Gene editing toward the use of autologous therapies in recessive dystrophic epidermolysis bullosa

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Recessive dystrophic epidermolysis bullosa (RDEB) is a disease caused by mutations in the *COL7A1* gene that result in absent or dysfunctional type VII collagen protein production. Clinically, RDEB manifests as early and severe chronic cutaneous blistering, damage to internal epithelium, an increased risk for squamous cell carcinoma, and an overall reduced life expectancy. Recent localized and systemic treatments have shown promise for lessening the disease severity in RDEB, but the concept of ex vivo therapy would allow a patient's own cells to be engineered to express functional type VII collagen. Here, we review gene delivery and editing platforms and their application toward the development of next-generation treatments designed to correct the causative genetic defects of RDEB. (Translational Research 2016;168:50–58)

Abbreviations: AAV = adeno-associated virus; AF = anchoring fibril; BMZ = basement membrane zone; C7 = type VII collagen protein; CRISPR = clustered regularly interspaced short palindromic repeats; DEJ = dermal-epidermal junction; gRNA = guide RNA; HCT = hematopoietic cell transplantation; iPSC = induced pluripotent stem cell; LV = lentiviral; MSC = mesenchymal stem cell; PAM = protospacer adjacent motif; PTCs = premature termination codons; RDEB = recessive dystrophic epidermolysis bullosa; RV = retroviral; RVDs = repeat variable diresidues; SCC = squamous cell carcinoma; SIN = self-inactivating; TALEN = transcription activator–like effector nuclease; ZFN = zinc-finger nuclease

INTRODUCTION

Epidermolysis bullosa (EB) represents a heterogeneous group of diseases characterized by errors in genes that encode the structural components of the skin. Clinical manifestations of EB primarily involve chronic blistering and poor wound healing of cutaneous and mucosal surfaces, with the severity of disease dictated by the specific underlying genetic mutation and degree of protein dysfunction. Although there are more than 20 subtypes of EB, one of the most severe forms is generalized severe recessive dystrophic epidermolysis bullosa (RDEB).[1] RDEB results from biallelic loss-of-function mutations within the collagen type VII gene (*COL7A1*) that lead to absent or deficient production of normal collagen type VII protein (C7). In healthy skin, keratinocytes and dermal fibroblasts secrete procollagen VII, which is processed into C7 and assembled into anchoring fibrils (AFs).[2] AFs provide the main structural connection between the papillary dermis and epidermal basement membrane zone (BMZ), thus providing a "biologic Velcro" at the dermal-epidermal junction (DEJ).[3] In patients with RDEB, dermal-epidermal integrity is compromised by the diminished presence of functional C7. This fundamental defect produces the characteristic RDEB clinical presentation of chronic and severe skin blistering; mitten deformities of the hands and feet; corneal

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erosions; oral, esophageal, and anal strictures; with variable involvement of the heart, kidneys, and bones.[4,5]

EPIDEMIOLOGY, GENETICS, AND HISTORICAL APPROACH TO TREATMENT

Affecting approximately 1 of 1,000,000 newborns in the United States, RDEB is often evident at birth and is associated with a life expectancy of 30 years in severe cases, with milder phenotypes exhibiting a median survival of 55–65 years.[6,7] In addition to severe pain and frequent failure to thrive, patients are at exceptionally high risk (≈80%) for developing aggressive forms of squamous cell carcinoma owing to the chronic remodeling and increased cell proliferation occurring at sites of mucocutaneous blistering.[4]

Genetics of the *COL7A1* locus and processing of C7 protein. The human *COL7A1* gene, located on chromosome 3p21, encompasses 32 kb of genomic DNA and contains 118 exons. The resultant messenger RNA (mRNA) transcript is 8.9 kb in length and is translated into a proα1 (VII) polypeptide composed of 2944 amino acids. Exons 1–28 of the mRNA transcript represent the N-terminal "noncollagenous" (NC-1) domain and exons 112–118 represent the C-terminal "noncollagenous" (NC-2) domain, whereas the intervening central "collagenous" triple helical domain contains varying stretches of bases coding for Gly-X-Y repeat sequences with disrupting noncollagenous regions throughout. Thus, each proα1 (VII) chain contains a central collagenous triple helical domain flanked by the NC-1 and NC-2 domains. Three proα1 (VII) chains polymerize to form a procollagen VII homotrimer, the secretory product of C7-producing cells. Procollagen VII homotrimers undergo extracellular processing to yield the functional C7 protein product, which assembles with another C7 molecule at the carboxy-terminal region to form antiparallel dimers with NC-1 domains facing opposing ends of the dimer. The NC-1 domains within each dimer are involved in forming adhesive bonds with extracellular matrix proteins (eg, collagen IV) that help stabilize AFs to the papillary dermis and BMZ of the epidermis. The triple helical regions in each dimer form cross-bonds with neighboring homotrimers, and the NC-2 domains are proteolytically cleaved after dimerization and before AF formation.[8-10] Antiparallel dimers then assemble to form AFs, which play a crucial role in the structural integrity of the DEJ.

Patients with RDEB are often compound heterozygotes, with biallelic premature termination codons (PTCs) being prevalent in the generalized severe subtype of RDEB (previously called Hallopeau-Siemens RDEB).[8,11-13] PTCs result from nonsense, frameshift, or splice-site mutations that cause truncation of the mRNA transcript and a nonfunctional protein that precludes normal AF formation. Patients with less severe subtypes (eg, generalized intermediate RDEB) typically have a PTC on one allele and a non-PTC mutation (eg, missense, deletion, insertion) on the other allele, which results in a partially functional polypeptide.

Clinical management of RDEB has classically involved measures aimed at palliative wound care and pain management. More recently, experimental therapies have been developed seeking to provide functional C7 protein at sites of involvement. The C7 product has been delivered either directly as a recombinant protein, or via introduction of allogeneic donor cells that synthesize the protein in vivo. Both approaches have shown some benefits, albeit nonuniform ones, in preclinical models and phase I human studies. The inability to achieve a complete therapeutic response provides impetus for developing more effective treatment options. These include improvements to existing recombinant protein and allogeneic cellular therapies, as well as genetically corrected autologous cellular platforms for C7 protein delivery.

Recombinant protein therapies. The use of human recombinant C7 protein as a potential treatment for patients with RDEB was first described in 2004 with the demonstration that intradermal injections of the protein could localize to the BMZ and produce functional AFs in murine and human RDEB skin models.[14] Owing to the relatively long physiological half-life of the molecule, C7 was shown to be present at the DEJ throughout the 3-month observation period. Subsequent studies extended these findings into a *Col7a1*$^{-/-}$ murine model, showing similar benefit.[15] Although these mice developed antibodies against the C7 protein, this did not preclude formation of AFs and improvement of the disease phenotype. Given the presumed advantage of a systemic intravascular delivery method—which could theoretically provide recombinant C7 protein to all mucocutaneous lesions rather than being restricted to injection sites—this same group provided evidence in 2013 that intravenous infusions of recombinant C7 could home to the DEJ and form AFs in an RDEB skin model.[16] Although these preclinical studies hold translational application and provide proof-of-concept for recombinant protein-based therapies, the likely need for repeated injections, with the associated costs and the possibility of anti-C7 antibody formation, may limit the efficacy of this approach.[17]

Allogeneic cellular therapies. The principle behind allogeneic cellular therapies in RDEB is that by introducing a wild-type donor cell capable of producing normal C7 protein, improved DEJ stability can be

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