Commentary: New advances in the development of therapies for treating inherited skin fragility disorders

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ABSTRACT

Inherited skin fragility disorders comprise a group of disorders, mainly designated as epidermolysis bullosa (EB), that are characterised by mechanical induced blistering and erosions of the skin and mucous membranes caused by mutations of gene coding for protein components of the dermal–epidermal junction zone. Patients with EB experience various degrees of recurrent skin blistering, widespread bullae, and erosions that characteristically heal with exuberant scarring and milia formation or lead to impaired non-healing wounds and the development of aggressive skin cancers. Advances in understanding the pathogenesis of EB in the last decade have led to the identification of several candidate genes and proteins and the development of several therapeutic strategies currently progressing to pre-clinical and clinical stages. This review will focus on the recent advances in the development of these new therapies for treating inherited skin fragility disorders (with particular focus on recessive dystrophic epidermolysis bullosa), which hold great promise for improving the quality of life for patients suffering from EB.

Keywords: Epidermolysis bullosa, inherited skin fragility disorders.

INTRODUCTION

Epidermolysis bullosa (EB) is a genetically heterogeneous group of skin diseases estimated at over 500,000 cases worldwide, comprising four main types: EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB) and Kindler Syndrome[1]. Over the last decade, more than 18 genes have been identified that contribute to the molecular pathology of these four types of EB, resulting in a large variety of mutations and these account for the phenotypic diversity of more than 30 subtypes[2]. The disease prevalence estimates from the national EB registry in the United States of America (USA), which is currently the most comprehensive registry including 3,280 patients from 1986–2002, which shows prevalence data (per one million population) for inherited EB: 4.6/million EBS, 0.4/million JEB and 0.9/million recessive dystrophic epidermolysis bullosa (RDEB) cases. This data excludes Kindler Syndrome and 10% of patients in this registry who could not be accurately separated into major disease types[3,4]. Total prevalence figures in the USA (8.2/million) are very similar to those reported in Italy (10.1/million) and Australia (10.3/million)[4,5]. Interestingly, the incidence data of new cases for inherited EB based on the national EB registry in the USA suggests much higher figures of 19.6/million live births[4], suggesting improved reporting of EB subtypes. Currently, there is no cure for EB and treatment options are limited to palliative care that is largely ineffective for treating the systemic, life-threatening pathologies associated with the most severe forms of EB[6]. Clinical features, diagnosis and management of different EB subtypes have previously been reviewed[7], including the summary of available dressings for Australian patients through the National Epidermolysis Bullosa Dressing Scheme[8]. Here we will focus on recent advances in the development of new therapies for treating inherited skin fragility disorders.

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Recent studies have investigated the views of EB patients, carers and health professionals to help set the research priorities for new therapies. These studies identified that wound care, itch and pain were the main patient concerns, closely followed by cancer and new future therapies[9,10]. These studies also emphasised the need for more randomised controlled studies addressing these priorities, including using outcome measures such as: blister count, wound healing, pain and validated scoring matrices[9,10]. However, emerging issues relating to the translation of scientific breakthroughs into the EB patient population include the low patient numbers and the need to use validated quality of life measures across all the different EB subtypes. In addition to overcoming the challenges of recruiting EB patients into clinical trials, which includes: high risk/small benefit for the patient, pain and travel time, there is also an increasing need for the proper design of clinical trials and the provision of better communication to EB patients and the community.

Current research efforts in EB reflect the need to develop therapies aimed at symptom relief for current patients as well as long-term development of therapy to cure the disease, aimed at future generations of patients. Main research efforts have focused on: gene therapy (involves introduction of the correct gene to the patient's skin so that the correct protein can be produced); protein therapy (involves introduction of the correct form of the faulty protein into the skin); cell therapy (involves introducing new cells, for example, fibroblasts or stem cells into the skin where they can then produce the functional protein); small molecules (involves molecules which can target a particular protein such as antibodies or siRNA); and formulations (involves topical applications of creams containing targeted therapies for improved healing of skin). Over the last five years there have been numerous advances in the development of treatment options covering a wide range of gene, cellular and protein therapeutic approaches, although none to date have been completely successful[11]. Due to disease severity, most approaches have focused on the development of new therapies for patients with RDEB, where mutations in the gene encoding type VII collagen (COL7A1) result in severely diminished or absent type VII collagen-anchoring fibrils which hold the skin layers together (Figure 1A–B)[12]. In the last few years there have been an increasing number of human clinical trials underpinning the development of new therapies for EB patients. It is now becoming clear that combined approaches have a real potential to alleviate the symptoms associated with EB. Localised therapeutic approaches under development include intradermal injections of missing protein, use of microneedles, and skin grafts; however, while these approaches show some efficacy in healing surface wounds, they do not treat all the physical manifestations of EB[13]. Long-term curative strategies are yet to be identified; however, a research consensus towards using multiple modalities targeting both "outside-in" and "inside-out" corrective approaches combining both localised and systemic strategies tailored to specific patient needs have been acknowledged as the gold standard by experts in the field (Figure 1C)[12].

GENE THERAPY

Advances in the development of cutaneous gene therapies for EB patients have the potential to be revolutionised by new techniques including Zinc fingers, TALENs and CRISP/Cas9 technologies, enabling gene targeting and editing without the need to use viral vectors. Current challenges in gene therapy and skin grafting for EB patients include selection of the best techniques for growing keratinocyte or keratinocyte/fibroblast grafts and optimal and safe gene correction methods[14].

Following pre-clinical studies in mice, which showed the safe and efficacious use of self-inactivating retroviral vectors expressing COL7A1, Phase I/II clinical trials are being performed. These trials use the autologous skin equivalents made from patient keratinocytes and fibroblasts genetically corrected with a safe retroviral vector expressing type VII collagen. These clinical trials are assessing the feasibility, safety and potential of genetically corrected skin equivalents to restore the dermal–epidermal junction in RDEB patients[15-17]. This European initiative, to develop efficient *ex vivo* gene replacement therapy for the permanent treatment of RDEB, has received Orphan Drug designation and currently five patients have been selected to be involved in this pilot clinical trial[18].

Gene editing of a patient's autologous cells has also been the focus of recent studies, including the use of combinational RNA-based approaches for high efficiency reprogramming of human somatic cells into stable, induced pluripotent stem cells and their potential use in RDEB therapy[2]. The principle of using gene-corrected, cultured keratinocyte autografts has been successfully demonstrated in one RDEB patient and current studies are now examining the safety and efficacy of this approach in Phase I clinical trials in RDEB patients[19]. While the use of induced pluripotent stem cells is still pending approval for clinical trials, researchers in both the USA and Europe are progressing the possibility of reprogramming patients' own cells and developing effective methods of gene therapy. Indeed, engineering the morphogenesis of pluripotent stem cells may unlock their potential for viable gene therapies that could transform the future of biomedical research.

PROTEIN THERAPY

Protein replacement therapies are currently the most appealing strategies for treating EB, despite the problems of limited durability of the extracellular matrix proteins in the skin and high amounts of protein required for achieving a therapeutic dose. To date, a number of mouse studies have demonstrated the efficacy of recombinant type VII collagen replacement therapy either intradermally, intravenously or topically[20-23]. Current research challenges include prolonging the durability of the protein in the skin and overcoming the high amounts of protein required to achieve a therapeutic effect. Most recently, studies have described the anti-fibrotic properties of type VII collagen regulating cell migration and TGF-β signalling, leading to inhibition of collagen lattice contraction, suggesting that excessive scarring observed in RDEB patients may be due to the absence of anti-fibrotic type VII collagen[22]. However, industry-funded clinical trials for the development of recombinant human type VII collagen (rhC7) protein replacement therapy have been halted due to toxicity associated with rhC7 (Dystrophic Epidermolysis Bullosa Research Association (DEBRA) America communicated data). Until this therapy is available in the clinic, currently approved medication (losartan and pirfenidone) which inhibit TGF-β signalling may be a strategy for improving the excessive scarring seen in RDEB patients, indeed losartan has been shown to reduce inflammation and TGF-β signalling in chronically injured forepaws of RDEB mice, resulting in decreased fibrosis and ameliorating long-term symptoms associated with skin blistering[24].

CELL THERAPY

Cell-based therapies represent an exciting focus of EB research with recent developments including the injection of allogeneic fibroblasts, whole bone marrow transplantation and injection of bone marrow-derived mesenchymal stromal cells[6]. Injection of allogeneic fibroblasts (or saline) to RDEB wounds resulted in upregulation of mutant and partially functional type VII collagen with accelerated wound closure and short-lived clinical outcomes[25,26]. In addition, this localised, injection-based therapy for severe RDEB patients is very painful and

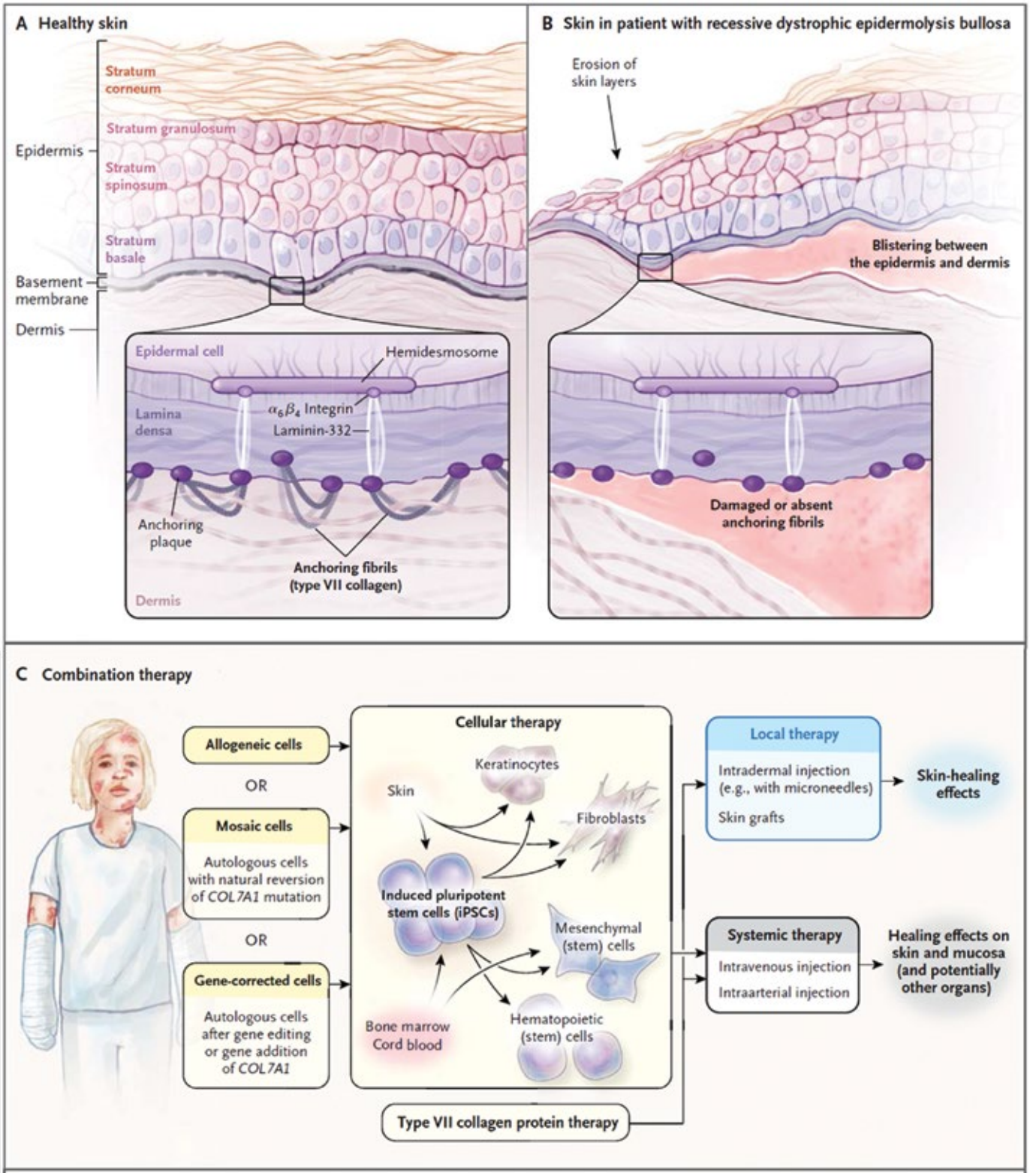

Figure 1: Combination therapy for EB. Compared to the strong adhesion of the skin layers in healthy skin (A), reduction or absence of type VII collagen anchoring fibrils observed in dystrophic EB patients (B) leads to excessive blistering between the epidermis and dermis. Many different therapeutic approaches are currently being investigated for EB patients (C) aimed at providing both localised and systemic relief of disease. Potential curative therapy will most likely involve combinational approaches of both localised and systemic therapies individualised to the specific patients. Figure adapted from Tolar and Wagener, 2015, and republished under licence approval from the New England Journal of Medicine (Licence number 3687981166270).

has practical limitations[25]. The successful transplantation of bone marrow-derived cells has been shown to restore type VII collagen in RDEB mouse skin grafts[27]. These studies are now in clinical development, with subcutaneous transplantation of healthy family donor-derived cultured bone marrow mesenchymal stromal cells to one severe RDEB patient showing initial promising results with no adverse effects and improved tissue regeneration[27]. Likewise, the use of genetically engineered primary autologous keratinocytes, in a Phase I clinical trial for RDEB patients, has also showed complete epidermal regeneration and type VII collagen expression following grafting, hence highlighting a potential durable, cell-based RDEB therapy in humans. To date, 26 patients have been treated with bone marrow transplantation and protocols are been continuously refined to reduce mortality and improve clinical responses[25,28]. For the cohort of engrafted individuals, 15 out of 19 RDEB patients showed partial biochemical and clinical improvement on the basis of COL7 expression, body surface area affected, and resistance to blistering[6].

In recent mouse studies, use of allogenic stem cells showed limited improvement, with EB mouse models with decreased blistering phenotypes, improved cutaneous and GI epithelisation, increased type VII collagen production and anchoring fibril formation leading to increased survival of RDEB mice[29]. Future studies will now focus on the use of unrestricted somatic stem cells from umbilical cord blood to improve the outcomes of children living with RDEB[30]. Human trials in two RDEB patients using allogeneic mesenchymal stromal cells delivered systemically reduced inflammation and improved wound healing[31]. Additionally, results from the EBSTEM study using donor mesenchymal stromal cells infusion in a small number of RDEB children resulted in a temporary improvement of clinical outcomes[25]. Future studies will now evaluate the effect of these approaches in adults and examine the role these cells play following infusion.

Collectively, these cell-based therapy approaches will help to understand the natural history of RDEB, the mode of action of cellular therapies, and the testing of innovative approaches in sufficiently powered randomised and placebo-controlled trials in the future. Progress in this area has raised the expectations of patients, clinicians and researchers that disease modification with different treatment modalities and improved quality of life are feasible goals. The strengths and weaknesses of different targeted therapies currently under investigation for EB patients are summarised in Table 1[2].

WOUND HEALING AND CANCER IN EB

Wound healing of blistered skin has been highlighted as an area of importance by both patients, health professionals and DEBRA[9,10]. Therapies that can provide symptomatic relief would be of great value to EB patients. Pre-clinical studies using antibodies to neutralise the Flightless protein, a negative regulator of wound healing, have shown promising results in an autoimmune mouse model of EB, resulting in decreased severity of skin blistering and improved recovery of skin barrier function post blistering in mice[32-34], suggesting that modulators of wound healing may offer important symptomatic relief in patients and could be used as another treatment modality for EB patients. Another recent pilot study in seven DEB patients has demonstrated that systemic treatment with granulocyte colony-stimulating factor (G-CSF) enhances wound healing, suggesting an alternative approach for DEB patients that do not respond to conventional therapies[35]. Likewise, a study that showed improved wound healing in RDEB could be achieved by using antimicrobial peptides, identified cathelicidin as a potential therapeutic target to improve healing in RDEB by regulating inflammation, and altering the biome composition of the wounds[36].

Patients with RDEB develop extensive skin blistering and scarring, which often leads to squamous cell carcinoma (SCC). The cumulative risk of developing SCCs in RDEB patients is 90.1% by the age of 55 years, with the most common site of involvement being a chronic wound followed by long-term cutaneous scar[37]. Studies have shown that type VII collagen can directly regulate the composition of matrix proteins which are secreted by dermal- and cancer-associated fibroblasts. These matrix proteins including: type XII collagen, thrombospondin-1, and Wnt-5A act in a paracrine manner to transformed tumour keratinocytes, hence creating a permissive environment for the development of aggressive cutaneous SCC in patients with RDEB[38]; however, the exact mechanism of SCC development in RDEB remains unknown. Recent emerging evidence suggests that the mechanism of cancer development in RDEB patients includes both cell autologous and non-cell autologous mechanisms[39]. The aggressive nature of SCC progression in RDEB patients is driven by both matrix composition and PI3K and PLK signalling pathways[40,41]. The use of the PLK1 inhibitor Rigosertib, a small molecule currently in Phase III clinical trials as an anti-cancer agent, could hold promise of therapeutic potential for the treatment of RDEB patients and highlights the need for targeted RDEB-SCC research. Furthermore, it is becoming increasingly clear that the cellular microenvironment in EB accounts for phenotypic variations between EB patients. Studies examining the role of matrix stiffness in the tumour microenvironment are determining if stiffness acts as a mutagen, leading to loss of tumour suppression and accelerating the aggressiveness of cutaneous SCCs[42].

LOCALISED AND SYSTEMIC THERAPIES IN CLINICAL DEVELOPMENT

Currently, there are a number of localised and systemic therapies in clinical development supported by industry partners. Fibrocell Science Inc. is developing a genetically modified autologous fibroblast cell therapy that is anticipated to improve skin function in RDEB patients by restoration of the COL7A1 gene[43], while Shire Pharmaceuticals Plc. is developing a systemic protein therapy focused on rhC7 for improved healing of RDEB patients[19]. The development of a localised topical therapy for EB patients is being led by Scioderm Inc., with clinical trials investigating the effect of Zorblisa™ (SD-101), a wound healing cream, currently in Phase III randomised, double-blinded, placebo-controlled trials in the USA and Europe. These studies have evaluated the safety and efficacy of Zorblisa™ in 130 patients with various EB subtypes, showing faster complete wound closure, reduction in body coverage of lesional skin, changes in itch and pain patterns with no adverse effects[44]. Another localised therapy under clinical development by Keroplast Technologies Ltd. is the use of KeragelT® (keratin gel), initially developed to reduce scarring[45]

Table 1: Strengths and weaknesses of targeted therapies currently in development for EB patients. Figure adapted from Has and Kiritsi, 2015, and republished under licence approval from Experimental Dermatology (Licence number 3687980121316).

Strategy		Pro	Contra
Gene therapy	• Gene correction	• Effective for topical treatment of chronic wounds	• Topical application • Safety concerns • Complex technology • Expensive
	• Antisense oligonucleotides	• Clinical trials for other genetic disorders	• Limited stability in the skin • High dosage required to achieve clinical benefit • Toxicity
	• DNA editing • Trans-splicing • siRNA	• Simple administration	• Experimental approaches • Low topical absorption in the skin • Only topical application
Cell therapies	• Bone marrow transplantation • Fibroblasts • MSC	• Established administration • Systemic application • Safe	• Short survival of cells in the skin • Low concentration of cells in unwounded skin • High amounts of cells required to achieve therapeutic protein concentrations • Immune reaction after repeated administrations probable
	• iPSCs	• Unlimited amount of autologous cells	• Highly complex technology • Safety concerns • Expensive
	• Revertant mosaicism	• Autologous cells which can be used as grafts or epidermal sheets • Simple administration • Safe • Inexpensive	• Only topical application • Revertant mosaicism must be present in the patient
Protein therapy		• Simple administration • Potential systemic application • Safe	• Limited durability of the protein in the skin • High amounts of protein required to achieve therapeutic dosage • Immune reaction after repeated administrations probable • Not suited for intracellular structural proteins
Drugs		• Established for other disorders • Simple administration • Target pathogenic mechanisms • Safe • Inexpensive compared to experimental therapies	• Unclear long-term effects • Do not address the disease cause

BMT, bone marrow transplantation; MSC, mesenchymal stem cells; iPSCs, induced pluripotent stem cells.

that is now being investigated for its benefits in hard-to-heal wounds, with initial clinical trials showing increased rates of healing, reduced tendency for blister recurrence and subsequent improvements in quality of life and reduced cost of care[46].

CONCLUSION

The development of new therapies for the treatment of EB is an urgent issue for patients and families who live with this condition. Here we have described the latest advances in technologies aimed at the treatment of EB. However, there are many challenges and technical limitations still faced by each therapeutic approach, including the following questions:

- Can current technologies generate sufficient amounts of protein required for disease modification?
- What is the most appropriate dosage, frequency and delivery option?

- Does therapy have a long-lasting effect and what are the effects of long-term application?
- What are the best reliable methods of therapeutic efficiency and safety concerns for different treatment approaches?

We hope future clinical studies will answer some of these questions while research defining the molecular pathways involved in the clinical phenotypes of EB will aid in the identification of novel approaches for successful EB therapies. While initial clinical trials for EB show great promise, further research is still needed to realise the practical application of these new therapies.

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