Efficient Non-Viral Ablation of COL7A1 Exon 73 Splice Acceptor for the Treatment of Dystrophic Epidermolysis Bullosa (DEB)

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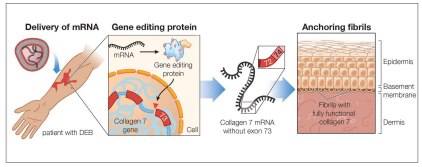
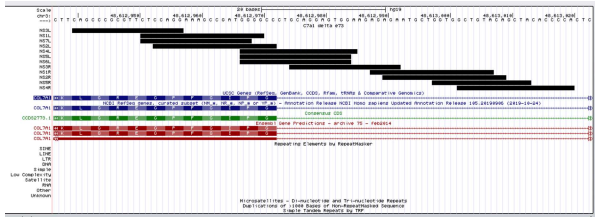
Rationale



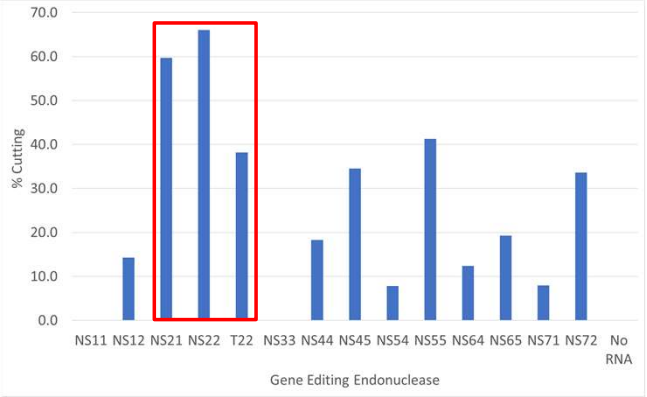
COL7A1 is a large gene, encompassing 118 exons, several of which are dispensable to protein function. Two exons, exon 73 and 80, are mutational hotspots. Consequently, omitting these exons through alternative splicing may have a great therapeutic effect. The use of antisense oligonucleotides (ASOs) to cause transient skipping of exon 73 and 80 have been shown to restore functional anchoring fibrils in patient-derived fibroblasts and keratinocytes by others. However, ASO-based approaches suffer from limited tissue penetration and require chronic dosing. Gene-editing approaches using TALENs and CRISPR-Cas9 are also being explored for the treatment of DEB. However, current gene editing approaches suffer from low efficiency, necessitating the use of selection markers that prevent *in vivo* application.

Modular construction of gene editing nucleases across COL7A1 splice acceptor site



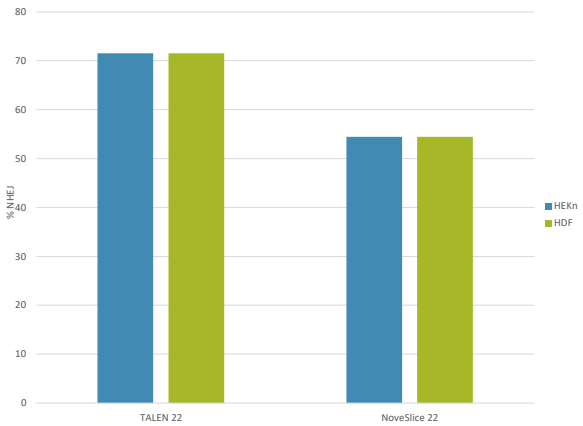
Left and Right hand sequences of the TALENs and NoveSlice™ proteins were aligned against the hg19 build of UCSC Genome Browser.

Cell-Free Target Cleavage Screening of RNA encoded Gene Editing Endonucleases



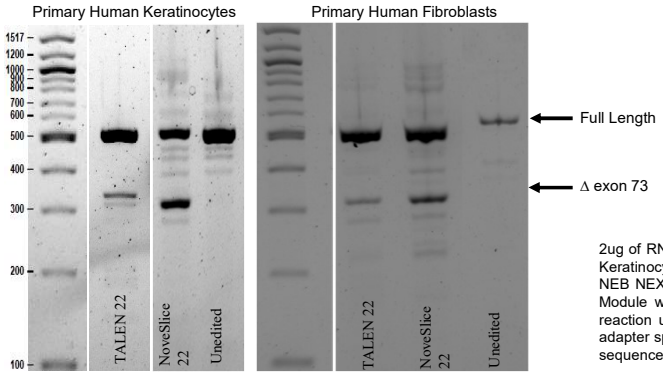
500ng total RNA encoded endonuclease (250ng RNA/hand) was added into a 25uL Rabbit Reticulocyte *In Vitro* Translation reaction along with 50ng of a 500bp COL7A1 DNA amplicon spanning exons 71 – 74. The reaction was cleaned and ran on a 2% gel. Band intensities were quantitated using ImageJ and plotted using Excel.

High Efficiency of COL7A1 editing in primary cells via RNA encoded Gene Editing Endonucleases



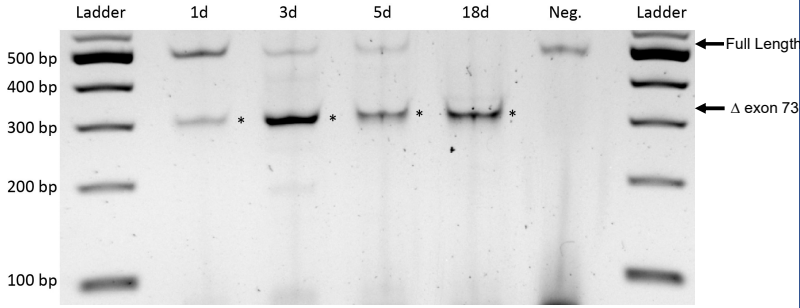
500ng total RNA encoded endonuclease (250ng RNA/hand) was transfected using ToRNAdo™ into 100,000 primary keratinocytes or fibroblasts, and extracted using Qiagen DNA micro kit. 5uL of gDNA was used in a 25uL PCR reaction, generating a 500bp band. 200ng was used in a T7E1 endonuclease mismatch assay. Band intensities were quantitated using ImageJ and plotted using Excel.

Delivery of RNA encoded Gene Editing Endonucleases show robust COL7A1 exon 73 omission in Primary Cells



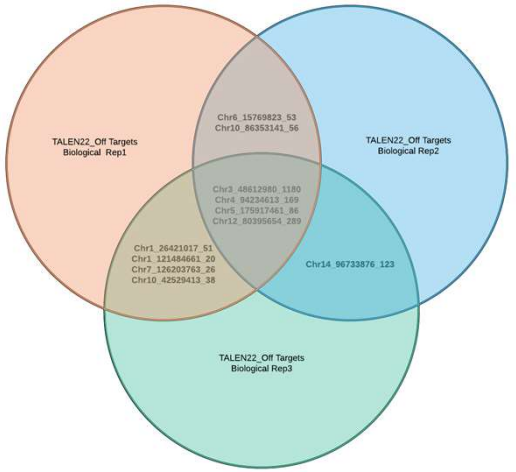
500ng total RNA (250ng RNA/hand) was transfected using ToRNAdo into 100,000 keratinocytes or fibroblasts, and extracted using Qiagen RNeasy kit. 2uL of total RNA was added into a Reverse Transcription reaction, using a primer specific to exon 71. 10% of the Reverse Transcription reaction was used in 50uL PCR reaction using COL7A1 primers spanning exons 71 through 75.

Keratinocytes show durable COL7A1 exon 73 omission for multiple weeks



2ug of RNA encoded TALEN 22 (1ug/hand) were transfected into 50,000 primary Keratinocytes. RNA was extracted using the Qiagen Rneasy micro kit 1,3,5 and 18 days following transfection. RT-PCR was performed on the edited RNA and analyzed for the lack of COL7A1 exon 73 (300 bp).

Genome wide Off Target Screening of Edited Primary Human Epithelial Keratinocytes



2ug of RNA encoded TALEN 22 (1ug/hand) and 0.2 ug of dsODN were transfected into 3 wells of 50,000 primary Keratinocytes. gDNA was extracted using the Promega Maxwell extraction device. 60ng of gDNA was added to the NEB NEXT UltraII Fragmentase Module, and the resulting reaction was added to the NEB NEXT UltraII Ligation Module with the GUIDE-Seq adapters. 10 ng of the adapted fragments were added to a linear amplification reaction using a dsODN specific primer. After linear amplification, all of the reaction was added to an Illumina adapter specific PCR. These libraries were quantitated via Kapa's Library quantification kit, and 10 pM library was sequenced on a MiSeq v3 kit with 2x300bp reads. Resultant data was analyzed using the GuideSeq pipeline.

Conclusions

We demonstrate efficient, non-viral ablation of the *COL7A1* exon 73 splice acceptor using gene-editing endonucleases delivered via mRNA. Also, we demonstrate durable exon omission in primary human cells over multiple weeks. We believe this approach overcomes the low-efficiency of previous gene-editing approaches, and can be used as broad exon omission platform. Therefore, this approach is being explored for clinical use in DEB as well as other diseases where exon omission can have a therapeutic effect

Acknowledgments

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