RaDiChal'21
FINAL

2021 Rare Disease Challenge

#butterflyeffect 7therapy

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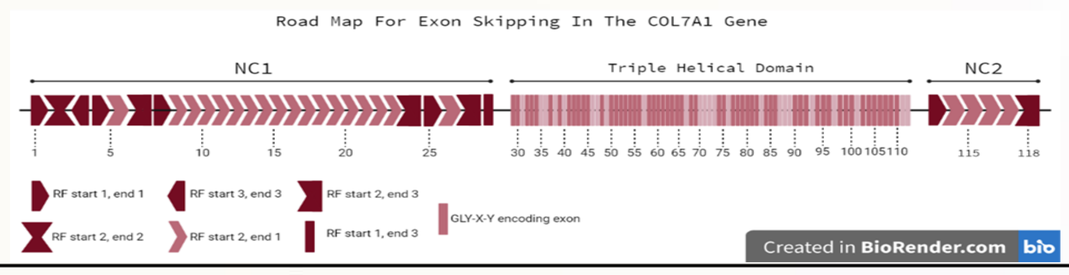
Abstract

Dystrophic Epidermolysis Bullosa is caused by mutations in the COL7A1 gene, which encodes Type VII collagen synthesized from keratinocytes and fibroblasts in the 3p21.31 chromosomal region, is caused by the loosening of the epidermis layer, after a mechanical effect such as pressure or trauma, which is defined as the mucosa in the subcutaneous spaces such as skin and mouth, genital area. It is a transitional and monogenic disease characterized by the formation or development of water-filled bubbles, defined as "bullae" of fluids leaking from the surrounding tissues into areas (such as the anal region, eyes, nose, respiratory tract, esophagus). Epidermolysis Bullosa, which has four different clinical forms, is usually observed on the skin. According to the Orphanet data, their incidence is respectively; Epidermolysis Bullosa Simplex (EBS) observed as 1.8 per 100,000 people, Junctional Epidermolysis Bullosa (JEB) observed as 0.17 per 100,000 people, Dystrophic Epidermolysis Bullosa (DEB) observed as 0.7 per 100,000 people. Finally, Kindler Syndrome is the rarest form which transitive autosomal recessive inheritance. The COL7A1 gene contains 118 exons and generally short introns between them. As a result of various mutations occurring in these exon regions, the disease was evaluated over many phenotypes. The classification of these phenotypes is made by considering criteria such as the exon in which the mutation occurs, the domain in which it is located, and its effect on protein expression. During the treatment studies of the subtypes, there are problems such as the exon skipping strategy not being suitable for skipping or the restriction enzyme not being in the genome and creating a risk of creating any problem in this case, and the exon region containing too much mismatch despite its high efficiency. In addition to those problems, one of the main problems is that specific strategies focused on nucleotide correction are a costly and time-consuming process to plan, create and reach more patients than other methods. The aim of the target project; A treatment strategy is being developed to cover Recessive Dystrophic Epidermolysis Bullosa and Dominant Dystrophic Epidermolysis Bullosa, which are caused by mutations in the COL7A1 gene, which encodes Type VII collagen synthesized from keratinocytes and fibroblasts in the 3p21.31 chromosomal region. Plan A, Plan A.1 and Plan B strategies designed to be interconnected. Dystrophic epidermolysis bullosa (DEB) is a severe inherited skin disorder characterized by fragility of the skin and mucous membranes because of loss-of-function mutations in COL7A1 encoding type VII collagen. Dystrophic epidermolysis bullosa (DEB) is a severe inherited skin disorder characterized by fragility of the skin and mucous membranes because of loss-of-function mutations in COL7A1 (OMIM *120120) encoding type VII collagen (C7). Mutations leads to aberrant synthesis ofC7 or defective assembly of the protein into Anchoring fibrils, resulting in poor epidermal-dermal adherence. DefectiveC7 leads to loss of adhesion between the epidermis and the dermis, resulting in severe blistering of the skin and mucosae after mild trauma. CO7A1 mutations leads to abnormally truncated polypeptides unable to form C7.These protein fragments become degraded within the cell leading to absent anchoring fibril production. A number of studies have indicated that MSCs can significantly affect wound healing, through cell differentiation and the release of paracrine factors, implying a profound therapeutic potential. Furthermore, it was reported that bone marrow-derived MSCs (BM-MSCs) supplement type VII collagen in vivo, which is one of the backgrounds to utilize allogeneic BM-MSCs for the treatment of RDEB. MSCs established from normal human and RDEB keratinocyte-derived cells analyzed their therapeutic potential as a strategy for the realization of in vivo MSCs from human fibroblasts therapies. The MSCs were derived from the bone marrow of healthy, unrelated individuals and injected intradermally. MSCs are colony-forming fibroblastic cells.



Social Impact

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Likes: 1461
Sharings: 70
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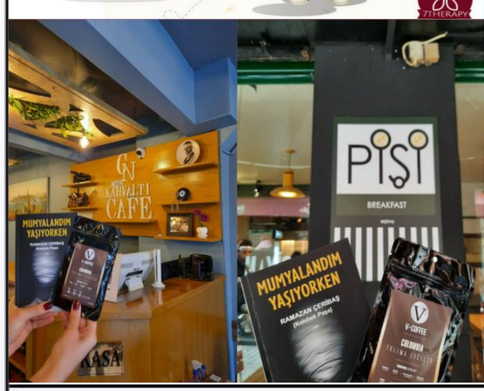
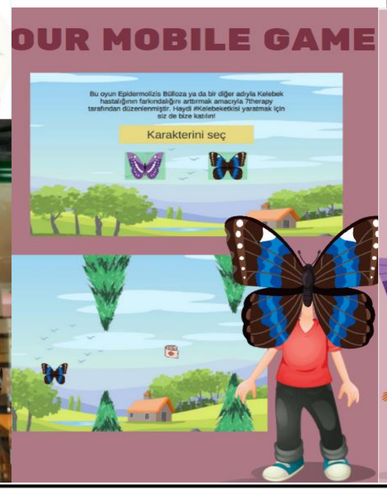
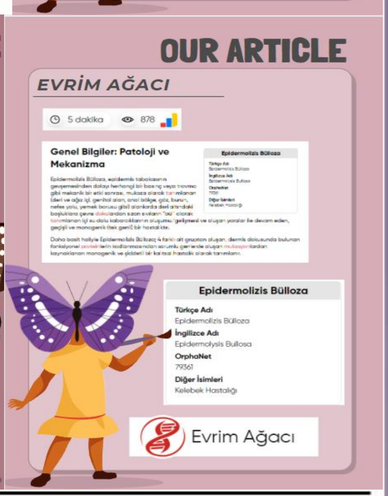
Starting from the chaos theory, we inspired by butterfly effect defined as small changes that can produce large and unpredictable results. In order to raise awareness, 4 friends set out on a small but effective path that we believe it will be effective.

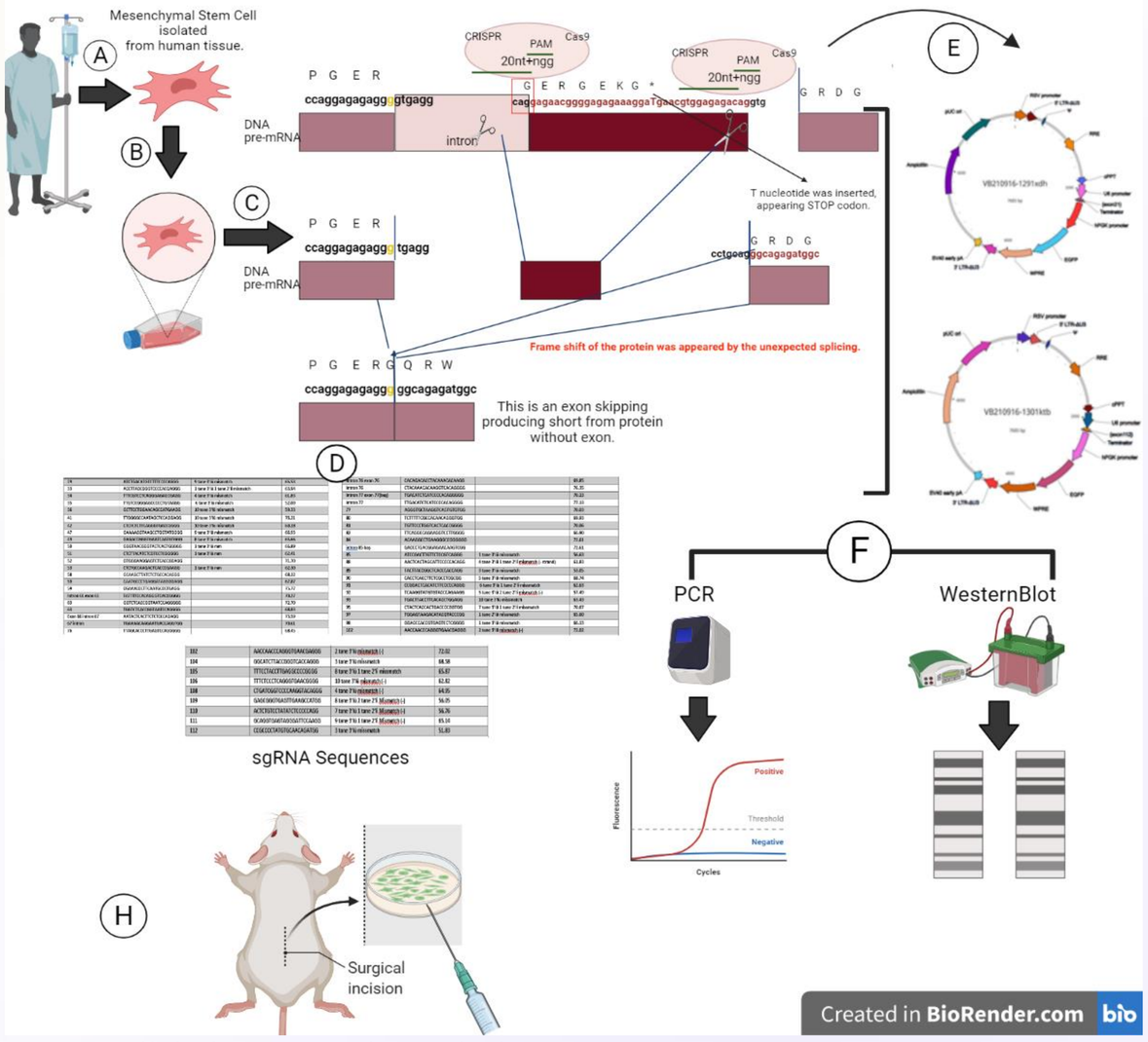






PLAN A

Projects



CRISPR-Exon skipping for THD, NC1 and NC2 In the chosen CRISPR-Exon skipping strategy for THD, NC1 and NC2 Domains, the NHEJ method leads to insertion or deletion generation. This method usually does not lead to correction of the mutated allele. Frameshift indels created with NHEJ can affect pre-mRNA splicing by deleting different regions of pre-mRNA splicing, as well as causing exon skipping. With Crispr/Cas9, short frameshift indels are introduced in exonic sequences to disrupt the frames in the mRNA reading. Double-strand breaks can be created with these indels by non-homologous end joining (NHEJ). An important application of the CRISPR/Cas9 system is to generate inactivating mutations in protein-coding genes by targeting single sgRNA sites to generate frameshifts. Most of the indels in protein-coding exons, except those with a size of a multiple of three, are assumed to be frameshift mutations that disrupt open reading frames. Frameshift indels are well suited for generating loss-of-function mutations in protein-coding genes. These mutated transcripts are recognized by the nonsense-mediated mRNA decay (NMD) machinery and are degraded or translated into truncated, nonfunctional proteins. In the THD, NC1 and NC2 domains, a single sgRNA creates small insertions or deletions at the intron and exon boundaries to disrupt the 3′ splicing site region. This causes exon skipping or stochastic exon skipping.

Target gene (RNA)	Target exon	Oligo name in literature	Oligo sequence /: Cocktail. -: weasel (connected).
Collagen type VII alpha 1 chain, COL7A1	70	h70AON1	CCACGCUCUCCAGGGAG
Collagen type VII alpha 1 chain, COL7A1	70	h70AON2	CUUCCAGGCUCUCCUCGC
Collagen type VII alpha 1 chain, COL7A1	70	h70AON2	CUUCCAGGCUCUCCUCGC
Collagen type VII alpha 1 chain, COL7A1	73	QR-313	CGUUCUCCAGGAAAGCCGAUG
Collagen type VII alpha 1 chain, COL7A1	73	QR-313	CGUUCUCCAGGAAAGCCGAUG
Collagen type VII alpha 1 chain, COL7A1	73	QR-313	CGUUCUCCAGGAAAGCCGAUG

PLAN A.1

Conclusion

Recently developed base editors, which are fusions of Mutant Cas9 (dCas9) with Aminoacyl transferase without DNA cleavage and cleavage activity, and cleavage reverse transcriptase without amino acyltransferase, have attracted much attention. In both designed treatment strategies, it was planned based on high efficiency rate and off-target effect. Developed treatments; It is a treatment approach with a wide mutation scale covering the NC1, NC2 and TH domains of the COL7A1 gene. Mutations common to both subtypes -RDEB, DDEB- were selected. A more controlled treatment study was planned by working ex-vivo.

PLAN A

CRISPR/Cas9 from analyzes for plan A based on the exon skipping strategy will be used for cleavage of target sites. This CRISPR/Cas9 will be used to destroy the splicing acceptor or splicing donor sites of selected exons.

Plan A.1

Unlike Plan A, exon skipping of mutated exon regions for the THD region via AON sequences, adding ORF sequences when necessary. It is planned to suppress or stop the expression of mutated exons.

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