Drugs of the Future 2009, 34(7): 575-578

CCC: 0377-8282/2009
DOI: 10.1358/dof.2009.34.7.1394149

INHERITED EPIDERMOLYSIS BULLOSA

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CONTENTS

ABSTRACT

Inherited epidermolysis bullosa (EB) is characterized by a lack of anchoring filaments that facilitate adhesion of the epidermal and dermal layers of the skin. This results in significant blister or sore formation in response to minor mechanical trauma, which has a detrimental impact on patient quality of life. While the standard of care tends to focus on wound healing/hygiene, a further understanding of the molecular and genetic events underlying skin separation has led to the identification of alternative treatment methods that may provide novel solutions for EB. This review focuses on the more up-to-date publications emerging from EB research, including advances in gene therapy, protein therapy, cell therapy, tissue engineering, the use of natural products and pharmacotherapeutics, highlighting ongoing clinical trials in the field.

INTRODUCTION

Epidermolysis bullosa (EB) is a group of autosomal inherited bullous disorders (inherited in both recessive and dominant manners) characterized by blister formation in response to minor mechanical trauma. People born with EB lack the anchoring filaments that facilitate adhesion of the outer (epidermis) and inner (dermis) layers of skin together. Therefore, any action that creates friction between the layers (like rubbing or pressure) will create blisters and painful sores. Several types of EB have been distinguished according to skin morphology and four main subtypes have been classified: dystrophic epidermolysis bullosa (DEB; sublamina densa basement membrane zone [BMZ] separation); epidermolysis bullosa simplex (EBS; intraepidermal skin separation); hemidesmosomal epidermolysis bullosa (HEB; blistering at the hemidesmosomal level in the most superior aspect of the BMZ); and junctional epidermolysis bullosa (JEB; skin separation in lamina lucida or central BMZ) (1-3).

According to a National EB Registry report published in 1999, 50 cases occur for every 1 million live births in the U.S. Complications can arise from blister formation in severe forms of the disorder, as scarring may cause deformities, fusion of the fingers and toes, and contracture deformities (for example, at the fingers, elbows and knees). Furthermore, generalized blistering caused by any subtype may be complicated by secondary infection, sepsis and death. If the mouth and esophagus are involved, blistering and scarring can lead to feeding and swallowing difficulties. The more severe forms of EB (HEB, JEB and DEB) may produce significant multiorgan involvement, and therefore more serious complications. In patients with EB that survive childhood, the most common cause of death in those with recessively inherited EB is metastatic squamous cell carcinoma (SCC) (4).

Advances in the molecular characterization of EB have facilitated the development of new tools for the rapid diagnosis of subtype-specific EB, for example mutations in the type VII collagen gene (*COL7A1*) (5-7). While the standard of care tends to focus on wound care, a further understanding of the BMZ and the genes and proteins responsible for its components has led to the identification of new treatment methods that may provide novel solutions for EB. Interestingly, a recent review of randomized, controlled trials for inherited EB illustrated little progress for traditional pharmacotherapies such as the oral tetracyclines or topical interventions (8). This article provides an up-to-date summary of the recent therapeutic approaches for inherited EB.

GENE THERAPY

Advances in the research of genetic mutations associated with EB subtypes have identified a wealth of targets for gene therapy.

Researchers have hypothesized that if EB stem cells are removed from a patient, genetically corrected and returned to a blistered area, they will have a selective growth advantage and the ability to restore normal functioning of the epidermis. Along these lines, investigators from Baylor College of Medicine have developed lentiviral vectors carrying the wild-type keratin-14 (*KRT14*) allele and

also vectors encoding small interfering RNA (siRNA) constructs that specifically cleave mutant *KRT14* alleles (in a bid to restore wild-type:mutant allele ratios) (10). Researchers from the University of Washington have also developed an adeno-associated virus (AAV) vector to target the mutated keratin allele. This technique works by inserting a selectable gene into a 5′ exon, allowing the cell to form a normal intermediate filament network (11).

Italian researchers have provided the first clinical evidence for successful transplantation of epidermal stem cells genetically modified using transcriptionally targeted gamma-retroviral vectors to overcome deficient components of the epidermal–dermal junction. Epidermal grafts were cultured following the transduction of epidermal stem cells from an adult patient affected by deficient beta 3 chains of human laminin 5 (*LAMB3*) JEB with a retroviral vector expressing *LAMB3* cDNA (encoding laminin 5 beta 3). The grafts generated and surgically transplanted remained stable up to follow-up (1 year) on the patient's leg area, with synthesis and proper assembly of normal levels of functional laminin 5 and the development of a firmly adherent epidermis without blisters, infections, inflammation or immune response (12).

Concerns over the use of a Moloney leukemia virus (MLV)-derived retroviral vector, which can have associated genotoxicity problems, in this study led to the recent characterization of an alternative gene transfer strategy. This new approach is based on self-inactivating (SIN) or long terminal repeat (LTR)-modified lentiviral vectors, in which transgene expression is under the control of different combinations of promoter–enhancer elements derived from the *KRT14* gene. This technique facilitated the generation of normal human skin in vivo, the reconstitution of normal levels of *LAMB3* in *LAMB3*-deficient keratinocytes and restoration of adhesion properties of epidermal stem cell-derived skin (13). The technique of genetically correcting skin has also been successfully demonstrated by French researchers using SIN vectors, utilized to introduce *COL7A1* cDNA into epidermal stem cells and dermal fibroblasts (14).

Japanese researchers are investigating the potential for antisense oligoribonucleotide (AON) therapy. These studies have generated an AON which modulates splicing at exon 70 of the *COL7A1* gene (a premature termination codon identified in Japanese DEB patients with mutation 5815delC). In vitro introduction of this AON into DEB keratinocytes harboring 5815delC is associated with restoration of type VII collagen expression, with further evidence for AON-induced type VII collagen at the BMZ in rats grafted with DEB keratinocytes and fibroblasts (15).

Researchers in Austria have investigated the potential of targeting the mutated plectin-1 gene (*PLEC1*), which underlies EBS with late-onset muscular dystrophy, using spliceosome-mediated RNA *trans*-splicing (SMaRT). Plectin is a widely expressed cytomatrix component involved in the attachment of the cytoskeleton to the plasma membrane. SMaRT utilizes endogenous splicing machinery to recombine two independent RNA molecules by *trans*-splicing, replacing the disease-causing portion of a pre-mRNA with an engineered sequence in an exon-specific manner. In cells derived from a compound heterozygous patient with a 3-bp insertion in exon 9 (1287ins3) and a nonsense mutation in exon 31 (Q1518X) of the *PLEC1* gene, transient transfection of EBS-MD fibroblasts with a 5′ pre-*trans*-splicing molecule encoding wild-type exons 2-9 led to specific replacement of the mutated 5′ portion of the endogenous *PLEC1* transcript through *trans*-splicing. This treatment reduced the levels of mutant mRNA and restored a wild-type pattern of plectin-1 expression. When EBS-MD fibroblasts were transfected with retroviral constructs, the level of full-length plectin-1 protein in the corrected fibroblasts increased by 58.7% (16, 17).

Further studies have also targeted the *COL7A1* gene via SMaRT technology. In primary and immortalized keratinocytes from a recessive DEB patient carrying two heterozygous nonsense mutations in *COL7A1* exons 14 and 104 (which induce collagen VII deficiency), retroviral transduction with a 3′ pre-*trans*-splicing molecule encoding wild-type *COL7A1* exons 65-118 restores functional collagen VII (18).

PROTEIN THERAPY

Protein therapy, which focuses on the therapeutic restoration of the disrupted protein itself (in place of genetic manipulation to restore protein function), is also under investigation in EB. Researchers from Thomas Jefferson University in the U.S. are working towards the replacement of the mutant chain of laminin 5 in JEB. Studies in human keratinocytes isolated from Herlitz-type JEB patients have shown that restoration of *LAMB3* via cytoplasmic delivery of recombinant full-length polypeptides can re-establish cellular adhesion (19).

Researchers from the U.S. and China have also demonstrated the potential for intradermal injection of recombinant human type VII collagen in a *Col7a1*$^{-/-}$ mouse model mimicking the features of human recessive DEB. Injected human type VII collagen was stably incorporated into the mouse BMZ, formed anchoring fibrils and corrected the DEB murine phenotype, as demonstrated by decreased skin fragility, reduced new blister formation and markedly prolonged survival (20).

CELL THERAPY

Fibroblast cell therapy has proven beneficial in in vivo and clinical studies of DEB. In a collagen VII hypomorphic mouse model, intradermal injection of wild-type fibroblasts resulted in de novo disposition of collagen VII and functional restoration of the dermal–epidermal junction. Treated areas were also resistant to induced frictional stress (21). A U.K. research collaboration went on to demonstrate that single intradermal injections of allogeneic fibroblasts to subjects with recessive DEB (n = 5) facilitates increased type VII collagen at the dermal–epidermal junction at 2 weeks and 3 months following injection, with evidence for regeneration of anchoring fibrils. These changes were associated with elevated *COL7A1* mRNA levels in the patients' skin (22).

Researchers from Japan, the U.K. and the U.S. are currently investigating the clinical potential of allogeneic human bone marrow cells from a healthy donor to replace defective or missing collagen type VII and reduce the symptoms of recessive DEB (23). To date, study observations have shown that three patients receiving busulfan (12.8-16 mg/kg), fludarabine (75 mg/m^2) and cyclophosphamide (200 mg/kg), followed by infusion of bone marrow or umbilical cord blood from an unrelated or matched sibling donor have marked clinical improvements, with decreased blister formation and associated elevations in collagen type VII and anchoring fibrils (24). Another

similar trial is currently recruiting at Columbia University (25), consisting of treatment with busulfan/fludarabine/alemtuzumab and allogeneic stem cells in recessive DEB.

TISSUE ENGINEERING

U.S. researchers have shown the clinical advantages of tissue-engineered skin (Apligraf®) in an open-label uncontrolled study in 15 patients with EB. Data showed that 82% (51 of 62) of the acute wounds were healed at the 6-week evaluation, 75% (27 of 36) remained healed at 12 weeks and 79% (11 of 14) remained healed at week 18. These symptomatic benefits were also associated with significant improvements in patient quality-of-life parameters (26).

NATURAL PRODUCTS

French researchers have demonstrated the protective effects of the major catechin in green tea, epigallocatechin-3-gallate (EGCG), in DEB. Their in vitro and ex vivo studies have indicated that EGCG is a good inhibitor of matrilysin (matrix metalloproteinase-7, MMP-7), which could be involved in epidermal detachment seen in recessive DEB, and provides good protection of collagen type VII and fibrillin-1 susceptible of being degraded by MMP-7 (27). The Centre Hospitalier Universitaire de Nice is currently planning a randomized, double-blind, placebo-controlled, crossover phase II study to assess the effects of EGCG (Polyphenon E®) in EB (28).

Sulforaphane, an antioxidant that can be obtained by eating cruciferous vegetables, has also been suggested to have antiblistering properties. Researchers from Johns Hopkins University in the U.S. have shown that sulforaphane activates the transcription factor Nrf2, which in turn has been shown to alleviate the blistering caused by *KRT14* deficiency in an EBS mouse model, correlating with *KRT17* induction in basal epidermal keratinocytes (29).

PHARMACOTHERAPIES

U.S. researchers have reported on the successful application of botulinum toxin type A (BTX-A) in a case study of EBS. They demonstrated that intradermal injections of BTX-A into the plantar aspect of the feet of a 43-year-old woman with multiple blisters and erosions on the soles of both feet provided a 64% reduction in blister count in the treated areas 3 months after injection, with reduced pain reported after just 2 weeks (30). As a result of this research, investigators from Uppsala University are currently recruiting for a double-blind, placebo-controlled phase II proof-of-concept study for BTA-X in EBS (31).

A randomized, double-blind clinical trial has been recently organized in the U.S. in collaboration with RegeneRx Biopharmaceuticals to determine whether daily topical administration of the polypeptide thymosin beta-4 can promote wound healing in patients with JEB and DEB (32). This stems from previous preclinical studies which suggested that thymosin beta-4 may be effective in promoting epithelial migration across wounds artificially induced on normal skin, both in rodents and in human volunteers (33).

Ongoing randomized, placebo-controlled phase II clinical trials are also investigating the wound-healing ability of the active component in 3% Alwextin® Cream, allantoin (34).

DISCLOSURE

The author states no conflicts of interest.

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