# Toward treatment and cure of epidermolysis bullosa

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Heritable forms of epidermolysis bullosa (EB), a heterogeneous group of skin fragility disorders, manifest with blistering and erosions with high degree of morbidity (1–3). The phenotypic spectrum is highly variable, and in the most severe cases the patients die within the early postnatal period or within a few months of life due to complications of fragile skin, such as infections, sepsis, dehydration, and metabolic alterations. Some of these cases are also associated with extracutaneous manifestations in the syndromic forms of EB, affecting the lungs, kidneys, and heart (4). There is no effective treatment for EB. In PNAS, Jacków et al. (5) report on a CRISPR/Cas9-mediated therapy approach for a form of EB.

Critical for understanding the molecular basis of EB is the recognition of adhesion complexes consisting of interacting proteins, which form a network responsible for keratinocyte cell–cell adhesion and stable association of the epidermis and the underlying dermis (Fig. 1). Currently, there are 21 distinct genes that have been demonstrated to harbor mutations in different forms of EB (6, 7). The topographic location of the mutant genes within the epidermis and the cutaneous basement membrane zone, the types and combinations of the mutations, and their consequences at the mRNA and protein levels collectively explain the tremendous phenotypic variability in this group of disorders.

One of the most severe forms of EB is the recessive dystrophic EB (RDEB), in which the most devastating cases manifest with extensive fragility with blistering and denudation of the skin resulting in extensive scarring and often associated with rapidly metastasizing cutaneous squamous cell carcinomas with high degree of mortality at a relatively early age (8–10). RDEB is also associated with extracutaneous findings, including scarring of the gastrointestinal track, particularly the esophagus, and blistering and erosions of the cornea (8). RDEB is caused by biallelic mutations in the *COL7A1* gene, encoding type VII collagen, the major if not the exclusive protein component of the anchoring fibrils (11). The *COL7A1* loss-of-function mutations
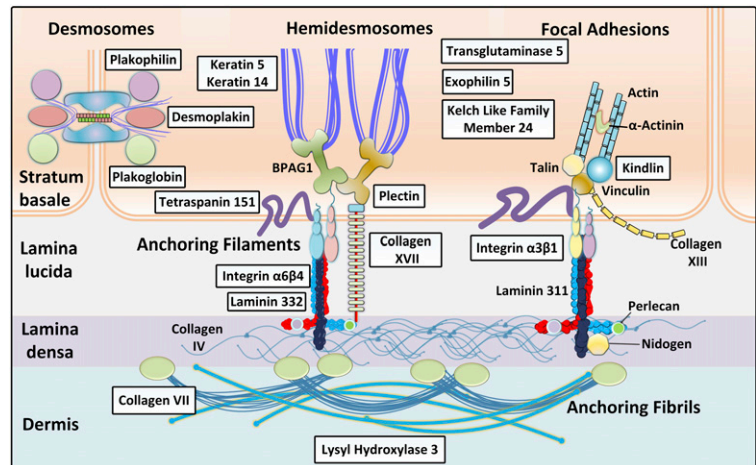
Fig. 1. Schematic representation of the cutaneous basement membrane zone depicting attachment complexes critical for stable cell–cell contacts and association of epidermis to the underlying dermis. The boxed proteins, which are encoded by 21 distinct genes, are mutated in different forms of EB, the prototype of heritable blistering skin disorders. Adapted from ref. 4, with permission from Elsevier.

are frequently nonsense mutations or small insertions or deletions leading to frameshift, but a number of missense mutations have also been reported (12, 13). In addition, there is a dominantly inherited form of dystrophic EB frequently associated with glycine substitution mutations, which interfere with the type VII collagen assembly into anchoring fibrils through dominant-negative mode of action (14).

Considering the clinical severity of RDEB and the fact that there is currently no specific or effective treatment beyond protection from trauma and extensive bandaging as well as prevention of infections, several studies have recently focused on developing new therapies for this devastating, currently intractable disorder (15–18). In PNAS, Jacków et al. (5) report extensive studies employing CRISPR/Cas9-based correction of *COL7A1* mutations in cells from 2 patients with RDEB. One of the patients had a homozygous frameshift mutation in exon 19 (c.2470insG),

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while the second one was a compound heterozygous for frameshift mutations in exons 19 and 32 (c.2470insG/c.3948insT). As the first step of their project, the investigators generated patient-specific inducible pluripotent stem cells (iPSCs). These cells were then subjected to clustered regularly interspaced short palindromic repeats (CRISPR)-associated nuclease/Cas9 (CRISPR/Cas9) homology-directed repair (HDR) of the pathogenic mutations. The researchers demonstrated that the CRISPR/Cas9-mediated HDR can introduce a corrected homologous sequence in the targeted gene and restore the normal gene sequence, which was shown by direct sequencing of the PCR products generated around the targeted region. The results revealed that ~10% of the clones had undergone biallelic correction, and 40% of them monoallelic correction of the COL7A1 mutation in exon 19. Sequencing also revealed that the areas at the target sequence were free of off-target mutations. Next, the gene-corrected iPSCs were differentiated into either keratinocytes, which were shown to become functionally mature in ~60 d, or fibroblasts, which after 31 d assumed characteristic morphology and expressed markers of mesodermal and fibroblastic differentiation, including type I and type III collagen as well as fibroblast-associated CD surface markers in a pattern similar to that of normal human fibroblasts. Importantly, the gene-edited fibroblasts derived from RDEB patients' iPSCs synthesized and secreted type VII collagen, which assumed its characteristic stable triple-helical conformation. Finally, the investigators built 3-dimensional skin equivalents from gene-corrected keratinocytes and fibroblasts, which were then grafted onto immunocompromised mice. Analysis at 2 mo postgrafting demonstrated robust expression of type VII collagen in the xenografts in a pattern resembling that in wild-type mouse skin, and importantly, transmission electron microscopy revealed the formation of anchoring fibrils. Thus, this work attests to the feasibility of a CRISPR/Cas9-mediated gene correction to develop autologous cell therapies for RDEB in the form of skin grafts for skin replacement or for local treatment of nonhealing wounds.

With the eventual goal of clinical translation of this approach, there are regulatory concerns that need to be addressed as treatment development moves forward. Although it is not possible to anticipate all of the potential regulatory concerns relevant to gene-editing–based therapeutics and iPSCs, the major issues at the present time include safety, efficacy, and quality control. Toward these aims, the work describes several improvements and approaches that represent important steps toward clinical translation. Such improvements include a highly efficient method for gene correction assisted by high-fidelity Cas9 (SpyFiCas9) nuclease, which was shown to have few if any detectable genome-wide off-target effects with a chemically modified synthetic guide RNA and single-strand DNA as a repair donor template for efficient gene editing (19). Another innovative feature of this work was the adoption of cell culture conditions devoid of animal products and, instead, using xeno-free, chemically defined culture media. The protocol developed by the investigators resulted in significantly higher reprogramming efficiency than has been reported previously, associated with enhanced safety by eliminating the undefined animal-derived components when developing cell-containing products for treatment of human diseases.

With the realization of the potential of CRISPR/Cas9-mediated genomic correction for human diseases, this technology has been tested preclinically in a number of disease models, including cells, both fibroblasts and keratinocytes, derived from patients with RDEB (19–24). These studies demonstrated HDR using different technical editing strategies and selection methods, including restoration of the reading frame of COL7A1 in cells carrying a frameshift mutation. In addition, an adenine-based editor ABE was shown to introduce a correct single-nucleotide substitution in primary fibroblasts and iPSCs with high efficiency without exogenous donor DNA template (20). The ability by Jacków et al. (5) to construct fully autologous skin equivalents made from gene-corrected keratinocytes and fibroblasts differentiated

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from iPSCs minimizes the risk of immune rejection, thus contributing to the maintenance of functional extracellular matrix and providing long-lasting survival of the graft. Collectively, the work by Jacków et al. significantly advances potential translation of these approaches toward clinical reality, providing an efficient and safe method for clinical application.

In summary, recent progress in development of treatments for EB gives hope to the patients, their parents, and their caregivers for future improvement in quality of life. CRISPR/Cas9-mediated gene targeting is one of the approaches that shows clear promise for potential breakthroughs, and the study by Jacków et al. has developed a systematic pathway leading from mutation correction in iPSCs to formation of functional skin equivalents that can be applied to patients suffering from RDEB. Theoretically, similar correction approaches could be applicable to other forms of EB with mutations in different genes. While the preclinical approaches developed here look promising, the translation of these findings to clinical treatment still faces critical questions. What size skin grafts can be transplanted to the patients and at what age? How long do the grafts remain functional in terms of skin stability and expression of the corrected protein? While the grafting procedures are largely targeting eroded areas of skin, how can we develop techniques for prevention of blistering and erosions by strengthening the dermal–epidermal junction of the normal-appearing skin in these patients? As the grafting targeted to the skin provides correction only of cutaneous findings, how will the treatment of extracutaneous manifestations be accomplished? Are there immunologic consequences to the application of grafts to the skin of patients, who in some cases have been completely devoid of the corresponding protein? Could complementary cell-, protein-, and gene-based correction approaches in combination improve the efficacy of the treatment? Answers to some of these questions will become available once early clinical trials have been initiated, but there is optimism in the field in light of recent grafting experiments with gene-corrected cells that demonstrated improvement in the functionality of the skin and overall quality of life of patients with EB (25–27).

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