the β3 chain. This globular domain is thought to be involved with the interaction between laminin 5 and other basement-membrane proteins, such as laminin 6. These mutations might therefore cause destabilization of the macromolecular network involved in adhesion at the dermo-epidermal junction (34). Reduced immunofluorescence staining of another hemidesmosome-anchoring filament component, the 180 kDa bullous pemphigoid antigen (also known as BP180, BPAG2 or collagen 17) in the skin of patients with generalized atrophic benign EB (GABEB), suggested that mutations in the BPAG2 gene might underlie this condition (36). A combination of premature codon termination on both BPAG2 alleles were shown in a patient with GABEB (37) who was clinically similar to the patients with laminin 5 disorder. The combination of a glycine substitution mutation in the helical domain of BPAG2 on one allele and an internal duplication on the other allele has been described in a junctional EB patient with fairly mild skin changes but severe dental abnormalities (38).

Immunohistochemical analysis showed reduced expression of the α6β4 integrin in the skin of patients with the form of EB associated with pyloric atresia (39-41). Staining for other antigens including laminin 5 was generally normal. The α6β4 integrin is a hemidesmosome-associated heteropolymer and receptor for laminin 5. Knockout mouse experiments with targeted removal of the α6 and β4 subunits showed that homozygous (-/-) mice manifested widespread epithelial separation from the underlying stroma and major ultrastructural changes in the hemidesmosomes (42, 43). Mutations in genes for the α6 and β4 chains (ITGA6 and ITGB4) have now been shown to underlie this form of EB (43, 44) (Figure 1). Not all proteins evidenced till now (uncein) play a role in the pathogenesis of various types of EB.

Dystrophic epidermolysis bullosa

The dystrophic forms of EB are characterized by skin fragility, blistering, scarring, nail changes and milia formation, and have either autosomal recessive or dominant inheritance. In contrast to EB simplex or junctional EB in which several genes are now recognized in the pathogenesis of these disorders, both autosomal dominant and recessive forms of dystrophic EB are caused by mutations in a single gene, COL7A1, which encodes the anchoring fibril protein, the type 7 collagen. Ultrastructurally, the level of blistering in dystrophic forms of EB is immediately below the lamina densa of the epidermal basement membrane, at a site normally occupied by anchoring fibrils (26). Quantitative electron microscopy and immune-electron microscopy have shown that anchoring fibrils in dystrophic EB are reduced in number, morphologically altered or completely absent (45, 46). Immunofluorescence staining of the skin of patients using anti-type 7 collagen antibodies showed that the normal bright, linear staining is absent in severe generalized recessive dystrophic EB, but present in dominant dystrophic EB (47-49). In the milder or more localized form of recessive dystrophic EB, immunoreactivity is present but often attenuated and in the inverse form of recessive dystrophic EB, and type 7 collagen is normally expressed but anchoring fibrils are structurally abnormal (50). These structural and immunohistochemical findings indicated that the gene, expressing type VII collagen, was the candidate gene for the recessive forms of dystrophic EB.

The most severe (Hallopeau-Siemens) form of recessive dystrophic EB is caused by premature termination codon mutations on both COL7A1 alleles (51-53). The effect is a severe reduction in mRNA as a result of nonsense-mediated decay leading to impaired synthesis of collagen 7 polypeptides for assembly into anchoring fibrils (3). Dominant dystrophic forms of EB have been shown to be associated exclusively with glycine substitutions within the triple helical collagenous domain of the type 7 molecule, characterized by a Gly-X-Y repeating amino-acid sequence (3, 54). In addition to their occurrence in classical forms of dominant dystrophic EB, glycine substitution mutations have been demonstrated in less common variants including Bart's syndrome (55), pretibial dystrophic EB (56) and EB pruriginosa (57).

Diagnosis and therapy

Developments in the molecular genetics of dystrophic EB and junctional EB have had a major impact on prenatal diagnosis. Several methods have been employed for prenatal diagnosis: amniocentesis, ultrasonography, fetoscopy, fetal skin biopsy, light and electron microscopy, enzymatic assay and antibody probes. Amniocentesis, usually performed at 16 week's gestation is a convenient and relatively safe method of obtaining amniotic fluid and cells for morphological, cytogenetic and biochemical analysis. Raised maternal se-

rum and amniotic fluid concentrations of α-fetoprotein have been reported in association with fetuses affected by EBS or by EB with pyloric atresia (58). Ultrasonography is a powerful tool for the detection of central nervous system and skeletal disorders (58, 59). It has been used successfully to diagnose bony abnormalities in a fetus affected with osteogenesis imperfecta (60).

Fetal skin biopsy has been used to diagnose EB (61, 62). The precise level of cleavage should then be determined by electron microscopy. Dermo-epidermal separation occurs in the lamina lucida of the epidermal basement membrane, and is associated with a hemidesmosome abnormality in lethal junctional EB, whereas in the dystrophic forms separation takes place beneath the lamina densa. Prenatal diagnosis of the Dowling-Meara form of EB simplex has also been made by light and electron microscopy, which revealed the intra-epidermal separation associated with clumping of keratin filaments (63). In the skin of patients with severe, recessive dystrophic EB there is absence or reduction of labelling with the LH 7.2 monoclonal antibody, which binds to the amino terminus (formerly thought to be the carboxy terminus) of type VII collagen (64, 65). This antigen is found in the lamina densa of epidermal basement membrane, as well as on the dermal ends of anchoring fibrils (66, 67). The LH 7.2 monoclonal antibody has been used for rapid prenatal diagnosis of recessive dystrophic EB using indirect immunofluorescence on skin samples from an 18-week fetus at risk for the disease (68). GB3 and 19DEJ-1 monoclonal antibodies have been used for the diagnosis of junctional EB (Herlitz form). GB3 antigen (laminin 5) expression appears normal in the form of junctional EB associated with pyloric atresia (69).

The differential diagnosis of EB comprises: bullous congenital ichthyosiform erythroderma, staphyloccocal scalded skin syndrome, bullous impetigo, incontinentia pigmenti, autoimmune bullous disease, such as pemphigus or herpes gestationis, acquired transplacental, aplasia cutis, focal dermal hypoplasia and Kindler's syndrome.

Mendes da Costa disease is often classified as a form of EB simplex. The pathogenesis of this rare X- linked disorder, reported in a Dutch family, is still poorly understood. Although blisters are seen, they are not trauma induced (70). Without further evidence, the classification of this disorder as a form of EB, is questionable (71).

Newer forms of treatment, including gene therapy have as their objective the introduction of normal alleles into somatic cells in recessive forms of EB, where two mutant genes exist. The introduced gene should be able to undergo transcription and translation and to synthesize an appropriate product: such as a basement-membrane protein, which then has to be secreted by the cells and incorporated into the skin basement-membrane in a way that will allow it to be functionally effective.

For autosomal dominant disorders, including dominant dystrophic EB or EB simplex the strategy is different. The objective is to try to deactivate the action of the mutant gene which usually exerts dominant negative effect on its normal paired allele. The introduction of preimplantation genetic diagnosis will broaden the available options in the overall management of those affected by EB.

Conclusions

Additional studies of normal and diseased skin (dermo-epidermal junction) should lead to an increased understanding of the ways in which individual structural components interact and function. Studies have shown that it is important not only to demonstrate any molecular abnormality but also its effect on the function of that structure, as well as the spatial orientation within the dermo-epidermal junction relative one to the other. The final result, we hope, will be a novel means of treatment for specific types of EB, but also for other diseases which are characterized by the presence of non-healing wounds.

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A U T H O R S ' A D D R E S S E S

Caius Solovan MD, PhD, Associate professor, University of Medicine and Pharmacy, Dermatology Department, Nicoara Moise 6, 300314 Timisoara, Romania
Maria Ciolan MD, resident in dermatovenereology, Dermatology Department, County Hospital, Marasesti 5, 300077 Timisoara, Romania
Liviu Olariu MD , same address