

## Recent Advances in the Molecular Pathology of Bullous Skin Disorders

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**Maintenance of an intact epidermis depends on secure adhesion between adjacent keratinocytes and between basal keratinocytes and underlying epidermal basement membrane. The major adhesion units that achieve this are the hemidesmosomes and desmosomes but when these structures are disrupted, for example by gene mutations or autoantibodies, the resilience of the epidermis is lost and blisters develop. Recently, there have been considerable advances in our understanding of the complex proteins and glycoproteins that contribute to maintaining keratinocyte adhesion via hemidesmosomes and desmosomes, as well as new insight into the molecular pathogenesis of several inherited and autoimmune blistering skin diseases. These new basic scientific data are clinically relevant, helping to improve patient management as well as providing a rationale for developing better and more specific treatments for patients with inherited or acquired blistering skin diseases. This review provides an update on the new information about the molecular pathology of hemidesmosomes and desmosomes that result in bullous skin diseases.**

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In human skin, there are several different types of keratinocyte cell junction, including hemidesmosomes and focal adhesions at the basement membrane zone as well as desmosomes, gap junctions, tight junctions and adherens junctions between adjacent keratinocytes<sup>1</sup>. All these junctional complexes are composed of intricate networks of proteins and glycoproteins, disruption of which can lead to skin fragility/blisters or abnormalities in epidermal differentiation or cell-cell communication. With relevance to blistering diseases, the main targets in skin are the hemidesmosomes and desmosomes (Figure 1). These structures can be targeted in two ways, either through inherited mutations in the genes that encode these proteins, or through acquired autoantibodies that attack specific proteins<sup>2</sup>. Depending on which protein is targeted, a particular clinical disease will result (Figure 2).

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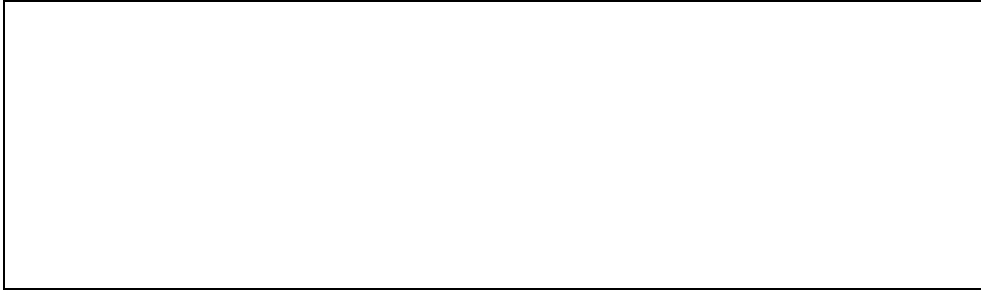
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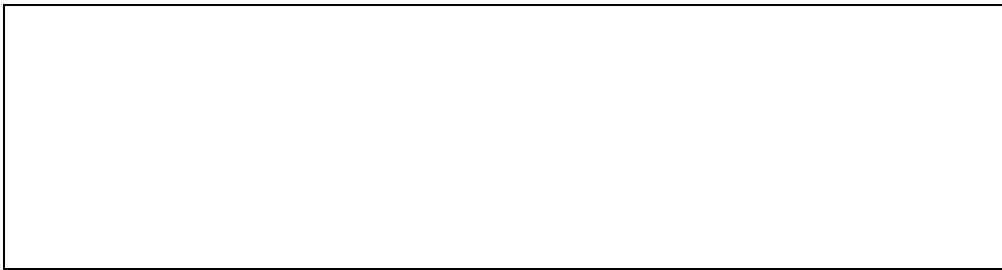
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*Figure 1. Transmission electron microscopy of (A) hemidesmosome and (B) desmosome within human skin. Each of these adhesion units consists of compact electron-dense plaques that contain networks of proteins involved in maintaining cell-matrix and cell-cell adhesion, respectively.*



*Figure 2. Skin structural proteins involved in hemidesmosomal or desmosomal adhesion may be targeted either by gene mutations or by acquired autoantibodies. For example, gene mutations in the type VII collagen gene result in the inherited blistering skin disorder, dystrophic epidermolysis bullosa, whereas autoantibodies to the type VII collagen protein lead to the acquired immunobullous disorder, epidermolysis bullosa acquisita.*

### **Structural composition of hemidesmosomes**

Hemidesmosomes are the major adhesion units at the dermal-epidermal junction. Ultrastructurally, they are composed of electron dense inner and outer plaques that bind to keratin filaments and also connect to epidermal basement membrane via anchoring filaments, which in turn bind to the lamina densa and anchoring fibrils in the superficial papillary dermis<sup>3</sup>. In effect, there is a continuous chain of linked proteins extending from keratin filaments inside basal keratinocytes through hemidesmosomes to the collagen fibres within the dermis<sup>4</sup>. Recently, understanding of the composition of the macromolecular chain of proteins involved in hemidesmosomal adhesion has improved and this has led to new insight into the pathogenesis of blister formation close to the dermal-epidermal junction (Figure 3).



*Figure 3. Illustration of some of the major structural components of a hemidesmosome, with a molecular chain extending from keratins 14/5 inside basal keratinocytes to type VII collagen in the superficial dermis.*

### **Inherited disorders of hemidesmosomes**

Mutations in the genes encoding proteins associated with hemidesmosomes result in the group of inherited skin fragility disorders known as epidermolysis bullosa (Figure 4). Thus far, mutations in 10 different genes have been identified. Targeted proteins include keratins 5 and 14, plectin, collagen types VII and XVII,  $\alpha 6\beta 4$  integrin and laminin 5<sup>5</sup>. The level of blister formation occurs close to the dermal-epidermal junction but the exact plane of cleavage (intra-epidermal, lamina lucida or sub-lamina densa) depends on precisely which gene/protein is mutated. In addition to skin blistering, several forms of epidermolysis bullosa have extracutaneous features, reflecting the tissue distribution of the abnormal protein. For example, plectin is present in skin and skeletal muscle and therefore plectin gene mutations result in epidermolysis bullosa associated with muscular dystrophy<sup>6</sup>.



*Figure 4. Illustration of the clinical disorders associated with either inherited gene mutations (left column) or acquired autoantibodies (right column) against specific proteins within hemidesmosomes.*

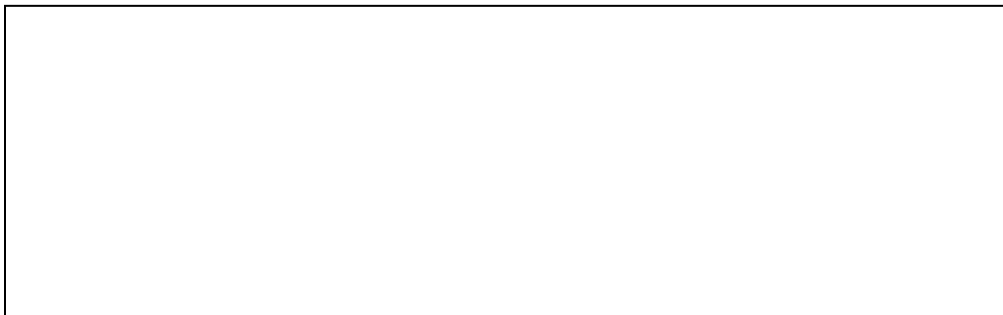
### **Acquired disorders of hemidesmosomes**

Many of the structural components within the basement membrane zone also serve as target autoantigens in the acquired sub-epidermal autoimmune blistering skin diseases (Figure 4). For example, specific epitopes within the type XVII collagen protein (also known as the 180-kDa bullous pemphigoid antigen) are recognised by autoantibodies in bullous pemphigoid<sup>7</sup>. Type XVII collagen antibodies may also be found in mucous membrane pemphigoid (the modern nomenclature for cicatricial pemphigoid),

pemphigoid gestationis and linear IgA disease (including chronic bullous disease of childhood). Other proteins targeted by autoantibodies include laminin 5 in mucous membrane pemphigoid and type VII collagen in epidermolysis bullosa acquisita or bullous systemic lupus erythematosus. Passive transfer studies have shown that several of these autoantibodies may be directly pathogenic (i.e. cause blisters) although inflammatory cells (such as neutrophils) and complement may be necessary to create the clinical disease scenario<sup>8</sup>.

### **Structural composition of desmosomes**

Desmosomes are intercellular junctions found in various tissues including skin, meninges, cardiac muscle and lymph nodes<sup>9</sup>. The main transmembranous proteins are the desmosomal cadherins (calcium-dependent adhesion glycoproteins) which comprise a family of desmoglein and desmocollin molecules, within which specific isoforms are expressed in a tissue-specific and differentiation-specific manner (Figure 5). Within the desmosomal plaque, the ultrastructurally dense region just inside the cell membrane, are proteins of the armadillo (eg. plakoglobin, plakophilin) and plakin (eg. desmoplakin, envoplakin, periplakin and epiplakin) families<sup>10</sup>. The main desmosomal plaque protein is desmoplakin which also provides anchorage to the keratin filament cytoskeleton. Some of the desmosomal proteins, also occur within other intercellular cell adhesion units, such as plakoglobin in adherens junctions. Many desmosomal proteins are also expressed in cell nuclei, often in tissues that are devoid of desmosomes, suggesting an alternative role in signalling rather than cell adhesion<sup>9</sup>.

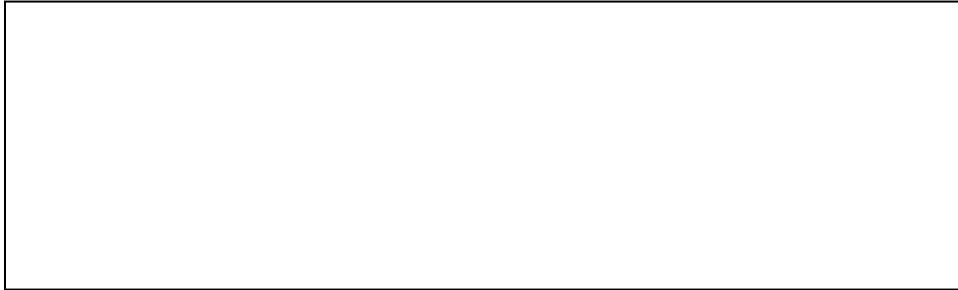


*Figure 5. Illustration of some of the major structural components of a desmosome, with a molecular chain extending from keratins inside adjacent keratinocytes, through a network of plaque proteins to the transmembranous cadherins that extend into the intercellular space.*

### **Inherited abnormalities in desmosomes**

Autosomal dominant or autosomal recessive mutations have now been identified in several components of desmosomes including plakophilin 1 and 2, desmoplakin, plakoglobin, desmoglein 1 and 4 and corneodesmosin (Figure 6). Clinically, some of these mutations result in skin blistering with loss of desmosome adhesion in skin<sup>11</sup>.

However, several inherited desmosome diseases have other features such as alopecia, hypotrichosis or woolly hair, as well as keratoderma. Some desmosome gene mutations lead to cardiac abnormalities (eg. arrhythmogenic right ventricular cardiomyopathy), often with no skin anomalies at all<sup>12</sup>. The spectrum of disease findings demonstrates that many desmosomal proteins also have important functions in cell signalling relevant to ectodermal development and conduction of cardiac impulses<sup>9</sup>.



*Figure 6. Illustration of the clinical disorders associated with either inherited gene mutations (left column) or acquired autoantibodies (right column) against specific proteins within desmosomes.*

### **Acquired diseases of desmosomes**

Pemphigus vulgaris and pemphigus foliaceus result from autoantibodies to desmoglein 3 and desmoglein 1, respectively (Figure 6). These antibodies directly lead to acantholysis and the pattern of epidermal blistering relates to the relative pattern of desmoglein expression in skin (desmoglein 3 is mainly within the lower epidermis, desmoglein 1 is more superficial)<sup>13</sup>. Other forms of pemphigus also involve anti-desmoglein antibodies, although some sub-types may also be associated with antibodies to certain desmocollins. Antibodies to several desmosomal proteins may also develop in patients with paraneoplastic antibodies, whether drug-induced or associated with benign or malignant tumours<sup>14</sup>. The presence of multiple anti-desmosomal antibodies may reflect the phenomenon of epitope spreading, in which prolonged inflammation can gradually lead to increased epitope exposure and a broad humoral immune response<sup>15</sup>.

### **New discoveries in inherited bullous skin diseases**

Identifying gene mutations in hemidesmosomes and desmosomes has helped establish the molecular basis of several inherited skin diseases. However, mutations in other junctional proteins have also been discovered. For example, the autosomal recessive disorder, Kindler syndrome, has been something of a mystery, perhaps representing an overlap between a form of dystrophic epidermolysis and an inherited poikiloderma syndrome. Now, however, we know that Kindler syndrome is a disorder of a new protein (called kindlin-1 or kindlerin, encoded by the gene *KIND1*) that binds actin microfilaments to the extracellular matrix via focal adhesion junctions at the dermal-epidermal junction<sup>16,17</sup>. Thus, Kindler syndrome represents the first bullous disease to involve a primary abnormality of the actin cytoskeleton.

### **New discoveries in acquired bullous skin diseases**

Histologically, the pattern of sub-corneal keratinocyte detachment in pemphigus foliaceus can often resemble the skin biopsy changes seen in bullous impetigo/staphylococcal scalded skin syndrome. Intriguingly, it has now been shown that both disorders involve disruption of the desmosomal protein, desmoglein 1<sup>18</sup>. In pemphigus foliaceus, autoantibodies target the extracellular EC1 domain, whereas the staphylococcal exfoliative toxin cleaves the extracellular portion of desmoglein 1 at a specific glutamic acid amino acid residue close to the EC3/EC4 domain junction<sup>19</sup>. Similar cleavage of desmoglein 1 can occur with staphylotoxins A, B and D, with the toxin acting like a serine protease in producing the acantholysis.

### **Benefits of new discoveries for inherited bullous diseases**

Defining the molecular basis of inherited abnormalities of hemidesmosomes and desmosomes has led to several direct translational benefits for patients<sup>20</sup>. For many bullous genodermatoses, it is now possible to make an accurate and rapid diagnosis (e.g. with skin immunohistochemistry), and then substantiated by gene sequencing and identification of pathogenic mutations. This has resulted in more accurate genetic counselling, more comprehensive multi-disciplinary clinical care, the feasibility of screening other family members for carrier status, and the possibility of DNA-based prenatal diagnosis for couples at reproductive risk for further affected children<sup>21</sup>. An increasing knowledge of the molecular pathology of inherited bullous skin diseases has also led to the development of trials of newer forms of treatment, including somatic gene therapy, although more detailed safety and efficiency studies need to be carried out before it can be established whether gene therapy really is going to be of major clinical value and relevance<sup>22</sup>.

### **Benefits of new discoveries for acquired bullous diseases**

The major current benefit of recent research for patients with acquired immunobullous diseases has been the introduction of better disease monitoring, notably with the development of several diagnostic ELISA kits<sup>23-25</sup>. These laboratory assays allow titres of antibodies to be determined very quickly and the values often have direct clinical relevance, allowing the dose of a particular patient's systemic immunosuppression to be modified according to the ELISA findings. ELISA tests for both hemidesmosomal and desmosomal antibodies are commercially available and their use, in conjunction with indirect immunofluorescence microscopy, has been helpful in improving the monitoring of patients in the clinic. Knowledge of particular antibody responses to certain proteins in immunobullous diseases is also leading to trials of targeted and specific immunotherapy. Although not yet developed for clinical use, it is hoped that this research will lead to new forms of immunomodulation that avoid many of the side-effects of current treatments such as systemic steroids<sup>26</sup>.

## CONCLUSION

**Over the last decade there has been a rapid expansion in basic research knowledge of the causes of many bullous diseases, both inherited and acquired. This has already had some beneficial impact on the management of patients in clinic but current efforts are now being directed towards the development of somatic gene therapy for inherited bullous skin diseases as well as more specifically targeted immunotherapy for the immunobullous disorders. It is to be hoped that the next 10 years of basic research will have even more translational benefits, as we strive to improve the healthcare of our patients with blisters.**

## REFERENCES

1. Garrod DR. Intercellular junctions in normal epidermis. *Exp Dermatol* 2004;13:652-3.
2. Hashimoto T. Skin diseases related to abnormality in desmosomes and hemidesmosomes--editorial review. *J Dermatol Sci* 1999;20: 81-4.
3. Mellerio JE. Molecular pathology of the cutaneous basement membrane zone. *Clin Exp Dermatol* 1999;24:25-32.
4. McMillan JR, Akiyama M, Shimizu H. Epidermal basement membrane zone components: ultrastructural distribution and molecular interactions. *J Dermatol Sci* 2003;31:169-77.
5. Uitto J, Richard G. Progress in epidermolysis bullosa: genetic classification and clinical implications. *Am J Med Genet C Semin Med Genet* 2004;131C:61-74.
6. Pfindner E, Rouan F, Uitto J. Progress in epidermolysis bullosa: the phenotypic spectrum of plectin mutations. *Exp Dermatol* 2005;14:241-9.
7. Van den Bergh F, Giudice GJ. BP180 (type XVII collagen) and its role in cutaneous biology and disease. *Adv Dermatol.* 2003;19:37-71.
8. Yancey KB. The pathophysiology of autoimmune blistering diseases. *J Clin Invest* 2005;115: 825-8.
9. Green KJ, Gaudry CA. Are desmosomes more than tethers for intermediate filaments? *Nat Rev Mol Cell Biol* 2000;1:208-16.
10. Yin T, Green KJ. Regulation of desmosome assembly and adhesion. *Semin Cell Dev Biol* 2004;15: 665-77.
11. McGrath JA, McMillan JR, Shemanko CS, et al. Mutations in the plakophilin 1 gene result in ectodermal dysplasia/skin fragility syndrome. *Nat Genet* 1997;17:240-4.
12. Gerull B, Heuser A, Wichter T, et al. Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy. *Nat Genet* 2004;36:1162-4.
13. Hanakawa Y, Matsuyoshi N, Stanley JR. Expression of desmoglein 1 compensates for genetic loss of desmoglein 3 in keratinocyte adhesion. *J Invest Dermatol* 2002;119: 27-31.
14. Anhalt GJ. Paraneoplastic pemphigus. *J Invest Dermatol Symp Proc* 2004;9:29-33.
15. Chan LS. Epitope spreading in paraneoplastic pemphigus: autoimmune induction in antibody-mediated blistering skin diseases. *Arch Dermatol* 2000;136:663-4.
16. Jobard F, Bouadjar B, Caux F, et al. Identification of mutations in a new gene encoding a FERM family protein with a pleckstrin homology domain in Kindler

- syndrome. *Hum Mol Genet* 2003;12:925-35.
17. Siegel DH, Ashton GH, Penagos HG, et al. Loss of kindlin-1, a human homolog of the *Caenorhabditis elegans* actin-extracellular-matrix linker protein UNC-112, causes Kindler syndrome. *Am J Hum Genet* 2003;73:174-87.
  18. Payne AS, Hanakawa Y, Amagai M, et al. Desmosomes and disease: pemphigus and bullous impetigo. *Curr Opin Cell Biol* 2004;16:536-43.
  19. Hanakawa Y, Schechter NM, Lin C, et al. Molecular mechanisms of blister formation in bullous impetigo and staphylococcal scalded skin syndrome. *J Clin Invest* 2002;110:53-60.
  20. McGrath JA. Translational benefits from research on rare genodermatoses. *Australas J Dermatol* 2004;45:89-93.
  21. Ashton GH, Eady RA, McGrath JA. Prenatal diagnosis for inherited skin diseases. *Clin Dermatol* 2000;18:643-8.
  22. Hengge UR. Progress and prospects of skin gene therapy: a ten year history. *Clin Dermatol* 2005;23:107-14.
  23. Giudice GJ, Wilske KC, Anhalt GJ, et al. Development of an ELISA to detect anti-BP180 autoantibodies in bullous pemphigoid and herpes gestationis. *J Invest Dermatol* 1994;102:878-81.
  24. Ishii K, Amagai M, Hall RP et al. Characterization of autoantibodies in pemphigus using antigen-specific enzyme-linked immunosorbent assays with baculovirus-expressed recombinant desmogleins. *J Immunol* 1997; 159: 2010-7.
  25. Bekou V, Thoma-Uszynski S, Wendler O, et al. Detection of laminin 5-specific autoantibodies in mucous membrane and bullous pemphigoid sera by ELISA. *J Invest Dermatol* 2005;124:732-40.
  26. Stanley JR. Pathophysiology and therapy of pemphigus in the 21st century. *J Dermatol*. 2001;28:645-6.