Raising the roof on epidermolysis bullosa (EB): a focus on new therapies

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Abstract

Epidermolysis bullosa (EB) is a complex group of genetic disorders producing various degrees of recurrent skin blistering and epidermal detachment from the basement membrane. Patients with this disease experience the loss of intact epidermis, disruptions of basement membrane adhesion units and altered cellular adhesion, migration and integrin expression. Wound healing in patients suffering from EB remains a major challenge to their survival because of infection risk and fluid loss.

There are four main types of EB each characterised by different levels of blistering formation at the dermal-epidermal junction (DEJ) (basal layer, lamina lucida, sub-lamina densa and various respectively) and different clinical phenotypes. Advances in the understanding of the pathogenesis of EB in the last 15 years have led to the identification of several candidate genes and proteins; however, present management of these diseases is still supportive and therapy symptomatic. Different avenues of therapy options being investigated, some of which are in clinical trials, include bone marrow transplant, gene therapy, cell-based therapy and protein-based therapy. Further research focused on the development of novel therapies may lead to improved quality of life for patients suffering from EB.

Introduction

The severity of blister expression in epidermolysis bullosa (EB) patients can range from mild blistering to severe bulla formation, erosions, scarring and mutilations. The disease affects one in 17,000 live births in all races, with an estimated 500,000 cases worldwide [1]. The disease has various forms caused by mutations in genes which code for structural proteins at the dermal-epidermal junction (DEJ), resulting in diminished adhesion of skin layers and blistering [2].

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Clinical features of EB

There are four main types of EB – EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB) and Kindler Syndrome – and their clinical spectrum of severity ranges from predisposition to blistering to severe morbidity and mortality [3]. The main subtypes of EB with different genes and proteins involved are illustrated in Table 1. Examples of clinical features associated with different EB subtypes are shown in Figure 1.

EBS is characterised by cytolysis of basal keratinocytes and mutations in *KRT5* and *KRT14* where blistering occurs intra-epidermally and wound healing takes place usually without scarring [4]. The main clinical features of EBS include generalised bleeding, superficial flaccid bullae and erosions.

JEB is the least common form of EB, in which the hemidesmosome-anchoring filament complex is weakened and genetic defects occur in one of the structural components, including integrin α6β4, collagen XVII and major basement membrane protein laminin-5 [5]. Clinical aspects of JEB involve disseminated blister formation at the DEJ, cleavage along the lamina lucida, polycyclic lesions, with blisters and post-lesional hyper-pigmentation [3]. The degree of severity in the EB disease can be seen in different subtypes of JEB which illustrate varying degrees of scarring. JEB Herlitz is usually

lethal within the first 2 years after birth, while JEB progressiva is mild and healing often takes place without scarring [3].

The third type of EB is DEB. Blistering occurs at the level of anchoring fibrils at the DEJ resulting in morphologically altered or absent anchoring fibrils – generalised dermal blistering occurs below the basement membrane leading to disabling mutilations, mucosal involvement and scarring [6]. Abnormalities of the anchoring fibrils and mutations in COL7A1, the gene encoding the major anchoring fibril protein collagen VII, underlie all DEB subtypes which can be recessive (RDEB) or dominant (DDEB). Other clinical features of DEB include severe blistering, nail dystrophy, scarring, erosions and atrophic areas on lower extremities [3].

Kindler Syndrome, an autosomal recessive genodermatosis, is the fourth recently-recognised main subtype of EB. This syndrome results from mutation of the gene encoding for protein kindlin-1, a component of focal contacts in basal keratinocytes, and clinically mimics different subtypes of EB, including JEB and DEB [7]. Kindlin-1 protein links the actin-cytoskeleton to the extracellular matrix and is involved in cell signalling, mediating the integrin dependent processes of cell adhesion, growth, migration, spreading, differentiation and apoptosis [8, 9]. The main clinical features of Kindler Syndrome include acral trauma-induced blistering, poikiloderma and photosensitivity, thereby distinguishing this subtype from other inherited EB subgroups [7].

Skin disadherence and poor wound re-epithelialisation are major clinical problems seen in EB patients and often result in the loss of movement and deformity. These are especially important considerations for children, where their growth places extra demands on healing wounds. In certain forms of EB, especially EB Hallopeau-Siemens subtype, early epidermal metaplasia may lead to development of squamous cell carcinoma which often results in amputation of the limbs [10]. It is also important to acknowledge that EB patients also suffer from blisters developing in the cells lining the mouth and the gastrointestinal tract, often resulting in oropharyngeal blistering and in severe cases may result in the obstruction of the upper airways. Gastrointestinal involvement is mainly seen in JEB and DEB subtypes, often resulting in reduced nutritional intake, contractures of the mouth, oesophageal strictures, dysphasia and gastro-oesophageal reflux [11].

Animal models of pathogenesis

Models are available for studying the pathogenesis of EB, including both *ex vivo* and animal models [12, 13]. A better understanding of the cellular and molecular events of wound healing is essential to designing novel therapies for treatment and better wound management associated with different EB subtypes [14]. Several knockout or transgenic mice also exist for studying DEJ components and cellular receptors. These include plectin, BP230, integrin chains α3, α6, β1 and β4, laminin-5 and collagen IV [3]. These animal models have helped us understand the pathophysiology of EB blistering diseases and have led to improved diagnosis and identification of novel genes and proteins involved in the molecular aspects of these diseases. Identification of specific gene mutations

Table 1. Main EB subtypes and associated genes and proteins involved.

Main EB type/subtype	Inheritance	Gene involved	Protein involved
EBS			
Suprabasal EBS	AD	PKP; DSP	Plakoplilin-1; Desmoplaktin
Basal EBS	AD	KRT5; KRT14; PLEC1; ITGA6; ITGB4	Kratin 5 & 14; Plectin; Integrin α6β4
JEB			
JEB-Herlitz	AR	LAMA3; LAMB3; LAMC2	Laminin-5
JEB, other	AR	LAMA3; LAMB3; LAMC2; COL17A1; ITGA6; ITGB4	Laminin-5; Type XVII collagen; Integrin α6β4
DEB			
Dominant DEB (DDEB)	AD	COL7A1	Type VII collagen
Recessive DEB (RDEB)	AR	COL7A1	Type VII collagen
Kindler Syndrome			
–	AR	KIND1	Kindlin-1

AD=autosomal dominant AR=autosomal recessive

have resulted in the development of DNA-based prenatal testing and genetic diagnosis for families at high risk of EB reoccurrence.

Diagnosis of EB

The principal problem in diagnosis of the most common varieties of EB is to distinguish different subtypes clinically; however, this is mainly the problem in the neonatal period. Secondary clinical symptoms usually start to develop in within first months and years of life, allowing clinical diagnosis of main EB categories [3]. Care must be taken not to confuse the condition for other skin disorders or conditions with similar clinical presentations.

Diagnosis of EB involves a combination of approaches, including examination of the family history, clinical examination, a skin biopsy for antigen mapping and electron microscopy and cell cultures and blood samples for mutation analysis [7]. Immunohistochemistry and electron microscopy usually allow differentiation of different subtypes but experience is required for reliable interpretations. Electron microscopy allows evaluation of skin separation, examination and semiquantitative assessment of different adhesion structures at the DEJ including desmosomes, keratin filaments, hemidesmosomes and anchoring fibrils. On the other hand, immunofluorescence involves antigen mapping against a panel of antibodies and interpretation of the fluorescence and specific proteins involved in structural weakness of the skin. Unfortunately, certain EB subtypes are very rare and molecular markers for these subtypes are yet to be investigates, making diagnosis difficult in some cases [3].

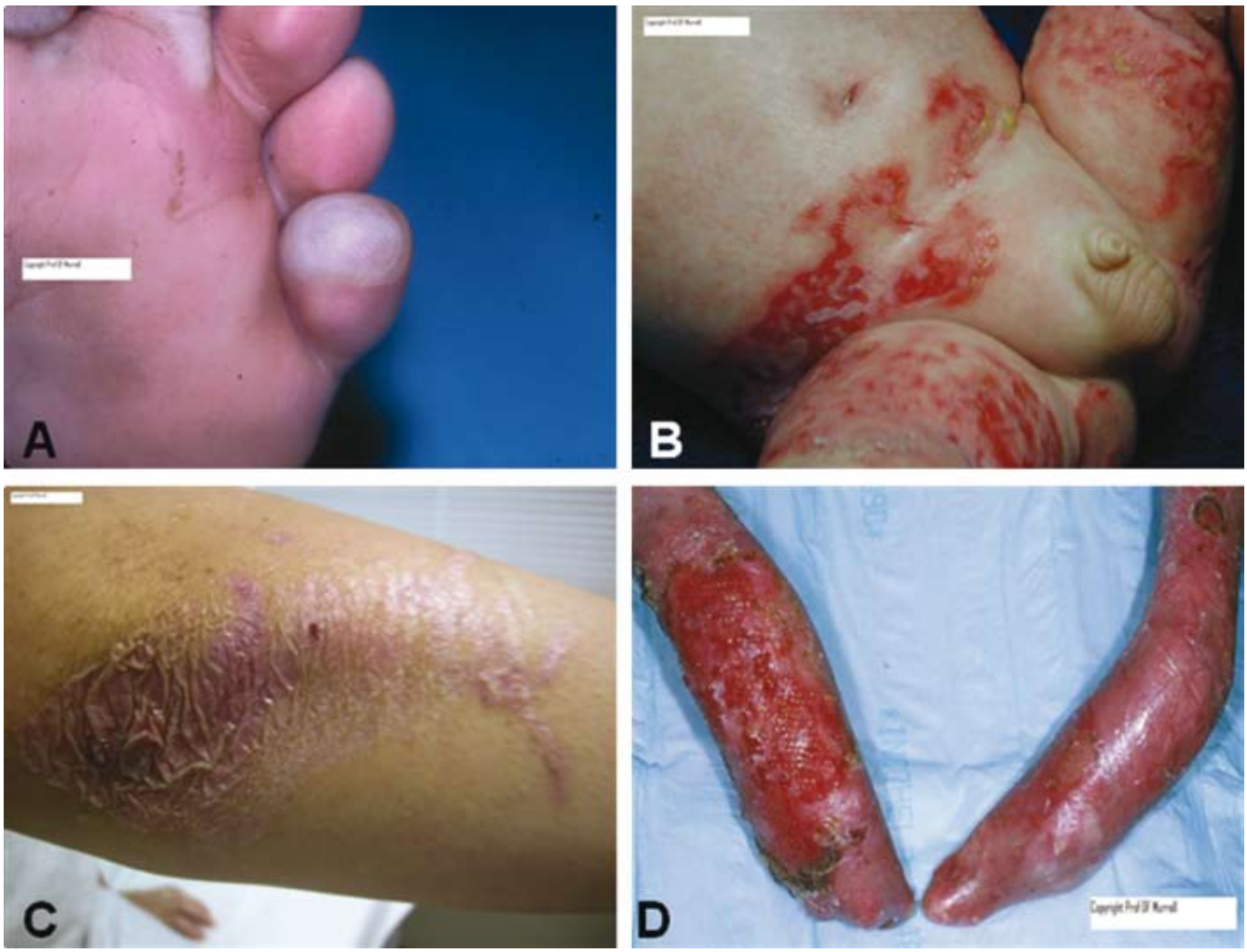

Figure 1. Clinical features associated with different EB subtypes (© Prof DF Murrell.)
A: Localised EBS on the feet.
B: Herlitz JEB on limbs and trunk.
C: Dominant DEB with atrophic scarring on elbow.
D: RDEB with pseudosyndactyly and squamous cell carcinoma.

Management of EB

Wound healing of EB patients remains a challenge and the development of new therapies and efficient wound management needs to be investigated. Current wound management includes topical applications to the wounds, lancing or de-roofing of blisters, removal of any necrotic material or fibres to enable efficient healing, minimisation of possible sources of friction and trying different types of protective padding [15]. A number of secondary complications often arise in EB patients and require separate treatments. These include anaemia, skin contractures, infections and growth retardation and malnutrition due to intraoral blisters, dysphagia and oespohageal erosions. Surgical release of contractures and skin grafting have been successful in some cases while growth retardation and anaemia are often reversed using gastroscopy tube feeding and blood transfusions respectively [3].

The optimal bathing regime for babies, children and adults with EB also remains to be firmly established, with no randomised trials of these; different home, hospital staffing and climates may affect clinics' recommendations. In colder climates, non-bathing and changing of individual limbs one at a time is in vogue for babies, only every few days, whereas in hotter climates daily baths and dressing changes are recommended as ideal. In Australia, the warmer climate causes increased sweating that may mean that infrequent dressing changes create a culture media under the dressings.

Salt baths prepared with swimming pool salt at the same pH as normal saline can reduce stinging for patients and enhance bathing compliance, as well as remove crusts that are a nidus for infection. Some older patients prefer showers, but the water force can damage some extensive wounds, whilst other individuals cannot stand due to the severity of their pseudosyndactyly, making bathing the only practical option.

Wound dressings

Clinical research has lead to the development of different wound dressings and ointments for the wound management of EB patients. Dressing choice varies between patients suffering from different types of EB depending on the need for mechanical protection, level of exudate and presence of colonisation.

Wound dressings containing medical grade honey have been suggested to have useful antimicrobial effects due to its low pH, high osmolality and low levels of hydrogen peroxide production [15]. Soft silicone dressing and foams currently available on the market offer good management of wounds in EB patients. However, further research is required for development of an 'ideal' dressing for EB patients which will promote healthy wound repair by increasing re-epithelialisation, maintaining appropriate moisture levels, being non-adherent and atraumatic, decreasing pain and being suitable for different body areas [15]. The use of an allogeneic cultured bi-layer of human skin origin containing both epidermal and dermal components resulted in rapid wound healing with no tissue rejection [16, 17].

Systemic approach

Systemic treatments for EB have so far been unreliable and have resulted in unpredictable side effects. In Sydney, Australia, a trial of allogeneic fibroblast cell therapy is underway [by DM] for patients with RDEB, designed to determine if it can improve wound healing. A pilot study by McGrath and colleagues showed that fibroblasts could increase collagen VII production in the non-blistered, non-wounded skin of RDEB patients, but did not assess wound healing or continue beyond 3 months [18].

Following studies of the possible amelioration of EB and reduced lethality by transfer of healthy normal bone marrow cells in the murine model of RDEB, one recent systemic treatment for RDEB currently under trial at the University of Minnesota involves transplant cultures of healthy donor cord blood cells and bone marrow into the patient's bloodstream [19]. This trial is still underway but it is hypothesised that healthy blood stem cells will migrate into the patient's skin and correct the genetic defect by depositing normal collagen VII which can then integrate into normal anchoring fibril synthesis.

A disease similar to DEB, EB acquisita (EBA), a rare auto-immune subepidermal blistering disease with antibodies targeting collagen VII, has been treated with systemic corticosteroids and other drugs including rituxibam, colchichine, dapsone as well as photopheresis and intravenous immunoglobulins (IVIG) therapy. However, clinical outcomes have varied and the evidence level is poor due to the lack of randomised control trials in EBA [20].

Gene therapy

Current research into novel treatments for EB patients has mainly focused on gene- and protein-based therapy [21-23]. EB is a good candidate for gene therapy as specific molecular defects have been identified in distinct genes expressed at the DEJ, and the majority of mutations are single-base pair substitutions, small insertions or deletions which may be altered by different gene therapy approaches [24].

Cutaneous gene therapy can be achieved by two different approaches, *in vivo* and *ex vivo*. *In vivo* approaches for gene therapy involves the introduction of genetic material into

the skin by injection, biolistic particle bombardment, topical application mediated by physico-chemical means (including liposomes), and *in vivo* electroporation [1]. An alternate *ex vivo* approach involves the removal of patient skin samples, *in vitro* proliferation of epidermal keratinocytes, introduction of genetic material into the cultured cells with gene transfer vectors (allowing a genetic and phenotypic shift and correction in adhesion properties of keratinocytes), and finally return of genetically modified cells in the form of a skin graft, as often performed for burns patients [25]. These methods rely on efficient targeting of stem cells with minimal apoptosis, integration of genetic material with the host genome without activation of oncogenes and no immune response in the patient. Current research is investigating the best options for integration of genetic material using both retroviral, lentiviral and non-viral means of gene delivery [25].

Correction of the genetic defect in JEB keratinocytes using retroviral vectors has been trialled and the first successful *in vivo* gene therapy in junctional non-Herlitz EB has recently been reported. Epidermal sheets of keratinocytes were transfected using a retroviral vector expressing normal laminin-5 [26]. Lentiviral vectors have, however, shown to be more suitable for *ex vivo* gene therapy as they affect both dividing and non-diving cells, have higher transduction efficiency and sustained gene expression *in vivo* [27]. Another significant challenge in gene therapy for EB is the systemic delivery of the therapeutic gene not only to the skin but also to the cells lining the mouth, gastrointestinal tract and other affected internal sites [10].

For treatment of recessive JEB or RDEB, gene therapy by an *ex vivo* approach targeting epidermal stem cells from EB skin seems most promising. For this to be achieved, stable integration of the transgene into the genome of the epidermal stem cells is required, followed by successful transcription, translation and synthesis of the appropriate basement membrane protein, its secretion and incorporation into the skin basement membrane, in a way that will allow it to be functionally effective [5]. Development of gene therapy approaches for DEB using retroviral vectors has been hindered due to difficulties in accommodating the entire 9-kilobase (kb) type VII collagen cDNA, and epidemiological studies of different genotypes and phenotypes of EB patients result in continual identification of novel collagen VII variants in patients suffering from DEB [6, 25]. For treatment of dominant disorders such as EBS or DEB, selective inhibition of the expression of the gene remains the only practical method. Researchers are investigating the use of siRNA to knock down the expression of genes and other antisense approaches including mutation specific ribozymes and RNA interference [1].

Alternative strategies based on non-viral gene therapy have also been reported, including sustainable gene correction of JEB via transposon mediated non-viral gene transfer [28, 29]. Other non-viral vector delivery gene therapy methods include spliceosome-mediated RNA trans-splicing (SMaRT) δC31 bacteriophage integrase and the Sleeping Beauty transposon system. Using the non-viral method of gene transfer, cotransfection of plasmids encoding δC31 integrase and human collagen VII resulted in the expression of collagen VII in DEB keratinocytes *in vitro* [10]. While these systems allow genetic correction in diseases where large genes are altered, mediate integration at specific sites of the genome and allow physiological expression of the missing protein, they result in poor gene transfection efficiency and require further research and development. Gene-based therapies offer an advantage of continuous protein production in the skin, and *ex vivo* gene therapy using retroviruses coupled with efficient skin grafting is a more promising option for epidermal gene therapy [10, 25].

Cell- and protein-based therapies

Besides gene therapy approaches, other potential developments for treating EB patients include cell- and protein-based therapies. Protein-based therapies have had some success in treating both DEB and JEB by direct intradermal injection of the missing protein, including collagen IV and laminin-5 respectively [10]. The use of cell-based therapy as a potential treatment of EB diseases has also been studied. Fibroblasts may be better candidates for cell therapy then keratinocytes as they are more easily cultured, expressed, exogenously delivered and tend to stay at site of delivery [10]. Indeed, skin grafts produced with gene-transferred fibroblasts have higher levels of collagen VII and anchoring fibril restoration compared to skin grafts with gene-corrected keratinocytes [27].

A recent study has shown that use of protein therapy for treatment of human patients with RDEB might be more feasible than originally thought. Using the recombinant human collagen VII injections, researchers were able to correct the disease phenotype in the murine model of DEB while avoiding the nature of the host immune response to protein therapy. Injected human collagen was stably incorporated into the DEB mouse basement membrane zone forming new anchoring fibrils which persisted for at least 2 months [28]. This study therefore suggested that use of protein therapy might be a technically simpler and safer procedure than other *in vivo* or *ex vivo* gene therapy approaches currently being investigated.

Consequently, potential therapies for treatment of DEB include *ex vivo* gene therapy, injection of the recombinant collagen VII in a protein-based therapy, or cell-based therapy with

intradermal injection of gene-corrected RDEB fibroblasts or normal healthy fibroblasts, where injected cells continuously export collagen VII alpha chain at the injected site [30]. Newly synthesised collagen VII exported by injected fibroblasts, in cell-based therapy, can then attach to the host basement membrane zone and support formation of novel anchoring fibril structure, correcting the DEB phenotype in a similar way to protein-based therapy [28, 30, 31].

Conclusion

The molecular pathways which define the different clinical EB phenotypes are not yet fully understood and unravelling these processes should identify potential new approaches and targets for improving wound repair and management of individuals suffering from EB. New EB therapies will most likely involve a combination of gene- or cell-based therapies and tissue engineering to improve the treatment of wounds. Multidisciplinary interactions of clinicians, scientists and nurses combined with different therapeutic approaches including skin grafting, best practice wound care, nutritional, pharmacological and psychological therapies will facilitate the optimal care of EB patients.

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