CONFLICT OF INTEREST
The authors state no conflict of interest.

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See related commentary on pg 742

Meganuclease-Mediated *COL7A1* Gene Correction for Recessive Dystrophic Epidermolysis Bullosa

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TO THE EDITOR

Dystrophic epidermolysis bullosa (DEB) is a rare and severe genetic skin disease inherited in a dominant (DDEB) or recessive (RDEB) manner, responsible for blistering of the skin and mucosa after mild trauma (Bruckner-Tuderman, 2010; Uitto et al., 1992). DEB is caused by a wide variety of mutations in *COL7A1* encoding type VII collagen, the major component of anchoring fibrils which form key attachment structures for dermal-epidermal adhesion (Hovnanian et al., 1997; Varki et al., 2007).

Gene correction approaches based on sequence-specific DNA double strand breaks (DSB)-mediated homology-directed repair (HDR) allow precise and accurate correction of mutations (Yanez-Munoz et al., 2006). They have the potential to restore stable expression and function of the defective gene and to reverse the disease phenotype

Abbreviations: DDEB, dominant dystrophic epidermolysis bullosa; DSB, double strand breaks; HDR, homology-directed repair; IDLVs, integration-deficient lentiviral vectors; MNs, meganucleases; RDEB, recessive dystrophic epidermolysis bullosa

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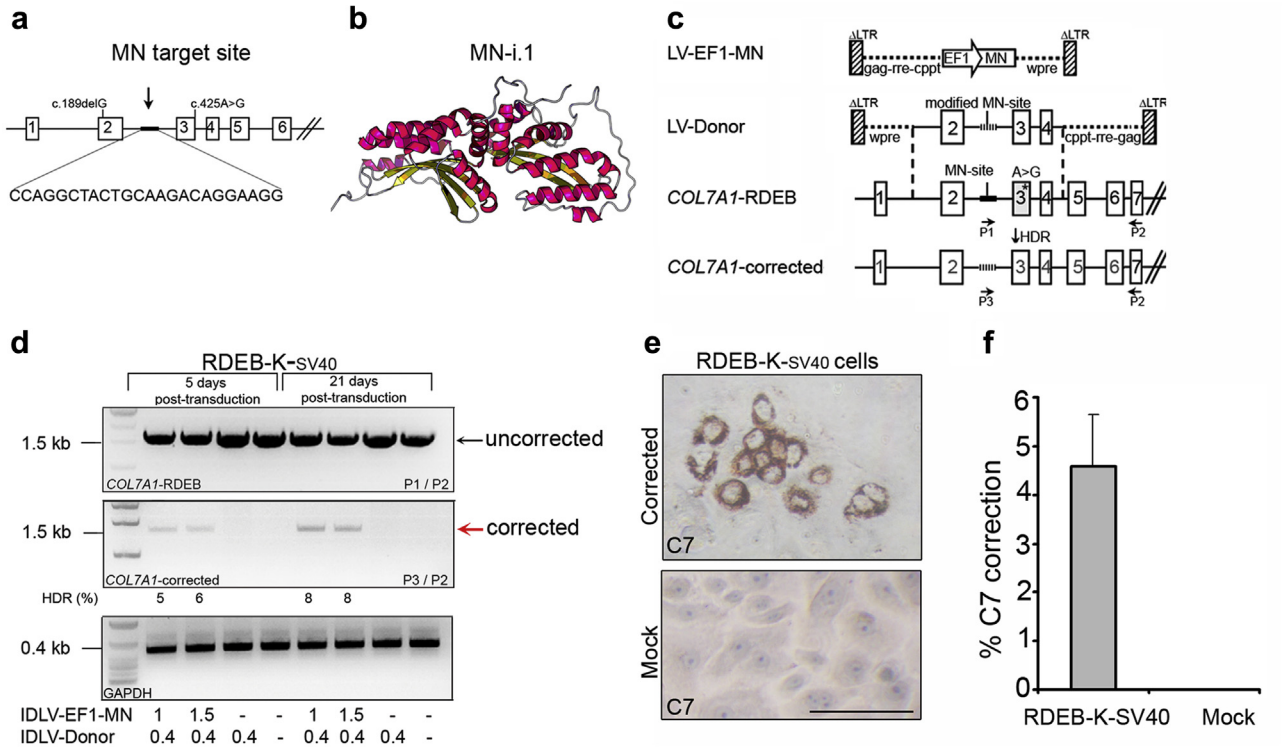

Figure 1. Evaluation of MN expression efficiency in HIV-derived lentiviral vectors and MN-mediated genetic correction of *COL7A1* Exon 3 in immortalized RDEB keratinocytes (RDEB-K-SV40 cells). (**a**) MN target site in intron 2 and corresponding sequence. (**b**) The predicted structure of the MN-i1 isoschizomer. (**c**) Top: design of the lentiviral vectors (LV-EF1-MN and the promoterless LV-Donor). Bottom: *COL7A1* encompassing mutated exon 3 and structure of corrected *COL7A1* after MN-mediated HDR. Arrows indicate allele-specific PCR primers. (**d**) Genomic PCR on the cells cotransduced with MN-i.1- and Donor-encoding IDLVs resulted in a specific 1.5 kb band, corresponding to the corrected allele when using P3/P2 primers. The GAPDH gene was used as the reference transcript. (**e**) Gene-corrected RDEB-K-SV40 cells were positively stained with the LH7:2 anti-C7 antibody. Untreated cells (mock) show negative staining. Scale bar = 100 μm. (**f**) Percentage of C7 rescue in bulk-transduced RDEB-K-SV40 cells. HDR, homology-directed repair; IDLVs, integration-deficient lentiviral vectors; MN, meganucleases; RDEB, recessive dystrophic epidermolysis bullosa.

(Genovese et al., 2014; Lombardo et al., 2007). Genome engineering tools such as meganucleases (MNs) have demonstrated their ability to target precise genomic locations in human primary cells without significant genotoxicity (Arnould et al., 2006; Redondo et al., 2008). Here, we demonstrate the feasibility of correcting *COL7A1* mutations through MN-mediated HDR. We used integration-deficient lentiviral vectors (IDLVs) (Coluccio et al., 2013) to achieve gene edition in unselected primary RDEB keratinocytes and fibroblasts.

In our study, four I-CreI-derived single-chain MN isoschizomers targeting the same sequence in intron 2 of *COL7A1* were generated (Supplementary Figure S1 online) to correct two mutations identified in patients with RDEB. The first RDEB null mutation in exon 2 (c.189delG; p.Lys6Trp*40) is located 302 bp upstream of the selected target site. The second mutation (c.425A > G; p.Lys142Arg) is a recurrent mutation located in exon 3 and is 630 bp downstream from the target site (Figure1a).

Among the four MN isoschizomers tested (namely MN-i.1, MN-i.2, MN-i.3, and MN-i.4), MN-i.1 (Figure 1b), displaying the highest expression efficiency (Supplementary Figure S2 online), was introduced in a lentiviral vector under the control of the cytomegalovirus or the EF1a promoter. Western blot analysis showed that only the EF1a promoter was able to drive significant levels of MN expression in an RDEB keratinocyte cell line (RDEB-K-SV40) (Supplementary Figure S3a and b online). No significant MN-related cytotoxicity was detected in a cell survival assay at the vector dose of 1 pg p24/cell (Figure S3c online). The capacity of MN-i.1 to induce site-specific DNA cleavage was assessed by documenting non-homologous end joining at the targeted region. The results revealed 9%, 7.5%, and 2.2% of modification at the target locus in RDEB-K-SV40 cells, primary keratinocytes (RDEB-K) and fibroblasts (RDEB-F), respectively, confirming that the MN is active in human primary cells (Supplementary Figure S4 online).

To achieve genetic correction by HDR, we designed and cloned a donor template (Donor), containing homology arms spanning genomic sequences between exon 1 and 5 (Figure 1c), in which we modified the MN recognition sequence, to prevent prolonged DNA exposure to DSB during transient MN expression after HDR (Supplementary Figure S5 online). We first assessed MN-mediated HDR in RDEB-K-SV40 cells in which the homozygous c.425A>G mutation leads to abnormal splicing, resulting in a complete absence of type VII collagen (C7) protein. RDEB-K-SV40 cells were cotransduced with increasing doses of IDLVs encoding MN-i.1 and Donor (IDLV-EF1-MN-i1: 1 or 1.5 pg-p24/cell, IDLV-Donor: 0.4 pg-p24/cell). Gene correction and C7 protein re-expression were investigated at 5 and 21 days after transduction. The presence of the corrected allele was detected by genomic PCR in bulk-transduced cells cotreated with the MN-i1- and Donor-encoding IDLVs. Gene correction efficiency