Epidermolysis bullosa (EB) – diagnosis and therapy

McMillan JR, Long HA, Akiyama M, Shimizu H & Kimble RM

Abstract

The hemidesmosome (HD)-anchoring filament complex comprises a multiprotein complex that aids the attachment of epidermal keratinocytes to the underlying basal lamina and dermis. The importance of the HD and its components is highlighted by genetic defects that cause congenital blistering skin diseases that are categorised under the epidermolysis bullosa (EB) group of disorders. EB disorders can be subcategorised into three main subtypes by the level of epidermal separation – within the basal keratinocyte (EB simplex – EBS), between the keratinocyte and underlying basal lamina (junctional EB – JEB), and separation beneath the basal lamina (dystrophic EB – DEB). HD-anchoring filament-related components – including keratins 5 and 14, plectin, α6ß4 integrin, collagen XVII, laminin 332 and collagen VII – have been demonstrated to harbour defects leading to EB disease. We summarise here the current understanding of the biological function of these HD-components and their involvement in EB in light of their functions in keratinocyte adhesion and also describe putative future therapeutic avenues that hold promise to alleviate the morbidity suffered by EB patients over the coming decades.

James R McMillan*† MSc PhD
Head of Burns Laboratory Research
The University of Queensland
Royal Children's Hospital Burns and Trauma Research Group, Paediatrics & Child Health
L/3 RCH Foundation Building
Royal Children's Hospital, Herston, QLD 4029
Email j.mcmillan@uq.edu.au

Heather A Long† BSc PhD
Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

Masashi Akiyama MD PhD
Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

Hiroshi Shimizu MD PhD
Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

Roy M Kimble MD FRCS
Royal Children's Hospital Burns and Trauma Research Group, The University of Queensland, Brisbane QLD

* Corresponding author
† These authors contributed equally to this work

Classification and diagnosis of epidermolysis bullosa (EB)

Epidermolysis bullosa (EB) comprises a closely linked group of genetic diseases characterised by skin separation between the epidermal and dermal layers (Figure 1). EB patients exhibit disruption to the rivet-like hemidesmosome (HD) junctions that provide a continuous structural link between the keratinocyte intermediate filament cytoskeleton and the underlying basal lamina and upper dermal matrix [1]. Disruption of important individual components involved in the maintenance of HD function (Figure 1) leads to loss of keratinocyte adhesion, the formation of epidermal weakness and blistering at sites of trauma [2,3].

The current basic system of EB classification, based on electron microscopy blistering findings, was first devised by Pearson *et al.* in 1962 [4]. EB disorders can be subcategorised into three main subtypes by the level of epidermal separation – within the basal keratinocyte (EB simplex –EBS), between the keratinocyte and underlying basal lamina (junctional EB – JEB), and separation beneath the basal lamina (dystrophic EB – DEB) (Figure 1) [3].

EB mutations have been identified in at least 10 distinct genes involved in maintaining epidermal keratinocyte linkage to the underlying dermal connective tissue [5] (Table 1). The clinical severity can range from relatively mild, localised blistering to widespread epidermal separation and can involve subsequent scarring and limb deformities ultimately leading to premature demise. These diseases can be disfiguring and,

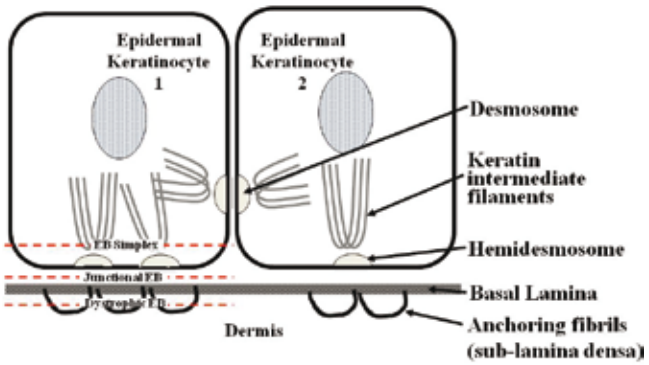

Figure 1. Schematic diagram illustrating the position of key ultrastructural features relevant to epidermal adhesion and basal keratinocyte structural integrity.

Desmosomes mediate keratinocyte contact between adjacent cells 1 and 2. Keratin intermediate filaments comprise the main structural cytoskeletal components of the epidermal keratinocytes and provide a structural link to desmosomal (cell-cell) and hemidesmosomal (HD) (cell-matrix) junctions. HDs are small discrete electron-dense structures that mediate adhesion to the underlying basal lamina and dermal matrix. Mediating the linkage of keratinocytes and basal lamina to the underlying collagen fibres in the upper dermis are long thin semicircular loops, anchoring fibrils that comprise collagen VII. The level of tissue separation observed in EB subtypes is shown on the left side of the diagram by the red dashed lines. EB simplex splits occur within the basal keratinocyte cytoplasm, JEB separation between the basal keratinocyte and the basal lamina (basement membrane) and DEB leads to separation beneath the basal lamina (in the sub-lamina densa region where anchoring fibrils are typically observed).

if the patient survives, there can be an increased risk of tumour development [6]. The clinical severity often depends on a combination of factors – the defective gene and the critical role of that gene product in epidermal function; the expression pattern and critical function of the defective gene in non-skin tissues; the type of mutation, with nonsense mutations generally leading to more severe disease and missense genetic defects leading to milder conditions; and the position of the genetic defect within or close to areas that affect critical protein domains [3, 7, 8]. Each EB subtype causes epidermal separation within subtly different levels of the dermal-epidermal junction and is dependent on the effect of the genetic defect on the specific protein function, the functional importance of the affected gene, and specific function of the defective protein domain encoded by the gene.

The recently revised EB classification system takes into account current advances in EB [5], as well as encompassing findings on the latest inherited skin diseases that affect epidermal adhesion, including Kindler Syndrome (KS). In KS, genetic defects lie in FERMT1 (formerly C20orf42 or KIND1 genes) that encode the focal adhesion junction-associated protein Fermitin Family Homologue 1 (FFH1, formerly known as kindlin-1/kindlerin). KS is included within the EB spectrum based on the presence of epidermal separation and mechanical fragility at sites of trauma [5] but as yet the protein

Table 1. Classification of EB and its candidate genes.

Main EB type/subtype	Candidate gene (encoded protein)
EB simplex (EBS) (intra-epidermal separation)	
Localised EBS (formerly Weber-Cockayne)	KRT5, KRT14 (keratin 5, 14)
Generalised EBS (formerly Köbner)	KRT5, KRT14 (keratin 5, 14)
Dowling-Meara EBS (DM-EBS)	KRT5, KRT14 (keratin 5, 14)
EBS with muscular dystrophy (EBS-MD)	PLEC1 (plectin)
EBS with pyloric atresia (EBS-PA)	PLEC1 (plectin), ITGB4, ITGA 6 (integrin ß4, α6)
Junctional EB (JEB) (separation within the lamina lucida)	
Herlitz JEB (HJEB)	LAMB3; LAMC2, LAMA3 (laminin 332)
Non-Herlitz JEB (nHJEB)	COL17A1(BPAG2), LAMB3 (laminin 332)
Pyloric atresia JEB syndrome (JEB-PA)	ITGB4, ITGA6 (integrin ß4 α6)
Dystrophic EB (DEB) (separation beneath the lamina densa)	
Dominant DEB (DDEB)	COL7A1 (type VII collagen)
Recessive DEB (RDEB, formerly Hallopeau-Siemens)	COL7A1 (type VII collagen)
Recessive DEB (RDEB, generalised other – formerly non-Hallopeau-Siemens)	COL7A1 (type VII collagen)

has not been shown to specifically localise to within the hemidesmosome cell junction, but is thought to be involved in epidermal keratinocyte adhesion.

Immunofluorescence antigen mapping [9] and transmission electron microscopy of EB freshly created blistered skin biopsies [10-15], when coupled with the use of specific monoclonal antibodies, provides the gold standard for EB diagnosis and helps identify the likely affected structural proteins. Figure 2 shows a detailed ultrastructural representation of the dermal-epidermal junction. Antigen mapping has traditionally relied on the staining of frozen sections from EB patient biopsies (harvested from recently trauma-induced blisters, never old lesions), with antibodies to specific basal lamina components – including bullous pemphigoid antigen 1, collagens IV, VII or XVII, laminin isoforms, and basal-specific cytokeratins – in order to determine the level (intraepidermal, intra-lamina lucida, sub-lamina densa) of epidermal separation [9].

Certain diagnostic antibodies are also useful for pinpointing the underlying defective genes. One of the best diagnostic antibodies for JEB and some forms of DEB is the 19-DEJ-1 antibody that recognises an unknown mid-lamina lucida component restricted to beneath HDs designated as uncein [16-18]. Uncein is a unique antigen that is completely absent in almost all subtypes of JEB. Several attempts have failed to determine the exact nature of this useful diagnostic antibody. These failures were most likely due to lack of uncein antigen reactivity after protein extraction. This has made the immunological and molecular characterisation of the antigen difficult. Current theory suggests that 19-DEJ-1 may recognise a conformational epitope related to collagens VII and/or XVII, laminin 332 and the α6ß4 HD integrin receptor (see Figure 3 for schematic representation of HD-associated antigens). Evidence for this hypothesis comes from a collagen XVII mosaic JEB-nH patient where the expression patterns of collagen XVII and uncein were identical on unaffected and affected skin sites [17]. Another similar diagnostically useful and again a conformationally sensitive antibody is the anti-amniotic GB3 antibody recognising the γ2 chain of laminin 332 [19-21] (Figure 3).

Prenatal diagnosis (PND)

Considerable progress has recently been made in elucidating the molecular pathology underlying several forms of inherited skin diseases. Clinically, these advances have led to better genetic counselling in many disorders and to the development of DNA-based prenatal diagnosis. One of the most immediate benefits has been the development of DNA-based prenatal diagnosis in pregnancies at risk for a recurrence of EB. EB prenatal testing has progressed from mid-trimester foetal skin biopsies [22, 23] to first trimester chorionic villus sampling in a much broader range of genodermatoses. Unfortunately, both foetal skin biopsy and chorionic villus sampling are invasive and therefore not completely risk-free and are therefore only generally available for the two most severely affected EB subtypes, JEB and DEB [13]. A further drawback of molecular testing using chorionic villus sampling is the need to know the precise genetic defect from an affected proband. In the absence of such molecular information, a foetal skin biopsy test remains the best alternative [22, 24-26].

Advances in *in vitro* fertilisation protocols and embryo manipulation technology have led to the feasibility of even earlier prenatal diagnosis through pre-implantation genetic

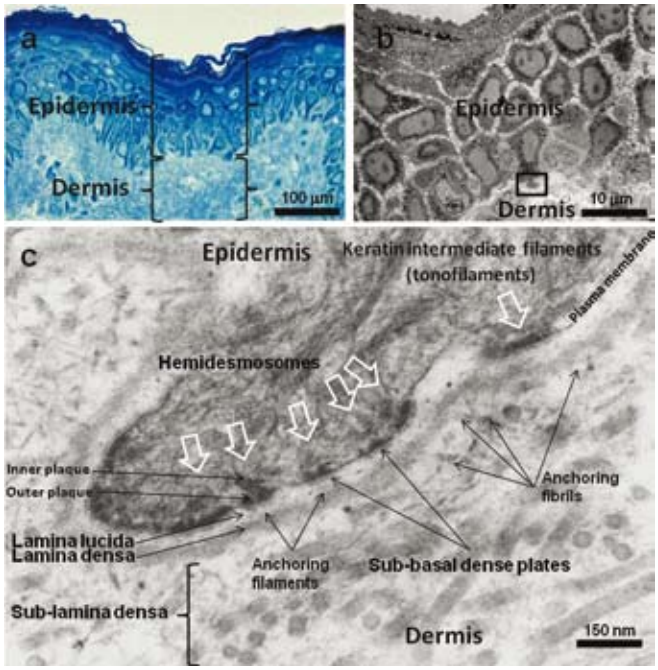

Figure 2. The ultrastructural features of normal human skin and the dermal-epidermal junction.

The upper layers of the skin comprise the epidermis and upper dermis (a). Ultrastructural observation of these layers reveals a thin, undulating layer dermal-epidermal between the epidermis and dermis (b). Higher magnification of the region within the black square (b) reveals the dermal-epidermal junction with epidermal basal keratinocyte containing numerous keratin intermediate filaments and the boundary of the cell marker by the plasma membrane (c). Intermittent focal sites of electron density, HDs (white arrows) are present along the membrane and internally can be separated into inner and outer plaques that are associated with keratin filaments. Beneath the HD is an electron dense line parallel to the membrane, the sub-basal dense plate that lies within the lamina lucida, through which anchoring filaments (labelled) traverse into the lamina densa. Protruding from the lamina densa (basal lamina) on its dermal side, are small semi-circular cross-banded anchoring fibrils that can be seen looping around dermal collagen fibrils in the sub-lamina densa region and re-inserting into the lamina densa. Scale bars (a) 100µm, (b) 10µm, and (c) 150nm.

diagnosis [22, 24-26]. This newest prenatal diagnostic technique involves a single blastomere biopsy from the 6-10 cell stage of the fertilised embryo followed by single cell DNA mutational analysis [22, 24-26]. Disease free embryos are then implanted into the uterus, thereby avoiding pregnancy termination associated with conventional methods. However, this technique has several drawbacks. Firstly the high cost and high level of technical expertise required is currently prohibitive, making the number of places limited. Secondly, the low success rates for deliveries together with the high costs make pre-implantation diagnosis an unattractive option. Nevertheless, there is a good chance that, with future technical advances, pre-implantation diagnosis will become much more widespread and will serve as a useful tool for EB and other severe genodermatoses.

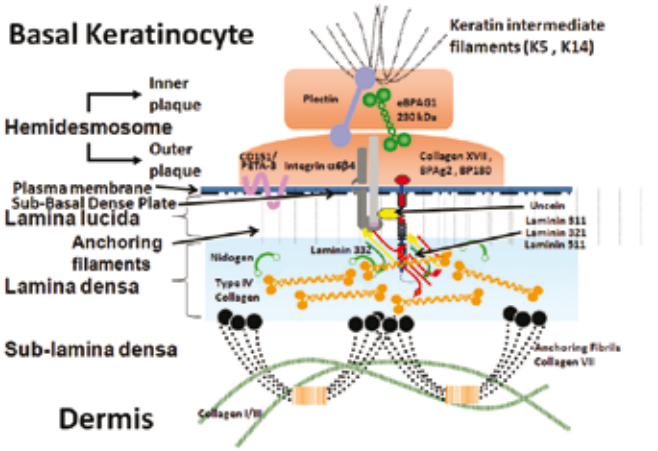

Figure 3. Schematic showing the molecular components of the HD and epidermal basal lamina.

Basal keratinocyte keratin intermediate filaments (K5 and K14) are associated with the HD inner plaque (at the top of the diagram in the upper orange box) where plectin (in purple) and eBPAG1 (in green) are localised and are thought to both be involved in binding keratins. Plectin spans the inner and outer plaque and binds the integrin ß4 subunit (in light grey). Present in the outer HD plaque are the transmembrane adhesion proteins collagen XVII (COL17) (BP180 in red) and the integrin α6ß4 (dark/light grey and red respectively). In addition there is the transmembrane tetraspanin or CD151, also known as PETA-3 (pink) and the 19-DEJ-1 antigen/uncein in the mid lamina lucida space (yellow oval). Within the lower lamina lucida and lamina densa regions are the three chains of laminin 332 (α3 chain in yellow, ß3 chain in green and γ2 chain in red), collagen IV (orange), nidogen/entactin (green semicircles) in addition to other laminin isoforms. Beneath the lamina densa (shown in aqua blue), the type VII collagen NC-1 domains (black circles) are attached to either end of anti-parallel anchoring fibrils that form loops that originate and terminate in the lamina densa enclosing dermal collagen I/III fibres.

Putative treatments for EB

While techniques like prenatal and pre-implantation diagnoses have their places in the clinical battle against EB, there are frequently feelings of despair for older patients with the more severe forms of JEB and DEB. Current treatments are limited to palliative, friction prevention and basic wound dressing and the use of some corticosteroid creams. Therefore research into long-term treatment options is important to improve the current poor patient outcome for EB patients. We will discuss possible long-term treatment for EB patients by briefly outlining how different forms of gene therapy have developed and how many small-scale clinical trials have shown potential.

Gene therapy approaches – *in vivo* verses *ex vivo*

There are three main strategies for gene therapy – *in vivo*, *ex vivo* and foetal (or *in utero*) gene therapy. These three areas have relative advantages and disadvantages. *In vivo* therapy, the direct application of a transgene to the patient's skin is relatively quick and easy to perform but drawbacks include safety issues and shorter-term transgene expression compared to *ex vivo* techniques. The *ex vivo* approach requires

the removal and expansion of cells and tissue *in vitro* and subsequent gene transfection using an appropriate vector. Foetal or *in utero* gene therapy is at the moment only experimental, but may prove beneficial for more severe genetic disorders with a high perinatal mortality as is the case for some JEB and DEB subtypes.

In vivo *gene therapy*

All methods for *in vivo* gene therapy have limitations in the long-term expression of the transgene and, as they are applied directly to patients, it is more difficult to monitor and control the extent of transgene introduction and genetic incorporation; however, these *in vivo* methods are quick and relatively cheap to carry out.

Ex vivo *gene therapy*

The *ex vivo* methods allow greater control of the extent of transgene and any erroneous effects can be clearly examined, limited and problems dealt with before gene-corrected cells are applied to patients. The use of patient's own cells reduces the possibility of an immune response and, as viral vectors are generally used, typically long-term expression can be maintained using these *ex vivo* techniques.

Foetal (in utero) *gene therapy*

Still in the theoretical and developmental stages in animal models, foetal or *in utero* gene therapy has the potential to provide significant benefits in the treatment of severe congenital diseases including the most severe genodermatoses. Due to the increased pluripotent or stem cell population and stem cell densities in embryonic and foetal tissue, *in utero* targeting theoretically dramatically improves the chances of sustained transgene expression. *In utero* gene therapy would be particularly beneficial for the most severe congenital disorders where the therapeutic window for transgene expression usually before or immediately after birth is relatively short and, if treated at the appropriate developmental stage, may help prevent the early onset of damage to the epidermis and other affected organs, significantly improving patient survival.

Expression systems

Many different types of viral vectors and systems have been exploited for gene targeting in skin. Each viral system has distinct advantages and disadvantages for gene transfer. The first gene therapy techniques employed adeno-virus and adeno-associated virus. These viruses lead to higher expression levels and supported larger transfer genes; however, their expression was transient (only up to 2 weeks) and several adverse immune responses to the adeno-viral vector were reported [27].

Retroviral vectors allow for more stable, long-term gene transfer through recombination with the host genome (months). However, this recombination occurs at random sites throughout the entire genome so the risk of disrupting vital house-keeping or oncogenes is increased and the maximal viral packaging size is smaller than that of the adeno-viral vectors. Immune responses against viral vectors are responsible for poor expression and adverse effects. Ongoing research is looking at methods for dealing with immune responses either through vector design or host immunosuppression.

Recently, the use of non-viral, transposable elements has been exploited for epidermal gene therapy. These non-viral vectors allow the stable integration of transgenes into chromosomes at specific sites via specific sequences allowing recombination within the genome. The elements – sleeping beauty retro-transposable elements [28], phiC31 integrase [29] and piggyBac [30] – have been shown to allow stable long-term gene expression *in vitro*. These elements do not contain any viral genes so they can be used more safely, making them more attractive for gene therapy. However, since transposable elements recombine into the host genome, they may disrupt genes that are required for normal cell growth and division [31]. The phiC31 integrase system has been shown to be successful in the long-term gene expression of LAMB3 in keratinocytes and is currently being tested for use in clinical trials for the treatment of Herlitz JEB (HJEB) [32].

Gene therapy for recessive DEB (RDEB) patients with collagen VII defects (cell therapy)

Cell therapy, using cells as factories for the production of specific proteins, can readily be exploited in the skin and has already been shown to have some therapeutic benefit, particularly with collagen VII. Collagen VII is a large protein which is expressed by both dermal fibroblasts but more normally by epidermal keratinocytes and is secreted into the extracellular matrix where it provides a link between the dermis and basal lamina, forming the major component of anchoring fibrils [32-34] (Figures 2c & 3).

Mutations in the collagen VII gene lead to a wide spectrum of DEB severity depending on the mutation location and type of mutation, often leading to premature stop codons or missense mutations and resulting in loss or reduced expression of collagen VII [35, 36] (Table 1). In the less severe dominant DEB (DDEB), the blistering tends to subside over time. In the more severe RDEB form (Hallopeau-Siemens variant, Table 1), the blistering is more widespread and accompanied by granulation tissue formation and subsequent fusion of digits on the hands and feet as a result of this excessive scarring, leading to severe morbidity and disfigurement. The

blistering occurs throughout life, is often accompanied by lesions in the oral and oesophageal membranes and has a tendency to develop malignant squamous cell carcinoma. The treatment of severe DEB is restricted to avoidance of friction and to palliative care with intermittent surgery to release contractures and oesophageal stenoses. The severity of RDEB makes collagen VII gene therapy a prime candidate for gene therapy development.

Other approaches have targeted fibroblasts, using gene-transferred DEB autologous fibroblasts into the dermis [37, 38]. Gene-transferred fibroblasts are more capable of producing sufficient levels of collagen VII to be clinically beneficial than gene-transferred keratinocytes [39] in skin grafts on SCID mice and so may make for a more attractive target for gene therapy in the case of DEB. Using cell therapy to treat other diseases such as protracted infectious diseases of the skin is also a distinct possibility. Beta defensins are small proteins that have microbicidal properties. Epidermis engineered to express one of the human beta defensins (HBD3) showed antibacterial activity, both in culture and on mouse grafts [40]. Conditions such as ulcers and other intractable infectious diseases could benefit from gene therapy that produces strong antibacterial protection.

The use of cells as gene factories is a strategy that is likely to benefit disorders where the gene product is exported from cells after synthesis, as in the case of collagen VII in fibroblasts and keratinocytes. For the majority of EB genodermatoses, however, the gene product is required within the cell or is limited to one specific cell type, therefore other methods or approaches for introducing and controlling transgene expression have been explored and employed.

Gene therapy for HJEB patients with laminin 332 defects

Considerable progress in recent years has gone into identifying underlying mutations in laminin 332 and the disease it causes, HJEB [10]. Laminin 322 (formerly laminin-5) comprises a complex of three laminin chains and is involved in linking collagen VII to the α6ß4 integrin (Figure 3) [2]. The Herlitz form of JEB, where there is severely defective expression of laminin 322, often leads to reduction in HD numbers and size, poor keratinocyte attachment and widespread blistering, leading to premature death within the first few months of life [1, 2, 9].

Recent evidence has highlighted a role for collagen XVII in both keratinocyte adhesion to collagen IV and in cell signaling during migration that appears to be important in hair follicle

stem cell maintenance and accounts for the marked alopecia and hair follicles abnormalities observed in both collagen XVII knock mice and non-HJEB (nHJEB) patients [41]. Non-HJEB (nHJEB), where there is partial expression of a mutated or truncated forms of laminin 322, causes recurrent blistering and scarring which severely impairs the quality of life of these patients [42].

Mutations in collagen XVII also lead to nHJEB. Collagen XVII is a constituent of the HD; as a transmembrane protein it is thought to play a role in direct cell matrix adhesion and in structure or stability of anchoring fibres at the dermal-epidermal junction. Often in many forms of JEB there is also incomplete alopecia and dental problems, such as enamel hypoplasia and dental caries. It also interacts with the α6ß4 integrins and BPAG1 proteins of the hemidesmosomal complexes (Figure 3). α6ß4 mutations have been shown to be causal in JEB associated with pyloric atresia (JEB-PA) and are usually fatal, though milder variants occur with some missense mutations [43, 44]. The treatments for JEB are limited to symptomatic or palliative treatment and blister prevention.

The discovery of a mutational hotspot (p. Arg635X) in the beta 3 chain of laminin 332 that affects approximately 50% of all HJEB patients has cleared the way for several groups to develop transgene vectors that target this gene defect [10, 45]. Ortiz-Urda *et al.* [32] have reported the successful integration of LAMB3 encoding the laminin beta 3 chain into primary keratinocytes from the severe Herlitz subtype of JEB using a plasmid encoding ϕC31 integrase. These keratinocytes were transplanted onto immunodeficient mice where human skin was produced with normal laminin 322 expression that exhibited no evidence of sub-epidermal blistering with normal HD assembly.

In a phase I/II clinical trial, primary keratinocytes were cultured from a patient suffering from non-lethal JEB affected by a point mutation in the LAMB3 gene (encoding LAM5-b3 chain) [46]. These primary keratinocytes were corrected using LAMB3 cDNA under the control of a monkey leukaemia virus promoter (MuLV-LTR). These corrected keratinocytes were transplanted as grafts back onto EB patients where they demonstrated a rescued phenotype and continued expressing the transgene for at least 1 year. The authors suggest that the transgene is present in the epidermal stem cell population, allowing the expression to continue during prolonged epidermal renewal. Furthermore, they were unable to detect any clonal expansion or selection of integration events *in vivo* which is a neoplastic risk associated with the use of monkey-derived retroviral vectors. Though still in the early stages, this apparently successful trial has demonstrated the powerful potential of targeting stem cells in the maintenance of long-term expression of transgenes in gene therapy.

Other gene mutations contribute to typically less severe forms of JEB (so-called nHJEB subtypes) [11, 12]. Collagen XVII mutations typically result in a null or deficient collagen XVII phenotype. Retroviral gene transfer of collagen XVII into nHJEB patient keratinocytes resulted in expression of protein at the dermal/epidermal junction with no evidence of blistering in reconstituted epidermis [47]. The production of a collagen XVII null mouse has proved to be essential for designing new collagen XVII transgenic therapies for nHJEB [48]. This mouse has been used to research the auto-immune disease bullous pemphigoid, which is typified by production of auto-antibodies against collagen XVII resulting in blistering in patients. Using the mouse collagen XVII null mouse, a humanised mouse expressing only human collagen was produced and the auto-antibodies against this protein were introduced via injection of human auto-antibodies into the mice [48].

Gene therapy for EBS patients with keratin and plectin defects

Keratins are cytoskeletal proteins and members of the intermediate filament super-family, with the existence of many keratin isoforms which are tissue and developmentally regulated. The cytokeratin mutations in EBS affect the structural integrity of the basal keratinocytes, leading to loss of normal cytoskeletal resilience and ultimate cell cytolysis. These mutations are generally dominant and can be severe but are also affected by significant phenotypic variation in disease severity. Mutations in the highly conserved residues lead to the most severe forms including Dowling-Meara EBS (DM-EBS), characterised by widespread blistering in response to minor trauma (Table 1). The Köbner form of EBS lies between these two in terms of disease severity and is associated with recessive mutations in the keratin genes.

Gene therapy approaches for these diseases differ from other genodermatoses due to the dominant negative effect of the mutations on the remaining, wild type, paired keratin bundles. Transgenic mouse studies have suggested that over-expression of the normal keratin copy can overcome these dominant mutations to a significant extent, but for specific keratins only [49]. Another intermediate filament protein, desmin (present in muscle), has also been used to restore the function of keratinocytes containing dominant negative mutations in K5 and K14. This technique could benefit a number of mutations as it does not depend on any site specific mutation in a particular keratin gene.

RNAi technology can overcome dominant gene disorders

Another approach is to target the mutant keratin directly using interference RNA and DNA specifically targeted against the mutant DNA. This approach provides probably

the most successful gene therapy technique for dominant gene disorders as this allows the normal gene product to function [50, 51].

Plectin mutations lead to forms of EBS with muscular dystrophy (EBS-MD), often incorporating other organ-like skeletal muscle due to the wide expression of plectin and its isoforms [3, 14, 15, 52]. Plectin is a large cytoskeletal linker protein, linking intermediate filaments to actin and microtubules. Gene therapy for plectin defects has employed techniques such as spliceosome-mediated RNA trans-splicing (SMaRT). SMaRT uses the endogenous spliceosome machinery to effectively excise mutant exons knocking out the mutant protein from the cells and is thus potentially beneficial in dominant negative disorders. This technology has been shown to correct mutations in collagen XVII in keratinocytes [53] and plectin mutations in a fibroblast model of EBS-MD [54]. The removal of the mutant plectin isoform allowed the retroviral transfected wild-type plectin to function correctly in the fibroblasts. These promising results show how this technology can be applied to other dominant negative disorders such as the keratinopathy group of disorders.

Stem cell targeting

Though many of the gene therapies strategies have already inadvertently targeted epidermal stem cells, facilitating the persistence of transgene expression through subsequent rounds of cell division, stem cell targeting is widely regarded as the key to achieving long-term transgene expression. However, difficulty in identifying epidermal stem cells and their markers in the interfollicular epidermal stem cell population remains a major barrier. Currently cells that are thought to be stem cells are selected on the basis of self-renewal potential and low terminal differentiation rates. The identification of p63 as a potential marker for keratinocyte stem cells may be of benefit [55]. Considerable efforts are being made to definitively identify epidermal stem cell markers (particularly adult interfollicular stem cells). If it becomes possible to use these markers to isolate or target the stem cell population, then long-term, stable and expression of transgenes should be possible.

Summary

In recent years, the initial results of gene therapy for some of the severest genodermatoses have been reported. Initial clinical trials using gene-corrected skin grafts in JEB patients have produced encouraging results and the development of new techniques for overcoming dominant gene disorders and the improved targeting of stem cell populations provide inspiration for future developments. New techniques or combinations of techniques to improve transgene expression, delivery and safety should also provide further beneficial approaches for the treatment of severe congenital human skin disorders. It is likely to be many years before these treatments become widely used due to safety issues; however, the initial benefits and prospects of epidermal gene therapy are now already beginning to be seen.

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References

1. McMillan JR, McGrath JA, Tidman MJ & Eady RA. Hemidesmosomes show abnormal association with the keratin filament network in junctional forms of epidermolysis bullosa. J Invest Dermatol 1998; **110(2)**:132-137.
2. McMillan JR, Akiyama M & Shimizu H. Epidermal basement membrane zone components: ultrastructural distribution and molecular interactions. J Dermatol Sci 2003; **31(3)**:169-177.
3. Sawamura D, McMillan JR, Akiyama M & Shimizu H. Epidermolysis bullosa: directions for future research and new challenges for treatment. Arch Dermatol Res 2003; **295 Suppl 1**:S34-42.
4. Pearson RW. Studies on the pathogenesis of epidermolysis bullosa. J Invest Dermatol 1962; **39**:551-575.
5. Fine JD, Eady RA, Bauer EA *et al.* The classification of inherited epidermolysis bullosa (EB). Report of the Third International Consensus Meeting on Diagnosis and Classification of EB. J Am Acad Dermatol 2008; **58(6)**:931-950.
6. Uitto J & Pulkkinen L. The genodermatoses: candidate diseases for gene therapy. Hum Gene Ther 2000; **11(16)**:2267-2275.
7. Eady R, McGrath J & McMillan J. Ultrastructural clues to genetic disorders of skin: the dermal-epidermal junction. [Review]. J Invest Dermatol 1994; **103(5 Suppl)**.
8. Mellerio JE, Smith FJ, McMillan JR *et al.* Recessive epidermolysis bullosa simplex associated with plectin mutations: infantile respiratory complications in two unrelated cases. Br J Dermatol 1997; **137(6)**:898-906.
9. McMillan JR, McGrath JA, Pulkkinen L *et al.* Immunohistochemical analysis of the skin in junctional epidermolysis bullosa using laminin 5 chain specific antibodies is of limited value in predicting the underlying gene mutation. Br J Dermatol 1997; **136(6)**:817-822.
10. Kivirikko S, McGrath JA, Baudoin C *et al.* A homozygous nonsense mutation in the alpha 3 chain gene of laminin 5 (LAMA3) in lethal (Herlitz) junctional epidermolysis bullosa. Hum Mol Genet 1995; **4(5)**:959-962.
11. McGrath JA, Gatalica B, Christiano AM *et al.* Mutations in the 180-kD bullous pemphigoid antigen (BPAG2), a hemidesmosomal transmembrane collagen (COL17A1), in generalized atrophic benign epidermolysis bullosa. Nat Genet 1995; **11(1)**:83-86.

12. McGrath JA, Gatalica B, Li K *et al.* Compound heterozygosity for a dominant glycine substitution and a recessive internal duplication mutation in the type XVII collagen gene results in junctional epidermolysis bullosa and abnormal dentition. Am J Pathol 1996; **148(6)**:1787-1796.
13. McGrath JA, McMillan JR, Dunnill MG *et al.* Genetic basis of lethal junctional epidermolysis bullosa in an affected fetus: implications for prenatal diagnosis in one family. Prenat Diagn 1995; **15(7)**:647-654.
14. McLean WH, Pulkkinen L, Smith FJ *et al.* Loss of plectin causes epidermolysis bullosa with muscular dystrophy: cDNA cloning and genomic organization. Genes Dev 1996; **10(14)**:1724-1735.
15. McMillan JR, Akiyama M, Rouan F *et al.* Plectin defects in epidermolysis bullosa simplex with muscular dystrophy. Muscle Nerve 2007; **35(1)**:24-35.
16. El Ghalbzouri A, Jonkman MF, Dijkman R & Ponec M. Basement membrane reconstruction in human skin equivalents is regulated by fibroblasts and/or exogenously activated keratinocytes. J Invest Dermatol 2005; **124(1)**:79-86.
17. Jonkman MF, Pas HH & Fine JD. Mosaic expression of uncein, linear IgA bullous dermatosis antigen and 180-kDa bullous pemphigoid antigen in generalized atrophic benign epidermolysis bullosa. Br J Dermatol 1998; **138(5)**:904.
18. Basarab T, Dunnill MG, Eady RA & Russell-Jones R. Herlitz junctional epidermolysis bullosa: a case report and review of current diagnostic methods. Pediatr Dermatol 1997; **14(4)**:307-311.
19. Schofield OM, Fine JD, Verrando P, Heagerty AH, Ortonne JP & Eady RA. GB3 monoclonal antibody for the diagnosis of junctional epidermolysis bullosa: results of a multicenter study. J Am Acad Dermatol 1990; **23(6 Pt 1)**:1078-1083.
20. Matsui C, Wang CK, Nelson CF, Bauer EA & Hoeffler WK. The assembly of laminin-5 subunits. J Biochem 1995; **270**:23496-23503.
21. Matsui C, Nelson CF, Hernandez GT, Herron GS, Bauer EA & Hoeffler WK. The gamma 2 chain of laminin-5 is recognised by monoclonal antibody GB3. J Invest Dermatol 1995; **105**:648-652.
22. McGrath JA, Dunnill MG, Christiano AM *et al.* First trimester DNA-based exclusion of recessive dystrophic epidermolysis bullosa from chorionic villus sampling. Br J Dermatol 1996; **134(4)**:734-739.
23. Rugg EL, Baty D, Shemanko CS *et al.* DNA based prenatal testing for the skin blistering disorder epidermolysis bullosa simplex. Prenat Diagn 2000; **20(5)**:371-377.
24. Fassihi H, Ashton GH, Denyer J, Mellerio JE, Mason G & McGrath JA. Prenatal diagnosis of Herlitz junctional epidermolysis bullosa in nonidentical twins. Clin Exp Dermatol 2005; **30(2)**:180-182.
25. McGrath JA. Translational benefits from research on rare genodermatoses. Australas J Dermatol 2004; **45(2)**:89-93.
26. McGrath JA & Handyside AH. Preimplantation genetic diagnosis of severe inherited skin diseases. Exp Dermatol 1998; **7(2-3)**:65-72.
27. Jooss K & Chirmule N. Immunity to adenovirus and adeno-associated viral vectors: implications for gene therapy. Gene Ther 2003; **10(11)**:955-963.
28. Geurts AM, Yang Y, Clark KJ *et al.* Gene transfer into genomes of human cells by the sleeping beauty transposon system. Mol Ther 2003; **8(1)**:108-117.
29. Groth AC, Olivares EC, Thyagarajan B & Calos MP. A phage integrase directs efficient site-specific integration in human cells. Proc Natl Acad Sci USA 2000; **97(11)**:5995-6000.
30. Wilson MH, Coates CJ & George AL, Jr. PiggyBac transposon-mediated gene transfer in human cells. Mol Ther 2007; **15(1)**:139-145.
31. Thyagarajan B, Olivares EC, Hollis RP, Ginsburg DS & Calos MP. Site-specific genomic integration in mammalian cells mediated by phage phiC31 integrase. Mol Cell Biol 2001; **21(12)**:3926-3934.
32. Ortiz-Urda S, Thyagarajan B, Keene DR, Lin Q, Fang M, Calos MP & Khavari PA. Stable nonviral genetic correction of inherited human skin disease. Nat Med 2002; **8(10)**:1166-1170.
33. Sakai LY, Keene DR, Morris NP & Burgeson RE. Type VII collagen is a major structural component of anchoring fibrils. J Cell Biol 1986; **103**:1577-1580.
34. Ryynanen J, Sollberg S, Parente MG, Chung LC, Christiano AM & Uitto J. Type VII collagen gene expression by cultured human cells and in fetal skin. Abundant mRNA and protein levels in epidermal keratinocytes. J Clin Invest 1992; **89(1)**:163-168.
35. Christiano AM, Greenspan DS, Lee S & Uitto J. Cloning of human type VII collagen. Complete primary sequence of the alpha 1(VII) chain and identification of intragenic polymorphisms. J Biol Chem 1994; **269(32)**:20256-20262.
36. Hovnanian A, Hilal L, Blanchet Bardon C, de Prost Y, Christiano AM, Uitto J & Goossens M. Recurrent nonsense mutations within the type VII collagen gene in patients with severe recessive dystrophic epidermolysis bullosa. Am J Hum Genet 1994; **55(2)**:289-296.
37. Ortiz-Urda S, Lin Q, Green CL, Keene DR, Marinkovich MP & Khavari PA. Injection of genetically engineered fibroblasts corrects regenerated human epidermolysis bullosa skin tissue. J Clin Invest 2003; **111(2)**:251-255.
38. Woodley DT, Krueger GG, Jorgensen CM *et al.* Normal and gene-corrected dystrophic epidermolysis bullosa fibroblasts alone can produce type VII collagen at the basement membrane zone. J Invest Dermatol 2003; **121(5)**:1021-1028.
39. Goto M, Sawamura D, Ito K *et al.* Fibroblasts show more potential as target cells than keratinocytes in COL7A1 gene therapy of dystrophic epidermolysis bullosa. J Invest Dermatol 2006; **126(4)**:766-772.
40. Sawamura D, Abe R, Goto M, Akiyama M, Hemmi H, Akira S & Shimizu H. Direct injection of plasmid DNA into the skin induces dermatitis by activation of monocytes through toll-like receptor 9. J Gene Med 2005; **7(5)**:664-671.
41. Qiao H, Shibaki A, Long HA *et al.* Collagen XVII participates in keratinocyte adhesion to collagen IV, and in p38MAPK-dependent migration and cell signaling. J Invest Dermatol 2009; 26 Accepted February 2009; doi:10.1038/jid.2009.20
42. Nakano A, Pfendner E, Hashimoto I & Uitto J. Herlitz junctional epidermolysis bullosa: novel and recurrent mutations in the LAMB3 gene and the population carrier frequency. J Invest Dermatol 2000; **115(3)**:493-498.
43. Vidal F, Aberdam D, Miquel C *et al.* Integrin beta 4 mutations associated with junctional epidermolysis bullosa with pyloric atresia. Nature Genet 1995; **10**:229-234.
44. Pulkkinen L & Uitto J. Hemidesmosomal variants of epidermolysis bullosa. Mutations in the alpha6beta4 integrin and the 180-kD bullous pemphigoid antigen/type XVII collagen genes. Exp Dermatol 1998; **7(2-3)**:46-64.
45. Ashton GH, Mellerio JE, Dunnill MG *et al.* A recurrent laminin 5 mutation in British patients with lethal (Herlitz) junctional epidermolysis bullosa: evidence for a mutational hotspot rather than propagation of an ancestral allele. Br J Dermatol 1997; **136(5)**:674-677.
46. Mavilio F, Pellegrini G, Ferrari S *et al.* Correction of junctional epidermolysis bullosa by transplantation of genetically modified epidermal stem cells. Nat Med 2006.
47. Seitz CS, Giudice GJ, Balding SD, Marinkovich MP & Khavari PA. BP180 gene delivery in junctional epidermolysis bullosa. Gene Ther 1999; **6(1)**:42-47.
48. Nishie W, Sawamura D, Goto M *et al.* Humanization of autoantigen. Nat Med 2007; **13(3)**:378-383.
49. Cao T, Longley MA, Wang XJ & Roop DR. An inducible mouse model for epidermolysis bullosa simplex: implications for gene therapy. J Cell Biol 2001; **152(3)**:651-656.
50. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K & Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 2001; **411(6836)**:494-498.
51. Elbashir SM, Lendeckel W & Tuschl T. RNA interference is mediated by 21- and 22-nucleotide RNAs. Genes Dev 2001; **15(2)**:188-200.
52. Smith FJ, Eady RA, Leigh IM *et al.* Plectin deficiency results in muscular dystrophy with epidermolysis bullosa. Nat Genet 1996; **13(4)**:450-457.
53. Dallinger G, Puttaraju M, Mitchell LG *et al.* Development of spliceosome-mediated RNA trans-splicing (SMaRT) for the correction of inherited skin diseases. Exp Dermatol 2003; **12(1)**:37-46.
54. Wally V, Klausegger A, Koller U *et al.* 5′ trans-splicing repair of the PLEC1 gene. J Invest Dermatol 2008; **23(3)**:568-574.
55. Pellegrini G, Dellambra E, Golisano O *et al.* p63 identifies keratinocyte stem cells. Proc Natl Acad Sci USA 2001; **98(6)**:3156-3161.