Lentiviral Engineered Fibroblasts Expressing Codon-Optimized *COL7A1* Restore Anchoring Fibrils in RDEB

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Cells therapies, engineered to secrete replacement proteins, are being developed to ameliorate otherwise debilitating diseases. Recessive dystrophic epidermolysis bullosa (RDEB) is caused by defects of type VII collagen, a protein essential for anchoring fibril formation at the dermal-epidermal junction. Whereas allogeneic fibroblasts injected directly into the dermis can mediate transient disease modulation, autologous gene-modified fibroblasts should evade immunological rejection and support sustained delivery of type VII collagen at the dermal-epidermal junction. We demonstrate the feasibility of such an approach using a therapeutic grade, self-inactivating-lentiviral vector, encoding codon-optimized *COL7A1*, to transduce RDEB fibroblasts under conditions suitable for clinical application. Expression and secretion of type VII collagen was confirmed with transduced cells exhibiting supranormal levels of protein expression, and ex vivo migration of fibroblasts was restored in functional assays. Gene-modified RDEB fibroblasts also deposited type VII collagen at the dermal-epidermal junction of human RDEB skin xenografts placed on NOD-*scid* IL2Rgamma^{null} recipients, with reconstruction of human epidermal structure and regeneration of anchoring fibrils at the dermal-epidermal junction. Fibroblast-mediated restoration of protein and structural defects in this RDEB model strongly supports proposed therapeutic applications in man.

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INTRODUCTION

Recessive dystrophic epidermolysis bullosa (RDEB) is a debilitating genodermatosis caused by loss-of-function mutations in *COL7A1* (Fine et al., 2014). Type VII collagen (C7) is essential for anchoring fibril (AF) formation at the dermal-epidermal junction (DEJ), and in RDEB, malformed, reduced, or absent AFs are a direct consequence of *COL7A1* mutations (Hovnanian et al., 1997). C7 is one of the main contributors of dermal-epidermal adhesion, forming "wheat-stack"-shaped, centrosymmetrically banded, semicircular loop structures known as AFs after antiparallel dimerization of two fibrils at their carboxyl (C)-termini (Burgeson et al., 1990). These can be seen extending from their amino (N)-termini that indirectly bind to hemidesmosomal α6β4 integrin via the bridging activity of laminin-332 in the lamina densa (Rousselle et al., 1997), where they protrude down to the papillary dermis encircling dermal type I and III collagen amongst other fibrous elements before terminating back in the lamina densa (Shimizu et al., 1997). Loss-of-function mutations in C7 lead to fragility of AF structures, thereby compromising the integrity of the DEJ resulting in severe sublamina densa blistering and tissue cleavage.

Clinically, skin blistering can follow even minor mechanical stress causing skin erosions from birth in many subtypes of RDEB. Moreover, chronic erosions with secondary infections that can progress to widespread, mutilating scars and joint contractures, and aggressive squamous cell carcinomas, typify the severe generalized forms of RDEB (Fine and Mellerio, 2009; Rodeck and Uitto, 2007). RDEB has a profound medical and socioeconomic impact on patients and their families (Tabolli et al., 2009). There are no curative therapies for RDEB, and supportive care, with daily dressings, meticulous wound care, nutritional support, and iron supplementation for chronic anemia are the mainstay of clinical management (Grocott et al., 2013; Mellerio et al., 2007).

Experimental therapies under development include recombinant C7 protein (Remington et al., 2008; Woodley et al., 2004, 2013), infusion of allogeneic mesenchymal cells (Conget et al., 2010), hematopoietic–stem cell transplantation (Tolar and Wagner, 2012; Wagner et al., 2010),

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Abbreviations: AF, anchoring fibril; DEJ, dermalepidermal junction; LV, lentiviral; RDEB, recessive dystrophic epidermolysis bullosa; C7, type VII collagen

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and gene therapies (Droz-Georget Lathion et al., 2015; Osborn et al., 2013; Sebastiano et al., 2014; Titeux et al., 2010). We have investigated the feasibility of ex vivo gene-modified cell-based delivery of C7 to restore AFs at the DEJ of affected skin. Although both keratinocytes and fibroblasts are involved in the production and secretion of C7, fibroblasts are generally more robust and easier to maintain in culture, making them an attractive target for such an approach (Goto et al., 2006). In addition, alternative approaches based on transduction of keratinocytes and production of engineered skin grafts may not be suitable for RDEB where the abnormal DEJ may compromise adhesion of engineered epidermal sheets. In previous studies, intradermal injections of allogeneic fibroblasts from healthy donors supported increased levels of *COL7A1* expression in patients with RDEB for several months (Nagy et al., 2011; Wong et al., 2008). However, a recent phase II double-blind randomized trial demonstrated the importance of intradermal control injections. These comprised placebo (vehicle only) reagents and resulted in similar levels of wound healing as with mismatched allogeneic fibroblasts (Venugopal et al., 2013). A significant difference between injection of vehicle and allogeneic fibroblasts was only noted at day 7 (of 28 days) in a separate trial (Petrof et al., 2013). Although the mechanism is unclear, a localized anti-inflammatory effect and upregulation of *COL7A1* from intradermal inoculation of the vehicle solution or injection needle itself (commonly used in scar remodeling) has been postulated (Nagy et al., 2011; Petrof et al., 2013; Venugopal et al., 2013). Irrespective of the mechanism, a major limitation of allogeneic injections is the immunological rejection of HLA-mismatched donor fibroblasts (Larcher and Del Río, 2015; Venugopal et al., 2013; Wong et al., 2008). An autologous approach using genetically modified RDEB fibroblasts should circumvent the risk of rejection and provide a source of locally synthesized C7. Previous reports have established the feasibility of modifying fibroblasts with a variety of vectors, including phage (Ortiz-Urda et al., 2003), gamma retrovirus (Goto et al., 2006; Titeux et al., 2010; Woodley et al., 2007), and lentivirus (Chen et al., 2002; Woodley et al., 2003), and local or systemic injection into recipient mice has provided varying degrees of evidence of restoration of skin integrity (Woodley et al., 2004, 2007). We have developed a self-inactivating-lentiviral (LV) platform combined with a human phosphoglycerate kinase promoter and codon-optimized *COL7A1* for the engineering of autologous RDEB fibroblasts and have shown definitive evidence of AF reconstruction at the DEJ in a human:murine xenograft model. The production and validation of good-manufacturing-practice compliant reagents and a robust process for manufacturing engineered fibroblasts have enabled the submission of applications for regulatory approval for first-in-man testing of this therapy.

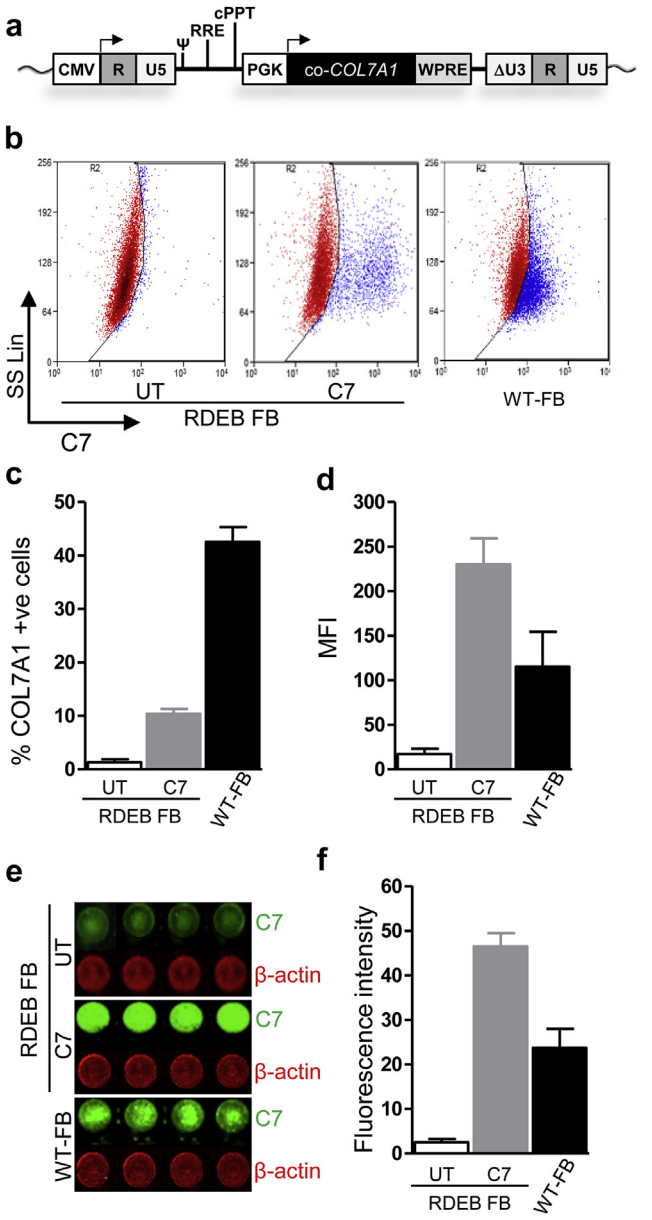

Figure 1. Expression of C7 in gene-corrected RDEB fibroblasts using a SIN-LV-*COL7A1* vector. (**a**) Configuration of pCCL-PGK-*COL7A1* lentiviral transfer plasmid shows a third-generation, split-packaging SIN vector with the deleted U3 region of the 3′LTR, internal PGK promoter, mutated woodchuck hepatitis virus posttranscriptional regulatory element (WPRE), and central polypurine tract (cPPT). Transgene *COL7A1* was codon-optimized (co-*COL7A1*) encoding the full-length *COL7A1* sequence. (**b**, **c**) Average expression of C7 in LV-*COL7*-transduced and untransduced (UT) primary RDEB-1 and -2 fibroblasts by intracellular staining and flow cytometry with corresponding mean fluorescence intensity (MFI) (**d**). (**e**) In situ expression of C7 in RDEB-1 and -2 LV-*COL7* fibroblasts using in-cell Western blotting (ICWB). Green lanes represent C7 expression; red lanes represent loading control (β-actin) expression with average immunoreactivity (**f**). LTR, long terminal repeat; LV, lentiviral; PGK, phosphoglycerate kinase; RDEB, recessive dystrophic epidermolysis bullosa; SD , standard deviation; SIN, self-inactivating. Error bars represent SD of four replicates.

RESULTS

Restoration of C7 expression in LV-*COL7A1*-transduced RDEB primary fibroblasts

Primary fibroblasts from patients with RDEB lacking C7 expression were transduced with a third-generation self-inactivating-LV vector encoding codon-optimized C7 (LV-*COL7*) under current good-manufacturing-practice compliant conditions using a single round of exposure at a multiplicity of infection 5 (Figure 1a). After 3 weeks of culture and expansion, flow cytometric analysis showed 9.3−12.8% of fibroblasts expressing C7 (Figure 1b−d), and this