From Marrow to Matrix: Novel Gene and Cell Therapies for Epidermolysis Bullosa

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Epidermolysis bullosa encompasses a group of inherited connective tissue disorders that range from mild to lethal. There is no cure, and current treatment is limited to palliative care that is largely ineffective in treating the systemic, life-threatening pathology associated with the most severe forms of the disease. Although allogeneic cell- and protein-based therapies have shown promise, both novel and combinatorial approaches will undoubtedly be required to totally alleviate the disorder. Progress in the development of next-generation therapies that synergize targeted gene-correction and induced pluripotent stem cell technologies offers exciting prospects for personalized, off-the-shelf treatment options that could avoid many of the limitations associated with current allogeneic cell-based therapies. Although no single therapeutic avenue has achieved complete success, each has substantially increased our collective understanding of the complex biology underlying the disease, both providing mechanistic insights and uncovering new hurdles that must be overcome.

Received 1 December 2014; accepted 11 March 2015; advance online publication 21 April 2015. doi:10.1038/mt.2015.47

BUTTERFLY CHILDREN

Children born with recessive dystrophic epidermolysis bullosa (RDEB), a severe inherited disorder of connective tissue, are often referred to as “butterfly children,” because the fragility of their skin can be compared to the delicateness of a butterfly wing. Individuals with RDEB endure chronic pain and daily challenges brought on by severe cutaneous and mucosal blistering, joint contractures, pseudo-dysaesthely, corneal abrasions, esophageal strictures, and impaired wound healing that contribute to significant morbidity and a shortened life span.¹–⁴ At present, palliative care is the only option widely available to RDEB patients, and it is limited to intricate and laborious bandaging, pain and itch control, and management of bacterial and fungal infection. Even with proper care, RDEB patients often develop chronic cutaneous infections and are prone to developing aggressive squamous cell carcinomas later in life.⁵–⁶

Recessive dystrophic epidermolysis bullosa (RDEB) is inherited in autosomal-recessive fashion.⁷,⁸ Generalized severe (GS) RDEB, the physical manifestations of the most severe form, is caused by mutations to the gene encoding type VII collagen (C7), COL7A1. Mechanistically, C7 is essential to the formation of anchoring fibrils that secure the epidermis to the underlying dermis at the dermal-epidermal junction.⁹–¹² In the absence of functional C7, these layers of skin do not adhere properly, and severe blistering occurs after minimal trauma. Subsequent wound healing is also impaired.¹³

Over the past several years, exciting progress has been made in the treatment of RDEB. In particular, allogeneic hematopoietic cell transplantation (HCT) has shown the ability to partially correct the systemic manifestations of RDEB-GS.¹⁴ Although these pioneering efforts are changing the collective perspective on RDEB treatment, the significant morbidity and mortality associated with HCT, along with the inability of other therapies to treat systemic symptoms, necessitate the development of novel therapies that overcome these limitations while still providing durable, effective amelioration of systemic RDEB pathology.

REPAIRING THE MATRIX WITH MARROW

The earliest efforts to treat the cutaneous manifestations of RDEB with cellular therapy employed intradermal injections of allogeneic fibroblasts and mesenchymal stromal cells (MSCs).¹⁵–¹⁹ Although these avenues of treatment show promise for restoring localized C7, they are not able to address the underlying systemic manifestations of epidermolysis bullosa (EB). Furthermore, as these cell populations do not contain self-renewing stem cells, the benefits are likely to be transient.

Hematopoietic cell transplantation (HCT) is the most widely employed stem cell therapy and the only one capable of providing durable and systemic delivery of donor cells upon transplantation.²⁰,²¹ Although HCT carries a proven track record in the treatment of hematological diseases and genetic enzymopathies, employing HCT to treat a disease of the extracellular matrix (ECM) initially flew in the face of prevailing wisdom. Nonetheless, well-documented examples of donor cell chimerism in the skin and mucosal epithelia of transplant recipients suggested that HCT could prove beneficial to patients with RDEB.²²–²⁵ Although cells of hematopoietic origin play important roles in mediating the inflammatory response to injury, evidence is accumulating that suggests they have a more direct role in skin repair.²⁶–²⁷

Initial studies involving the transfer of marrow cells from 8-week-old green fluorescent protein-positive mice into the...
circulation of day 13 col7a1−/− embryos (embryonic bone marrow transplant (E-BMT)) demonstrated the capacity of bone marrow cells to contribute dermal fibroblasts and prolong survival.28 As the fetal microenvironment is believed to foster cellular plasticity, it was not immediately clear that the results obtained via E-BMT could be reproduced in postnatal recipients. To address this question, subsets of marrow-derived cells were transplanted into neonatal col7a1−/− mice, with the result that bone marrow-derived cells migrated to the skin, mediated C7 deposition, and prolonged the survival of col7a1−/− mice.29 These preclinical studies paved the way for the first human trials using HCT to treat RDEB-GS. The children with RDEB-GS also exhibited significant donor chimerism in the skin after HCT, which coincided with C7 deposition at the cutaneous basement membrane and improvement in skin integrity and wound healing as seen in an image from clinical trials showing phenotypic correction and C7 deposition (Figure 1).14 While these results are undoubtedly promising, a number of questions remain regarding the cell type or types responsible for mediating the deposition of C7 after HCT.

Bone marrow-derived fibroblast-like cells capable of migrating to wounds and depositing ECM proteins were identified as early as 1994.30 These so-called fibrocytes are bone marrow-originating cells that express both hematopoietic surface antigens, such as CD34 and CD45, as well as a number of ECM proteins.31 Based on their shared expression of several monocyte and macrophage surface antigens, fibrocytes are thought to arise from a subpopulation of monocytes, and their formation can be promoted by the presence of inflammatory cytokines.32 Upon injury, fibrocytes are involved in modulating the inflammatory response,33 promoting tissue remodeling and angiogenesis,34 and inducing α-smooth muscle actin-positive myofibroblasts from fibroblasts via the secretion of paracrine factors such as platelet-derived growth factor (PDGF) and transforming growth factor-β1.35 Interestingly, lineage-tracing studies in a hematopoietic-specific Vav1-cre model identified a previously undescribed fibrocyte population lacking prototypical hematopoietic surface markers (such as CD45 and CD11b), suggesting that a subset of early blood progenitors is able to give rise to wound-resident cells that may not be readily discernable from dermal fibroblasts by simple surface antigen phenotype.36 It is yet to be explored whether donor-derived fibrocyte populations play a role in C7 deposition after transplant.

Beyond the relatively well-defined fibrocyte, it has been shown that bone marrow-derived cells are capable of engrafting into hair follicles and undergoing transdifferentiation into keratinocytes after transplantation.37,38 Although transdifferentiation of hematopoietic progenitors has been suggested,23,39 in the studies reporting this phenomenon, the transplanted population was either not well defined or too heterogeneous to definitively prove a hematopoietic origin. Indeed, when highly purified hematopoietic stem cells (HSCs) were transplanted, there was little evidence of transdifferentiation, indicating that any such event is extremely rare in vivo.40 It is unknown whether the active wounds and consequent inflammatory milieu present in RDEB-GS patients receiving HCT create an environment permissive to the transdifferentiation of hematopoietic progenitors.

There is stronger evidence to suggest that non-hematopoietic bone marrow-derived cells such as MSCs are capable of integrating into diverse tissues and are responsible for generating keratinocytes present in epithelial tissues after bone marrow transplant (BMT).41–43 Not surprisingly, MSCs share a similar gene-expression profile with dermal fibroblasts, including the expression of ECM proteins such as C7.44 Recently, a previously undescribed bone marrow-resident population identified as lineage marker negative, PDGF receptor α-positive (Lin-PDGFRα+) was shown to home to skin grafts and generate functional keratinocytes capable of depositing C7.45 Importantly, the authors identified the high-mobility group box protein HMGB1 as playing a key role in the homing of bone marrow-derived Lin-PDGFRα+ cells to sites of cutaneous injury. Follow-up studies by the same group demonstrated that these lineage marker (CD3e, CD11b, B220, TER-119, Ly-6G, and Ly6C) negative, PDGFα+ mesenchymal cells homed to RDEB skin grafted onto wild-type mice in a SDF-1α/CXCR4 axis-dependent
manner, suggesting that manipulation of either the stromal-derived factor 1 or HMGB1-signaling pathways could be used to improve trafficking of donor cells to the skin posttransplant.66

CELLS MADE TO ORDER: THE PROMISE OF INDUCED PLURIPOTENCY AND CELLULAR PLASTICITY
The demonstration that somatic cells could be reprogrammed to pluripotency has opened new doors in the field of regenerative medicine.47,48 Induced pluripotent stem cells (iPSCs) represent, in principle, a nearly inexhaustible source of somatic cell types for tissue regeneration and *ex vivo* modeling of genetic disease (Figure 2).49–55 Skin cells from RDEB patients, as well as the closely related junctional form of EB (JEB), can be reprogrammed to pluripotency, thereby providing new tools with which to investigate the mechanisms underlying EB pathology *in vitro*.52,53 Perhaps, the most direct route to iPSC-based cell therapies for RDEB comes via the frequent occurrence of somatic mosaicism in RDEB patients.54,55 Keratinocytes from these healthy patches of skin can be used directly,56–58 or they can be reprogrammed to pluripotency and subsequently used as a source of autologous cells for therapeutic intervention.59,60 These naturally gene-corrected iPSCs can be differentiated into C7-producing fibroblasts and keratinocytes, as well as three-dimensional skin equivalents that are well suited for localized treatment of chronic wounds.61–65 Ideally, iPSC could be utilized to generate epidermal stem cells capable of regenerating all components of adult skin,66 and indeed progress is being made in this area.67 Nevertheless, as skin-resident cell types derived from iPSC will not be sufficient to address the systemic pathology associated with RDEB, it is necessary to identify alternative strategies to overcome this issue.

Although iPSC can be readily differentiated into nearly all mature hematopoietic lineages *in vitro*, derivation of HSC capable of long-term reconstitution has not yet been demonstrated.68–72 However, two recent studies demonstrated the formation of HSC from iPSC within teratoma, suggesting that this conversion is possible if the appropriate environmental cues are present.73,74 The identification of new small molecule modulators of HSC differentiation and expansion offers fresh opportunities in the efforts to derive HSC from iPSC.75–79 Intriguingly, several studies have begun to identify methods for direct conversion of somatic cells into hematopoietic stem and progenitor cells, bypassing pluripotency altogether.80–82 While undoubtedly exciting, these methods of transdifferentiation require genetic manipulation that is not suitable for translational efforts, although future modifications that do not rely on multiple integrated vectors could overcome these issues. In the interim, methods for the generation of iPSC-derived non-stem subsets of C7-producing hematopoietic cells could prove beneficial, as these cells could conceivably provide systemic, albeit transient, delivery of C7.

Another option to address the systemic manifestations of RDEB with iPSC could come via the delivery of iPSC-derived non-hematopoietic MSCs. Although strategies for the differentiation of iPSC to MSC have been reported,83–85 it is unclear whether the iPSC–MSC described in these studies are similar to the mouse Lin–PDGFRα+ mesenchymal cells in their capacity to migrate to wounds and mediate C7 deposition. Further characterization of iPSC–MSC in the setting of RDEB is warranted and, if necessary, modified differentiation protocols to generate cells tailored for this specific purpose should be developed.

To realize the full benefit of reprogramming technology in RDEB, it needs to be combined with gene correction—such as with viral-mediated gene addition or with gene-editing strategies—to allow customized autologous cellular therapies tailored to the needs of each individual patient.

**GENE THERAPY: CUTTING TO THE CURE**
Despite the long and successful track record of HCT in the treatment of genetic disease,86–89 there remain significant limitations to the procedure. The requirement for a human leucocyte antigen-matched donor prevents the use of HCT in some cases, and even when a matched donor is available, the procedure itself carries a significant risk of morbidity and mortality.90–93 Gene-correction and reintroduction of autologous cells would overcome many of the limitations associated with allogeneic cell therapies. A number of viral- and transposon-based vectors have been used to reintroduce wild-type C7 into RDEB and JEB cells.94–97 Long-term follow-up (6.5 years) of a patient who received retrovirally corrected epidermal stem cell grafts for JEB shows restoration of skin integrity and no clinically obvious adverse effects.98 Although several recent reports have demonstrated the safety of lentiviral- and retroviral-mediated gene therapies in humans,99–102 there remains appreciable trepidation due to previous adverse events in trials using retroviral vectors.103 Beyond the risk of insertional mutagenesis, the long-term effects of supraphysiological expression of ECM proteins are unknown. Considering the evidence that perturbation of the expression of ECM proteins can impact the cellular microenvironment,104 it might be preferable to develop gene-editing strategies that correct the causative mutation *in situ*. Toward this end, Melo et al.105 report successful homologous recombination (HR)-mediated gene correction of a mutation in

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**Figure 2** Potential strategies to implement next-generation therapies in the treatment of recessive dystrophic epidermolysis bullosa. In this diagram, mutant or naturally gene-reverted cells are isolated from the patient, and mutant cells are gene corrected. These cells are then either used directly or reprogrammed in to induced pluripotent stem cells (iPSCs). If gene correction of primary cells is not possible, the gene correction step can be performed at the iPSC stage. The gene-corrected iPSCs are subsequently differentiated into hematopoietic stem/progenitor cells and/or mesenchymal stem cells for systemic administration, or keratinocytes and/or fibroblasts for localized application.
Figure 3 Teratoma immunofluorescence shows the dermal-epidermal junction indicated with white arrows. The immunofluorescence markers are as follows: blue, 4,6-diamidino-2-phenylindole (DAPI) nuclear stain; green, cytokeratin 5; and red, type VII collagen. Images are representative images from at least three animals. Antibody staining was performed from a single master mix on the same day using identical microscopy settings. Data taken from ref. 109.

the LAMAS3 locus in primary human keratinocytes using a highly recombinogenic adeno-associated virus (AAV-DJ) vector, thus limiting the risk of insertional mutagenesis.

The rapid advancement in designer nuclease technologies has ushered in an era where HR in human cells is not only possible, but also reaching the efficiency required for therapeutic application.106–108 As a proof of concept, transcription activator-like effector nucleases were utilized to mediate homologous repair of COL7A1 in patient fibroblasts with minimal off-target activity (Figure 3).109 These gene-corrected fibroblasts were selected and subsequently reprogrammed to iPSC and shown to be capable of generating three-dimensional skin structures with appropriate deposition of C7 protein. More recently, the demonstration of HR-mediated gene correction in human-repopulating HSC has opened doors to autologous HCT for RDEB.110

Moving forward, the clustered regularly interspaced short palindromic repeats and associated nucleases (CRISPR/Cas) system should allow for expedited reagent generation, making it highly suited to gene-correction strategies for the large number of unique mutations associated with EB.111–113 While the safety profile of designer nuclease technologies is still being assessed, future modifications will likely enhance efficiency and specificity, and as such these reagents might represent the future of gene therapy for RDEB.

Although recent proof-of-concept studies using both iPSC and gene-editing technologies demonstrate their immense potential to treat RDEB, a substantial effort will be required in order to move these strategies from the bench to the bedside. Toward this end, methods for reprogramming and gene modification under good manufacturing practices have been demonstrated.114 Subsequent efforts should focus on thorough characterization of gene-corrected iPSCs and the safety of their derivative cell types in vivo.

THE FUTURE OF REGENERATIVE MEDICINE: IMPLICATIONS FOR RDEB TREATMENT

Simultaneous and significant advances in the fields of cellular reprogramming and genome engineering have set the stage for entry into an era of revolutionary advances in regenerative medicine, a field in which the delicate balances between the genome, the cell, and the patient must be carefully and methodically explored. This is particularly relevant to the treatment of RDEB, where the complex and systemic manifestations of the disease will likely require combinatorial therapies in order to achieve the ultimate goal.115 For example, although HCT provides systemic long-term benefits and creates immune tolerance to donor C7 protein, some individuals with severe, chronic wounds may further benefit from the localized delivery of skin-resident cells or skin grafts to augment healing. Fibroblasts and/or keratinocytes from the HCT donor could be employed to enhance repair in these situations, as even microchimerism has been shown to mediate immunological tolerance and acceptance of grafts.116 Further, for corneal abrasions, one of the most debilitating clinical features of RDEB-GS, limbal stem cell populations could prove beneficial.117,118 Eventually, autologous gene-corrected iPSC could be used to generate all of the aforementioned cell types on demand. This could be an ideal situation, as only a single gene-modification step would be necessary and only the iPSC clones with a validated safety profile and lack of off-target mutations would be retained for use.114 Although this review has focused primarily on cell- and gene-based therapies for RDEB, future treatment strategies will also likely incorporate the use of recombinant C7 alone or boost the therapeutic effects of cell-based interventions.119–121 Pharmacological interventions could also prove beneficial, whether via the restoration of wild-type C7 expression from premature termination signals122 or via the promotion of narrow stem cell mobilization to cutaneous wounding post-BMT.123 Which combinations of these therapies will ultimately result in a cure for RDEB is yet to be determined; however, it is certain that the lessons learned along the way will have a widespread impact on the future of regenerative medicine.

REFERENCES


