GENE EDITION-MEDIATED DELETION OF A PATHOGENIC MUTATION-CONTAINING COL7A1 EXON 80 IN EPIDERMAL STEM CELLS OF RDEB PATIENTS ALLOWS REGENERATION OF NORMALLY ADHESIVE HUMAN SKIN

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Abstract

Recessive Dystrophic Epidermolysis Bullosa (RDEB) is a skin fragility disorder associated with mutations in *COL7A1* gene. A highly prevalent mutation in Spanish RDEB patients' cohort, c.6527insC, is located in exon 80 and results in a frame shift causing a premature termination codon. Homozygous carriers of this mutation have no Collagen VII (C7) protein at the dermal-epidermal junction. We had previously designed TALENs expressed by adenoviral vectors (Ad-TALENs) to generate indels in the vicinity of the c.6527insC mutation with the potential to restore *COL7A1* gene reading frame and Collagen VII protein expression in an immortalized keratinocyte cell line (Chamorro et al., *Mol Ther Nucleic Acids*. 2016, Apr 5;5:e307). After infecting primary patient keratinocyte cultures with these Ad-TALENs, clones (n=136) with epidermal stem cell features were isolated and analyzed phenotypically for restored C7 expression. All C7 positive clones (n=13) were shown to carry different reading frame-correcting indels. Selected clones were expanded and cells used to produce skin equivalents that were grafted onto immunodeficient mice to generate gene edited human skin. A single base pair deletion at the dsDNA break site generated by our TALENs was found to be the most common indel in genotyped clones. This deletion causes frame recovery but results in four amino acid changes in the C7 protein that disrupt the Gly-X-Y pattern, characteristic of the triple helix-forming domain, and hinders protein secretion. Skin equivalents generated with these clones displayed C7 at the basal layer of epidermis, but not at the basement membrane zone, and exhibited blisters at the dermal-epidermal junction. A clone carrying a bigger deletion encompassing exon 80 entirely was found to express a truncated C7 lacking exon 80 encoded sequence. Skin equivalents generated with this clone regenerated human skin displaying C7 at the basement membrane zone, dermal-epidermal adhesion as a consequence of anchoring fibrils formation. Our results indicate that it is possible to edit *COL7A1* in primary patient stem cells, isolate edited clones and use them to generate functional human skin with therapeutic potential. We also demonstrate the structural functionality of C7 lacking exon 80 encoded sequence and therefore the utility of exon-deleting strategies to restore ColVII protein deposition at the dermal-epidermal junction and revert the RDEB phenotype.

Introduction

COL7A1 mutation c.6527insC at Exon 80 is highly recurrent in the Spanish population. A gene editing strategy targeted to that mutation could be beneficial for a large cohort of patients

Materials and Methods

Previously designed TALENs expressed by adenoviral vectors (Ad-T6 and T7) generate indels in the vicinity of the c.6527insC mutation with the potential to restore *COL7A1* gene reading frame and C7 protein expression (Chamorro et al., *Mol Ther Nuc Ac* 2016, Apr 5;5:e307). These TALENs were used with primary RDEB keratinocytes

Results I: Characterization of COL7A1 edited clones

Fig 1. (A) Genotype, phenotype and skin regeneration from clones carrying *COL7A1* reframing indel (deletion) c.6512delG. Cells restore expression of C7 but with a substitution of 4 aminoacids. (B) Clones are capable to achieve long-term skin regeneration upon grafting but the faulty C7 remains retained in the basal cells. Therefore, fragility is not corrected

Results II: Characterization of a COL7A1 ΔE80 clone

Fig 1. (A) Genotype, phenotype and skin regeneration from clones carrying *COL7A1* reframing indel (deletion) c.6512delG. Cells restore expression of C7 but with a substitution of 4 aminoacids. (B) Clones are capable to achieve long-term skin regeneration upon grafting but the faulty C7 remains retained in the basal cells. Therefore, fragility is not corrected

Conclusions

- TALENs-induced, NHEJ-mediated reframing of mutant (frameshifted) *COL7A1* is relatively efficient.
- In our setting, the majority of NHEJ-derived indels appear to be 1 bp deletions at or close to the TALENs DNA breaking point.
- Most frequent single bp deletions led to faulty aminoacid substitutions.
- Less frequent larger deletions, able to eliminate splice acceptor sequences (seen in the immortalized cell line) or fully delete Exon 80, occur.
- Isolation of stem cell clones carrying the proper reframing event (e.g. ΔE80) is laborious but feasible.
- Long-term skin regeneration with these clones has been achieved.
- Improved gene editing tools enabling precise targeting of the causing mutation or to efficiently delete Exon 80 may accelerate the road to clinical applications.

Funding

Funded by grant from Debra International and Debra Austria