ELSEVIER SAUNDERS

Epidermolysis Bullosa: Part II – Diagnosis and Management
GUEST EDITOR: Dédée F. Murrell, MA, BMBCh, FAAD, MD

DERMATOLOGIC CLINICS

CONSULTING EDITOR: Bruce H. Thiers, MD

Volume 28 • Number 2 • April 2010   theclinics.com

Fibroblast-Based Cell Therapy Strategy for Recessive Dystrophic Epidermolysis Bullosa

W.F. Yan, MBBS, MSc[a,b],
Dédée F. Murrell, MA, BMBCh, FAAD, MD[a,b,*]

KEYWORDS

- Fibroblasts • Dystrophic epidermolysis bullosa
- Cell therapy

Dystrophic epidermolysis bullosa (DEB) is a severe skin fragility disorder associated with trauma-induced blistering, progressive soft tissue scarring, and increased risk of skin cancer. DEB is caused by mutations in the *COL7A1* gene which result in reduced, truncated, or absent type VII collagen, and anchoring fibrils at the dermal-epidermal junction (DEJ). Although various topical wound-healing agents have been examined, including autologous cultured keratinocytes and skin bioequivalents, none has shown unequivocal benefits in the treatment of dystrophic forms of EB. Alternative approaches are needed.

CLINICAL MANIFESTATIONS

The spectrum of disease is very wide, ranging from minor dystrophy in the nails to the more severe forms in which there is a significant fragility of the skin with recurrent wounds, infection, scarring, deformity, failure to thrive, and premature death from squamous cell carcinoma.[1] The most severe varieties of the disease characteristically begin at birth with both cutaneous and mucosal involvement. Severe generalized blistering produces erosions that heal slowly, with atrophic scarring and milia formation. Nails become dystrophic and are lost early in life. Repeated cycles of blistering followed by scarring lead to digital fusion and mittenlike epidermal encasements of the fingers and toes, and flexural contractures of the knees, elbows, and wrist joints. Other features include esophageal stenosis,[2] dilated cardiomyopathy,[3] fractures,[4] and dental deformities.[5] Recurrent denudation of the skin, with chronic inflammation, causes anemia and growth retardation in severe recessive dystrophic epidermolysis bullosa (RDEB).

CLASSIFICATION OF RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA

RDEB is classified into 2 subtypes, namely generalized severe (RDEB-GS) and generalized, other; the former lacks anchoring fibrils, whereas the latter exhibits reduced or rudimentary-appearing anchoring fibrils.[6] RDEB-GS is easily one of the most devastating, chronic diseases known to humanity. Characterized by mechanical fragility and repeated blister formation within potentially all epithelial-surfaced or lined structures, patients with generalized subtypes of RDEB who survive recurrent bacterial sepsis during early infancy are at high risk of later developing one or more severe complications, including profound growth retardation, multifactorial anemia, esophageal strictures, corneal scarring or blindness, poststreptococcal glomerulonephritis and renal failure, and progressive mutilation and loss (pseudosyndactyly) of the fingers and toes.[7]

[a] Department of Dermatology, St George Hospital, Gray Street, Kogarah, Sydney, NSW 2217, Australia
[b] University of New South Wales, Sydney, NSW 2050, Australia
* Corresponding author. Department of Dermatology, St George Hospital, Gray Street, Kogarah, Sydney, NSW 2217, Australia.
E-mail address: d.murrell@unsw.edu.au

ETIOLOGY AND PATHOGENESIS

Two possible mechanisms for blister formation have been proposed: (1) destruction of dermal connective tissue by excessive amount of a protease,[8] and (2) a defective structural protein in the dermis that is responsible for the normal integrity of the skin at the epidermal-dermal junction.[9–11] Electron microscopy shows destruction of collagen fibrils in association with blister formation, as well as phagocytosis of collagen by macrophages in the skin adjacent to clinical blisters.[12]

The pathogenesis of DEB and structure of collagen VII are reviewed by Bruckner-Tuderman[13] and Uitto.[14] The purpose of "cell therapy" for RDEB is to increase the amount of collagen VII in the basement membrane zone (BMZ) to heal wounds and prevent further wound formation. Many DEB patients have multiple wounds with a large area of involvement, and all of these areas ideally should be treated, along with internal blistering such as may appear in the esophagus.

Compared with gene therapy, cell therapy has many advantages. The most important feature is that fibroblast cell therapy is safe and easy to work with. Several experiments have been performed with allogeneic fibroblasts, autologous fibroblasts, or parent donor fibroblasts in mouse models and in one study in RDEB patients in recent years. Only minor side effects such as temporary erythema, pruritus, mild hypertrophic scarring at the injection sites, and local skin inflammation have been found in some cases. All of these side effects resolved spontaneously.[15] Fibroblasts are more robust and easier to propagate than keratinocytes,[16] are less susceptible to growth arrest and differentiation, and can be frozen, packaged, and stored. Fibroblasts can be used by intradermal injection into skin and no special subsequent wound care is required. Furthermore, unlike stem cell transplantation, in which human leukocyte antigen (HLA) typing is important because the degree of HLA compatibility between donor and recipient will influence the outcome of the transplant, the donor of fibroblasts can be unrelated to the patient. Graft-versus-host disease did not occur when allogeneic fibroblasts were injected in the pilot study of 5 patients.[15] These advantages of fibroblast-based cell therapy give hope to RDEB patients and their families for improving therapy in the near future.

TARGET CELLS CHANGED FROM KERATINOCYTES TO FIBROBLASTS

Keratinocytes and dermal fibroblasts both express collagen VII, but keratinocytes are the primary source of collagen VII in the developing skin.[17,18] Many investigators have used keratinocytes as the target cells of RDEB gene therapy. Of note, intradermal injection of normal human fibroblasts or gene-corrected RDEB fibroblasts alone were found capable of synthesizing and secreting stable deposition of type VII collagen in mouse DEJ, thus contributing to the formation of anchoring fibrils.[19,20]

FIBROBLAST-BASED CELL THERAPY IN MOUSE STUDIES
Intradermal Injection of RDEB Fibroblasts Overexpressing Type VII Collagen

In 2003, RDEB fibroblasts overexpressing collagen VII (RDEB+ fibroblasts), RDEB fibroblasts, and normal fibroblasts were injected into mouse intact skin and the expression of collagen VII in mouse skin was determined. Compared with normal fibroblasts, which only produce a low level of collagen VII centered around murine dermal hair follicles, and RDEB fibroblasts, which yielded undetectable protein, the results showed that RDEB+ fibroblasts can produce correctly localized collagen VII at the epidermal-dermal junction, which can be stable for 16 weeks.[21]

Intradermal Injection of Normal Allogeneic Human Fibroblasts

Based on the supposition that the administration of actual fibroblasts would result in more sustained type VII collagen deposition, gene-corrected RDEB fibroblasts and normal human fibroblasts alone were administered to immunodeficient mouse skin or transplanted human skin equivalent. The expression of new human collagen VII by gene-corrected fibroblasts were detected stably for at least 4 months, and formation of anchoring fibrils was present in the mouse DEJ after injection. Nevertheless, unlike what had been found by Ortiz-Urda, this study also found that normal human fibroblasts are capable of producing, secreting, and depositing collagen VII at the DEJ as effectively as gene-corrected fibroblasts, which seems to be dependent on the number of cells injected, that is, 5×10^6 cells versus 1×10^6 cells.[19,21]

Bruckner-Tuderman's group succeeded in creating a mouse model for RDEB that expressed collagen VII at about 10% normal level, and their phenotype closely resembled characteristics of RDEB.[22] Intradermal injection of wild-type (WT) fibroblasts resulted in restoration of the DEJ, and increased deposition of collagen VII and resistance to induced stress compared with untreated areas. Recently, a long-term study of

fibroblast-based cell therapy has been performed on a mouse model for RDEB. Collagen VII expression at the DEJ was increased by 3.5- to 4.7-fold for at least 100 days after intradermal injection of WT fibroblasts, and injected fibroblasts were the major source of newly deposited collagen VII, although injected fibroblasts gradually become apoptotic within 28 days. Skin integrity and resistance to mechanical forces also improved for at least 100 days. This preclinical test paves the way for human clinical trials.[23]

Intravenous Injection of Normal Human Fibroblasts

Severe RDEB patients usually have widespread lesions and multiple wounds spanning large areas as well as internal esophageal erosions; hence, a systemic delivery mode for cell therapy would be ideal. One problem with therapy involving intradermal injection of fibroblasts is that many intradermal injections need to be performed into numerous wound sites for each patient. An alternative strategy might be to inject allogeneic fibroblasts into the patient's circulation that home to the skin wounds and deposit the transgene product. A recent study showed that intravenously injected normal human fibroblasts home to the sites of wounded human RDEB skin engrafted onto immunosuppressed mice, and continually synthesize and secrete collagen VII to the BMZ of the human skin, forming anchoring fibril structures.[24,25] Thus, the mouse had a heterogeneous population of anchoring fibrils made up of both mouse collagen VII α chains and human collagen VII α chains. Of note, the investigators found that collagen VII delivered to the wound sites significantly enhanced wound healing. This study provides the first demonstration of the potential use of intravenously injected normal fibroblasts to restore collagen VII in DEB patients who have multiple open wounds.

FIBROBLAST-BASED CELL THERAPY FOR RDEB PATIENTS

Among the current cell therapies for RDEB using skin fibroblasts are the use of allogeneic fibroblasts (cultured from the patients' parents or unrelated individuals) or autologous fibroblasts (cultured from the patients themselves). Specifically, investigators evaluated the clinical benefits that may accrue from a single intradermal injection of these cells in subjects with RDEB. To assess potential clinical benefits in humans, Wong and colleagues[15] gave single intradermal injections of allogeneic fibroblasts to nonwounded skin on the backs of 5 subjects with collagen VII positive

RDEB. These investigators noted a 1.5- to 2-fold increase of type VII collagen at the DEJ at 2 weeks and at 3 months following injection, and a 1.5-fold increase of anchoring fibrils. Molecular analysis suggested that the major effect of allogeneic fibroblasts is to increase the recipients' own *COL7A1* mRNA levels, with greater deposition of mutant type VII collagen at the DEJ and formation of additional rudimentary anchoring fibrils. No significant immune reactions were observed in skin biopsies, and none of these patients developed autoantibodies to collagen VII.

In a clinical trial of allogeneic cultured dermal substitute for the treatment of skin wounds in patients with RDEB, Hasegawa and colleagues[26,27] employed an allogeneic cultured dermal substitute (CDS) prepared by plating normal human fibroblasts on a double-layer spongy matrix of hyaluronic acid (HA) and Atelo-collagen (Col) to treat RDEB patients. During a 6-week treatment, abundant granulation was found on the wound surface within a week and epithelialization began from the margins of the ulcer after 4 weeks. This study indicated that cryopreserved human fibroblasts in the CDS are able to release vascular endothelial growth factor and fibronectin, which in turn induce prompt granulation in the wound beds. These data demonstrated the feasibility of fibroblast-based therapeutic approaches in a preclinical setting, and lay a basis for further dissection of quantitative and qualitative details associated with development of a clinically applicable therapy regimen.

McGrath's group have been conducting an open-label study of cultured foreskin fibroblasts obtained from Intercytex to RDEB wounds with encouraging results (EB 2009, Vienna, oral communication). The authors' group has conducted a double-blind randomized placebo-controlled trial of allogeneic fibroblasts versus their transport media in paired symmetric wounds in RDEB, demonstrating efficacy and safety of the technique.[28]

SUMMARY AND FUTURE OVERVIEW

RDEB is caused by recessive mutations in the human type VII collagen gene. The current lack of specific treatment for RDEB is the impetus to develop fibroblast-based cell therapy strategies that have many advantages including technical ease of use, wide application, and minor side effects. Over the past 10 years, tremendous progress has been made in fibroblast-based cell therapy for RDEB. Fibroblast-based cell therapy can dramatically restore stable collagen VII at the DEJ and normalize the substructure changes of DEB for at least a few months. Even though the mechanism and the duration of newly produced

collagen VII at the DEJ are still unknown, cell therapy provides a new effective approach to therapy for RDEB.

REFERENCES

1. Mallipeddi R. Epidermolysis bullosa and cancer. Clin Exp Dermatol 2002;27(8):616–23.
2. Okada T, Sasaki F, Shimizu H, et al. Effective esophageal balloon dilation for esophageal stenosis in recessive dystrophic epidermolysis bullosa. Eur J Pediatr Surg 2006;16(2):115–9.
3. Fine JD, Hall M, Weiner M, et al. The risk of cardiomyopathy in inherited epidermolysis bullosa. Br J Dermatol 2008;159(3):677–82.
4. Fewtrell MS, Allgrove J, Gordon I, et al. Bone mineralization in children with epidermolysis bullosa. Br J Dermatol 2006;154(5):959–62.
5. De Benedittis M, Petruzzi M, Favia G, et al. Oro-dental manifestations in Hallopeau-Siemens-type recessive dystrophic epidermolysis bullosa. Clin Exp Dermatol 2004;29(2):128–32.
6. Fine JD, Eady RA, Bauer EA, et al. The classification of inherited epidermolysis bullosa (EB): report of the Third International Consensus Meeting on Diagnosis and Classification of EB. J Am Acad Dermatol 2008; 58(6):931–50.
7. Fine JD, Johnson LB, Weiner M, et al. Chemoprevention of squamous cell carcinoma in recessive dystrophic epidermolysis bullosa: results of a phase 1 trial of systemic isotretinoin. J Am Acad Dermatol 2004;50(4):563–71.
8. Takamori K, Ikeda S, Naito K, et al. Proteases are responsible for blister formation in recessive dystrophic epidermolysis bullosa and epidermolysis bullosa simplex. Br J Dermatol 1985;112(5):533–8.
9. Uitto J, Pulkkinen L, McLean WH. Epidermolysis bullosa: a spectrum of clinical phenotypes explained by molecular heterogeneity. Mol Med Today 1997;3(10):457–65.
10. Korge BP, Krieg T. The molecular basis for inherited bullous diseases. J Mol Med 1996;74(2):59–70.
11. Chan LS, Fine JD, Hammerberg C, et al. Defective in vivo expression and apparently normal in vitro expression of a newly identified 105-kDa lower lamina lucida protein in dystrophic epidermolysis bullosa. Br J Dermatol 1995;132(5):725–9.
12. Tidman MJ, Eady RAJ. Evaluation of anchoring fibrils and other components of the dermal-epidermal junction in dystrophic epidermolysis by a quantitative ultrastructural technique. J Invest Dermatol 1985;84:374–7.
13. Bruckner-Tuderman L. Dystrophic epidermolysis bullosa: pathogenesis and clinical features. Dermatol Clin 2010;28(1):107–14.
14. Chung HJ, Uitto J. Type VII collagen: the anchoring fibril protein at fault in dystrophic epidermolysis bullosa. Dermatol Clin 2010;28(1):93–105.
15. Wong T, Gammon L, Liu L, et al. Potential of fibroblast cell therapy for recessive dystrophic epidermolysis bullosa. J Invest Dermatol 2008;128(9):2179–89.
16. Chen M, Woodley DT. Fibroblasts as target cells for DEB gene therapy. J Invest Dermatol 2006;126(4):708–10.
17. Ryynänen M, Ryynänen J, Sollberg S, et al. Genetic linkage of type VII collagen (COL7A1) to dominant dystrophic epidermolysis bullosa in families with abnormal anchoring fibrils. J Clin Invest 1992; 89(3):974–80.
18. Ghazizadeh S, Taichman LB. Virus-mediated gene transfer for cutaneous gene therapy. Hum Gene Ther 2000;11(16):2247–51.
19. Woodley DT, Krueger GG, Jorgensen CM, et al. Normal and gene-corrected dystrophic epidermolysis bullosa fibroblasts alone can produce type VII collagen at the basement membrane zone. J Invest Dermatol 2003;121(5):1021–8.
20. Goto M, Sawamura D, Nishie W, et al. Targeted skipping of a single exon harboring a premature termination codon mutation: implications and potential for gene correction therapy for selective dystrophic epidermolysis bullosa patients. J Invest Dermatol 2006;126(12):2614–20.
21. Ortiz-Urda Susana, Lin Qun, Green Cheryl L, et al. Injection of genetically engineered fibroblasts corrects regenerated human epidermolysis bullosa skin tissue. J Clin Invest 2003;111(2):251–5.
22. Fritsch A, Loeckermann S, Kern JS, et al. A hypomorphic mouse model of dystrophic epidermolysis bullosa reveals mechanisms of disease and response to fibroblast therapy. J Clin Invest 2008;118(5):1669–79.
23. Kern JS, Loeckermann S, Fritsch A, et al. Mechanisms of fibroblast cell therapy for dystrophic epidermolysis bullosa: high stability of collagen VII favors long-term skin integrity. Mol Ther 2009, Jun 30 [online].
24. Woodley DT, Remington J, Huang Y, et al. Intravenously injected human fibroblasts home to skin wounds, deliver type VII collagen, and promote wound healing. Mol Ther 2007;15(3):628–35.
25. Uitto J. Epidermolysis bullosa: prospects for cell-based therapies. J Invest Dermatol 2008;128(9):2140–2.
26. Hasegawa T, Suga Y, Mizoguchi M, et al. Clinical trial of allogeneic cultured dermal substitute for the treatment of intractable skin ulcers in 3 patients with recessive dystrophic epidermolysis bullosa. J Am Acad Dermatol 2004;50(5):803–4.
27. Yamada N, Uchinuma E, Kuroyanagi Y. Clinical trial of allogeneic cultured dermal substitutes for intractable skin ulcers of the lower leg. J Artif Organs 2008;11(2):100–3.
28. Yan WF, Venugopal SS, Frew JW. Igawa S, Ishida-Yamamoto A, Murrell DF: Investigation of cell therapy for generalized severe recessive dystrophic epidermolysis bullosa by intradermal allogeneic fibroblasts randomized against placebo injections. J Invest Dermatol 2009;129:S28.