REVIEW

Advances in Gene/Cell Therapy in Epidermolysis Bullosa

Eva M. Murauer,[1] Ulrich Koller,[1] Graziella Pellegrini,[2] Michele De Luca[2] and Johann W. Bauer[3]

[1]Laboratory for Epidermolysis Bullosa Research, EB House Austria, University Hospital of Dermatology, Paracelsus Medical University Salzburg, Salzburg, Austria

[2]Center for Regenerative Medicine "Stefano Ferrari," Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy

[3]University Hospital of Dermatology, Paracelsus Medical University Salzburg, Salzburg, Austria

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In the past few years, substantial preclinical and experimental advances have been made in the treatment of the severe monogenic skin blistering disease epidermolysis bullosa (EB). Promising approaches have been developed in the fields of protein and cell therapies, including allogeneic stem cell transplantation; in addition, the application of gene therapy approaches has become reality. The first *ex vivo* gene therapy for a junctional EB (JEB) patient was performed in Italy more than 8 years ago and was shown to be effective. We have now continued this approach for an Austrian JEB patient. Further, clinical trials for a gene therapy treatment of recessive dystrophic EB are currently under way in the United States and in Europe. In this review, we aim to point out that sustainable correction of autologous keratinocytes by stable genomic integration of a therapeutic gene represents a realistic option for patients with EB. (doi: 10.2302/kjm.2014-0013-RE; Keio J Med 64 (2) : 21–25, June 2015)

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Epidermolysis bullosa (EB) is a genetically heterogeneous skin disease with an estimated 500,000 cases worldwide; it is characterized by chronic fragility and blistering of the skin, mostly induced by minimal trauma.[1,2] The various forms of EB are caused by mutations in genes coding for protein components of the dermal–epidermal junction zone. As a result of the mutations, the proteins produced in skin cells are either functionally impaired or absent. The absence of the protein or impairment of its proper function leads to diminished resistance of the skin to mechanical stress and shearing forces. EB has been classified into four main types according to the level of blister formation in the skin: EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB), and Kindler Syndrome.[3]

Currently, no general curative treatment is available for all EB forms, although novel therapeutic strategies ranging from protein-based[4–6] and fibroblast-based[7–9] therapies to allogeneic bone marrow transplantation[10] hold promise for significant advances. Because a single gene defect is usually responsible for the disease phenotype, one focus lies on development of cDNA and RNA therapies using viral or integrating nonviral strategies.[9,11–18] Many of these studies have targeted primary keratinocytes *ex vivo* and have shown that a phenotypic correction of their adhesion properties is feasible *in vitro* and in skin grafts on mice.

Nonviral strategies using nucleases such as zinc-finger nucleases (ZFN), transcription activator-like effector nuclease (TALEN), or the CRISPR/Cas9 technology for gene editing have the advantage that transgenes can be integrated site-specifically into an endogenous locus of choice.[19] However, these technologies still have to be further investigated to develop methods facilitating the

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Reprint requests to: Eva M. Murauer, PhD, Laboratory for Epidermolysis Bullosa Research, EB House Austria, University Hospital of Dermatology, Paracelsus Medical University Salzburg, Strubergasse 22, 5020 Salzburg, Austria, E-mail: e.murauer@salk.at

identification of off-target effects. In addition, techniques have to be improved in terms of efficiency prior to clinical application in patients.

Thus, *ex vivo* skin gene/cell therapy using human epidermal cells in combination with retroviral cDNA correction is considered to be an attractive means for treatment of EB patients. Although *ex vivo* transplantation therapy cannot fully cure all symptoms of EB, e.g., the involvement of mucous membranes, it has some major advantages over *in vivo* applications. First, keratinocytes can be easily expanded in culture from small skin biopsies, and *ex vivo* protocols can be employed to select epidermal stem cells prior to gene transfer, leading to a long-lasting, if not permanent, and complete correction of the diseased skin. Epidermal sheets made from corrected stem cells can then be grafted back onto the patients following well-established procedures used in the treatment of burn injuries.[20,21]

In this way, several of the major clinical hallmarks of EB can be combated. Once the graft has taken, there is no more loss of body fluids, and this reduces the loss of proteins and peptides. Closure of wounds further improves the defense against microbial infections and might prevent cancer formation, because long-standing wounds are prone to development of the cancer that occurs in junctional and dystrophic variants of EB.[22] Further, the efficiency of gene transfer can be confirmed prior to delivery to the patient.[23–25] A general concern associated with the use of retroviral vectors is the potential for genotoxicity resulting from random integration of the transgene in the genome.[26] Here, *ex vivo* gene therapy offers the advantage that direct administration of vectors to the patient is avoided, and preliminary safety studies can therefore be performed in cell culture.

Thanks to advanced research and improvements in safety profiles, *ex vivo* gene therapies based on retroviral transduction of the full-length cDNA of a truncated protein have become a realistic treatment for EB. In 2006, Mavilio et al. reported the first successful *ex vivo* gene/cell therapy approach in a patient suffering from JEB caused by mutations in the *LAMB3* subunit of the laminin-332 protein.[15] Laminin-332 is made up of three subunit polypeptides encoded by the genes *LAMA3*, *LAMB3*, and *LAMC2*. Most patients with JEB harbor mutations within these genes. JEB can be divided into two clinical forms: Herlitz (H-JEB) and non-Herlitz (nH-JEB). A diagnosis of H-JEB is accompanied by the death of the patient within the first year of life, whereas patients with nH-JEB suffer no negative effect on their overall lifespan. Generally, a genotype/phenotype correlation is apparent in both JEB variants. In H-JEB, premature termination codons in both alleles of one of the laminin-332 genes are the reason for the severe phenotype of the disease. In contrast missense mutations in genes encoding for laminin-332 are commonly the cause of nH-JEB.[2,27]

In the first phase I/II clinical trial for junctional EB, epidermal stem cells from a nH-JEB patient were genetically corrected using a retroviral vector expressing full-length *LAMB3* cDNA. Epidermal grafts created from the corrected cells were transplanted onto the patient's legs, resulting in the development of fully functional and non-blistering skin.[15] The study revealed that the survival of a small number of skin stem cells transplanted onto wounded sites was sufficient to restore normal skin function. A follow-up period of more than 8 years has demonstrated sustained synthesis of the target *LAMB3* protein, together with firm adhesion of the epidermis. There has been no evidence of blistering, inflammation, tumor formation, or immune response in the grafted area.[28]

In July 2014 we adopted the same gene therapy protocol for an Austrian JEB patient in collaboration with the Center for Regenerative Medicine in Modena, Italy (**Fig. 1A**), for which we got approval from the Austrian authorities in 2011. This is the second use of *ex vivo* skin gene therapy targeting autologous epidermal stem cells after that of Mavilio et al.; it is also the first good manufacturing practice (GMP)-guided *ex vivo* gene/cell therapy for junctional EB.[15] Five skin sheets (**Fig. 1B**), each measuring 5 × 7 cm, were grafted onto wounded areas on the patient's thighs. We are currently evaluating the patient according to the protocol in the 1-year post-transplantation follow-up.

Two scientifically similar approaches developed by a research consortium in Europe and in the USA are underway to test the effectiveness of gene therapy for severe recessive dystrophic epidermolysis bullosa (RDEB).[16,29] Professor Alfred Lane and his team in Stanford University, California, have recently started a clinical trial with one RDEB patient using retroviral-mediated transfer of full-length cDNA encoding type VII collagen. In a similar way to our approach, six 5 × 7 cm sheets prepared of gene-corrected epidermal keratinocyte epidermis were grafted onto prepared wounds on the patient's arms. At the first follow up 30 days after transplantation, the grafts had taken well and the grafted areas did not show blister formation. Further, the initially very low type VII collagen expression in the patient's skin had increased in the grafted areas.

The outcome of these pilot studies will reveal whether patient-derived keratinocytes that are gene corrected *in vitro* can reconstitute the normal adherence function of EB skin in the long term without severe side effects. The major challenge lies in the long-term expression of the transgene, because transient amplifying cells of the epidermis undergo self-renewal every 3–4 weeks. To achieve a long-lasting therapeutic effect, epidermal stem cells have to be targeted. As a result of continuous wounding and scarring, EB patients harbor a very limited number of epidermal stem cells, and this number might decrease with the age of the patient.

Another issue regarding safety in cutaneous gene therapy trials that must be addressed is the potential risk of

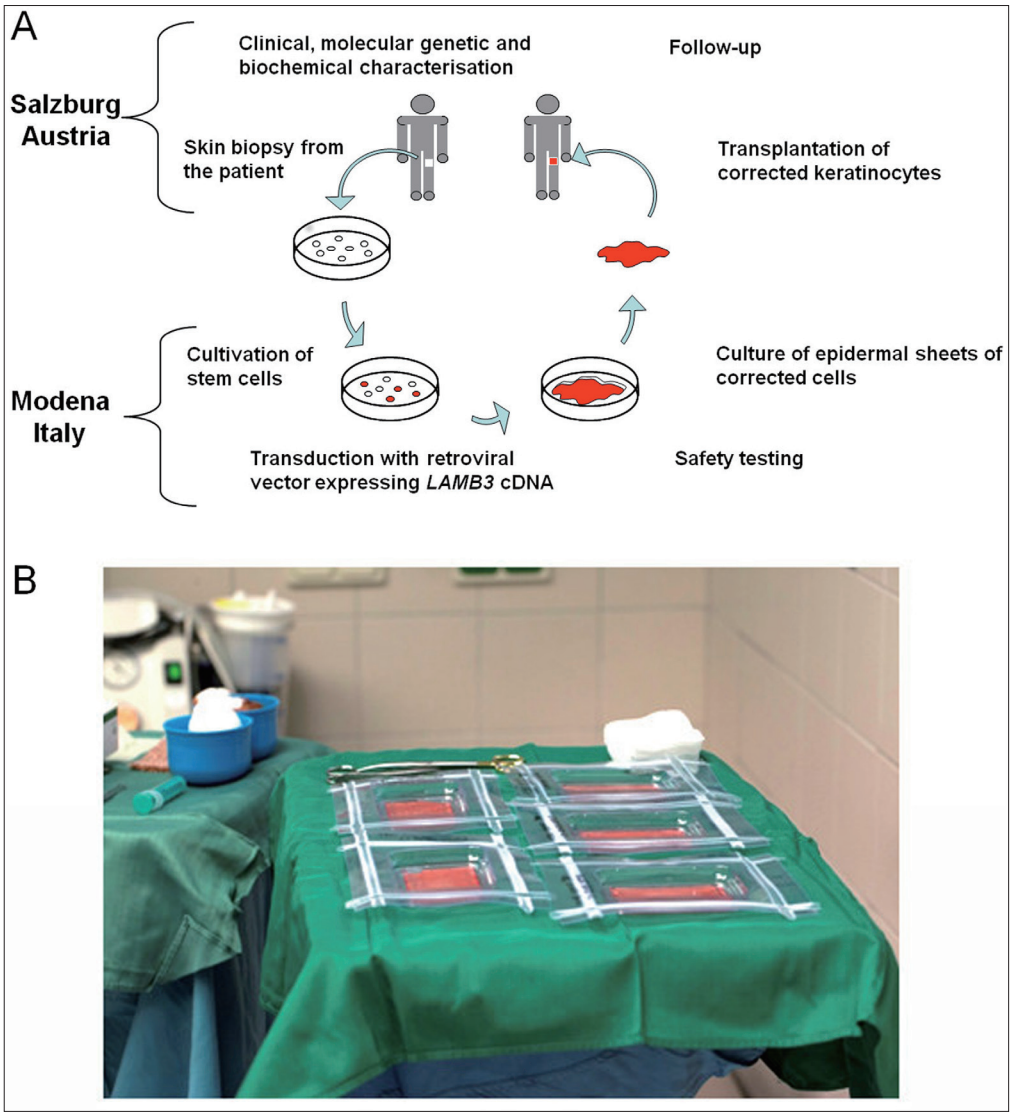

Fig. 1 (A) Principle steps of the GMP-guided *ex vivo* gene/cell therapy for junctional EB. (B) Five skin sheets, prepared from gene-corrected keratinocytes, before grafting onto the patient.

adverse immunological responses against the newly expressed protein. This eventuality has to be considered, especially in patients with null-mutations expressing no residual protein.[23,30] Studies are underway to answer the outstanding question of possible antigen-specific immune responses as well as the development of protocols to prevent such reactions prior to therapy (Gratz, personal communication).

Some individuals with EB, however, have skin patches that do not blister and show increased expression of the protein that is not expressed in most areas of their skin.[31,32] This phenomenon, termed revertant mosaicism, or natural gene therapy, might prevent immune reactions against the newly synthesized protein resulting from gene therapy. Furthermore, the presence of reverted "healthy" stem cells in the patient's own skin can be exploited to develop a cell-based therapy without the need of gene correction. However, attempts to expand revertant keratinocytes in culture from a patient with nH-JEB, create a skin graft, and transplant it back onto skin wounds were not successful because of the small number of revertant cells in the skin graft.[33] Therefore, protocols need to be developed to efficiently select revertant keratinocytes in culture to produce grafts containing adequate numbers of revertant stem cells to secure functional repair of the skin.

Thus, the grafting of gene-corrected skin stem cells is still the most promising approach for permanent treatment of chronic wounds in EB patients. The long-term results of the above-described ongoing studies will pave

the way to make *ex vivo* gene/cell therapy a realistic option for patients with EB and other genetic skin diseases.

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