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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Recessive Dystrophic Epidermolysis Bullosa (RDEB) is a skin fragility disorder due to mutations in *COL7A1* gene. A highly prevalent mutation in Spanish RDEB patients' cohort, c.6527insC, is located in exon 80. Homozygotic carriers of this mutation have no Collagen VII (C7) protein. We had previously designed TALE Nucleases (TALENs) expressed by adenoviral vectors (Ad-TALENs) with the potential to restore C7 expression in an immortalized keratinocyte cell line. Primary patient keratinocyte were infected with these Ad-TALENs, and several clones with epidermal stem cell features isolated and analyzed phenotypically. All C7 positive clones were shown to carry different reading frame-correcting indels. Selected clones were expanded, used to produce skin equivalents and grafted onto immunodeficient mice to generate gene edited skin. A single base pair deletion found to be the most common indel in genotyped clones causes frame recovery but resulted in four amino acidic changes in C7 that disrupt the characteristic Gly-X-Y pattern of the triple helix-forming domain, hindering protein secretion. Skin equivalents generated with these clones displayed C7 at the basal layer of epidermis, but not at the basement membrane, and exhibited blisters at the dermal-epidermal junction. However, 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, dermal-epidermal adhesion and anchoring fibrils formation. We conclude that is possible to edit the *COL7A1* gene 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 aminoacids and therefore the utility of exon-deleting strategies to restore C7 deposition and revert the RDEB phenotype.