RARE DISEASE CHALLENGE
RaDiChal'21

PROJECT PRELIMINARY REPORT

TEAM NAME

EBee-Cure

TEAM MEMBERS

Sude ARSLAN, Rüya ÜÇEL, Hanbike EFE, Nur Sima ÇİL, Eren ZAMAN

TARGETED RARE GENETIC DISEASE

Recessive Dystrophic Epidermolysis Bullosa

TEAM LOGO

1. Summary of the Project (Project Description)

Recessive Dystrophic Epidermolysis Bullosa (RDEB) is a subtype of the rare genetic disease Epidermolysis Bullosa (EB) which is caused by the mutations in COL7A1 gene that encodes for type VII collagen. Due to the mutations occuring in the gene, the gene is not able to encode for sufficient amount of anchoring fibrils. This leads to the formation of blisters on skin and in mucosal tissue.

The core of our treatment project involves PolyPurine Reverse-Hogsteen Hairpin (PPRH) structures which are triplex-forming oligonucleotides. Using these hairpin structures, we aim to permanently fix the point mutations in the COL7A1 gene so that the production of functional type VII collagen and anchoring fibrils can be achieved.

The Polypurine core of the PPRH links to the polypyrimidine tracts present in the double-stranded DNA while the extension sequence of structure creates a D-loop (displacement loop) with the strand and allows for recombination without any nuclease activity. Since there is no nuclease activity in recombination, there are hardly any off-target effects of the treatment.

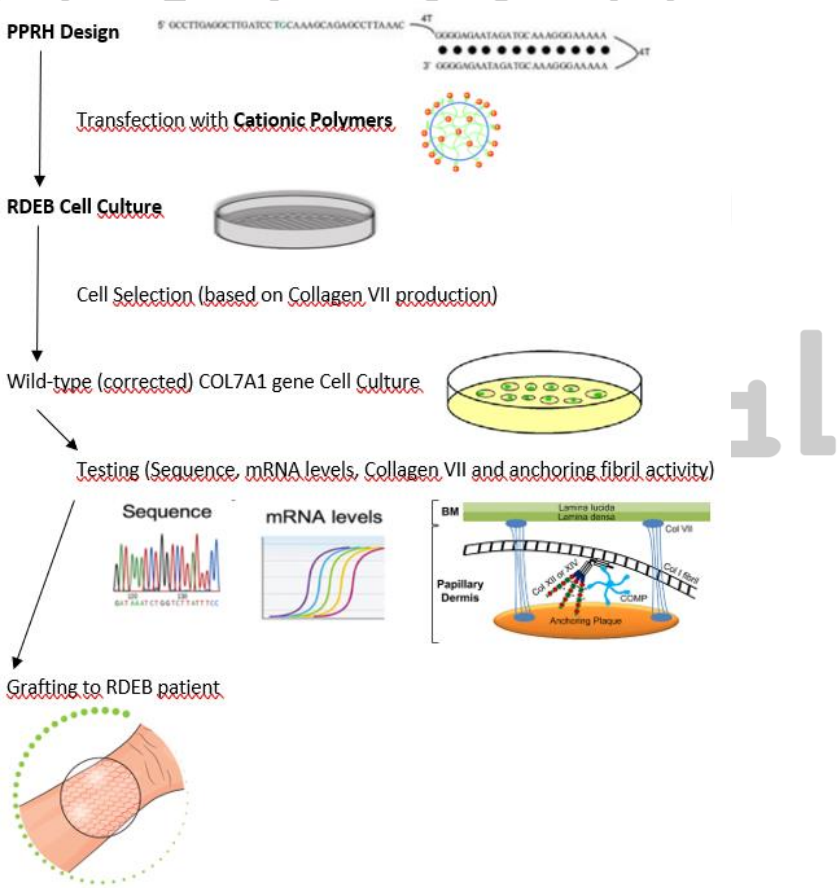

Figure 1: Steps for RDEB gene therapy with PPRH.

2. Problem:

It is estimated that Epidermolysis Bullosa (EB), which is a rare genetic disease, affects approximately 500.000 people worldwide. Although a substantial number of people live with EB, accurate and permanent treatment has not been found yet. Moreover, very few people know about EB. In general, existing treatment methods can be applied only to reduce patients' symptoms. EB has lots of subtypes such as EB simplex, Dystrophic EB, Kindler Syndrome. There can be many mutations causing a single type of EB. This situation has caused different cases in the same EB subtype, thus the current treatment methods can be applied only to a limited number of patients. Furthermore, the lack of knowledge about rare diseases and Epidermolysis Bullosa have negative effects on patients' social and psychological life.

3. Solution

With our treatment project in which the PPRH structure will be used for RDEB for the first time ever, personal PPRH structures can be designed for different patients depending on the type of mutations they possess in their COL7A1 gene. One of the biggest advantages of this method is that one can fix all the mutations present in a 20-50 bp section of the DNA in one go. Additionally, via our social awareness activity, we aim to get as many people as we can reach to know about EB and for all EB patients to live a more relaxed, socially-active life.

4. Source

1) "Autosomal recessive generalized dystrophic epidermolysis bullosa, intermediate form"(2021,May).Orphanet.https://www.orpha.net/consor/cgi-bin/OC_Exp.php?lng=en&Expert=8984

2) Eichstadt, S., Tang, J. Y., Solis, D. C., Siprashvili, Z., Marinkovich, M. P., Whitehead, N., ... & Sarin, K. Y. (2019). From Clinical Phenotype to Genotypic Modelling: Incidence and Prevalence of Recessive Dystrophic Epidermolysis Bullosa (RDEB). Clinical, cosmetic and investigational dermatology, 12, 933.

3) Kulalı, F., Özbakır, H., Kundak, S., Kalkanlı, O., Eriş, D., Çolak, R., Yıldız, M., Özdemir, S., Yıldırım, T., & Çalkavur, Ş. (2019). Epidermolizis Bülloza: Olgu Serisi. Jinekoloji-Obstetrik ve Neonatoloji Tıp Dergisi, 16(2): 69-73.

4) Vanden Oever, M., Twaroski, K., Osborn, M. J., Wagner, J. E., & Tolar, J. (2018). Inside out: regenerative medicine for recessive dystrophic epidermolysis bullosa. Pediatric research, 83(1-2), 318–324. https://doi.org/10.1038/pr.2017.244

5) Titeux, M., Mejía, J. E., Mejlumian, L., Bourthoumieu, S., Mirval, S., Tonasso, L., ... & Hovnanian, A. (2006). Recessive dystrophic epidermolysis bullosa caused by COL7A1 hemizygosity and a missense mutation with complex effects on splicing. Human mutation, 27(3), 291-292.

6) Uitto, J., Bruckner-Tuderman, L., Christiano, A. M., McGrath, J. A., Has, C., South, A. P., ... & Robinson, E. C. (2016). Progress toward treatment and cure of epidermolysis bullosa: summary of the DEBRA International research symposium EB2015. Journal of Investigative Dermatology, 136(2), 352-358.

7) Lin, Y., Chen, X. J., Liu, W., Gong, B., Xie, J., Xiong, J. H., ... & Yang, Z. L. (2012). Two novel mutations on exon 8 and intron 65 of COL7A1 gene in two Chinese brothers result in recessive dystrophic epidermolysis bullosa. Plos one, 7(11), e50579.

8) Das, B. B., & Sahoo, S. (2004). Dystrophic epidermolysis bullosa. Journal of perinatology, 24(1), 41-47.

9) Bruckner-Tuderman, L. (2019). Newer treatment modalities in epidermolysis bullosa. Indian dermatology online journal, 10(3), 244.

10) Poulton, J., Finsterer, J., & Yu-Wai-Man, P. (2017). Genetic counselling for maternally inherited mitochondrial disorders. Molecular diagnosis & therapy, 21(4), 419-429.

11) Shinkuma, S. (2021). Advances in gene therapy and their application to skin diseases: A review. Journal of Dermatological Science.

12) Osborn, M. J., Newby, G. A., McElroy, A. N., Knipping, F., Nielsen, S. C., Riddle, M. J., ... & Tolar, J. (2020). Base editor correction of COL7A1 in recessive dystrophic epidermolysis bullosa patient-derived fibroblasts and iPSCs. Journal of Investigative Dermatology, 140(2), 338-347.

13)Supp, D. M., Hahn, J. M., Combs, K. A., McFarland, K. L., Schwentker, A., Boissy, R. E., ... & Lucky, A. W. (2019). Collagen VII expression is required in both keratinocytes and fibroblasts for anchoring fibril formation in bilayer engineered skin substitutes. Cell transplantation, 28(9-10), 1242-1256.

14) Izmiryan, A., Ganier, C., Bovolenta, M., Schmitt, A., Mavilio, F., & Hovnanian, A. (2018). Ex vivo COL7A1 correction for recessive dystrophic epidermolysis bullosa using CRISPR/Cas9 and homology-directed repair. Molecular Therapy-Nucleic Acids, 12, 554-567.

15) Hainzl, S., Peking, P., Kocher, T., Murauer, E. M., Larcher, F., Del Rio, M., ... & Koller, U. (2017). COL7A1 editing via CRISPR/Cas9 in recessive dystrophic epidermolysis bullosa. Molecular Therapy, 25(11), 2573-2584.

16)Takashima, S., Shinkuma, S., Fujita, Y., Nomura, T., Ujiie, H., Natsuga, K., ... & Shimizu, H. (2019). Efficient gene reframing therapy for recessive dystrophic epidermolysis bullosa with CRISPR/Cas9. Journal of Investigative Dermatology, 139(8), 1711-1721.

17) Rodríguez, L., Villalobos, X., Dakhel, S., Padilla, L., Hervas, R., Hernández, J. L., ... & Noé, V. (2013). Polypurine reverse Hoogsteen hairpins as a gene therapy tool against survivin in human prostate cancer PC3 cells in vitro and in vivo. Biochemical pharmacology, 86(11), 1541-1554.

18) Bremer, J., van der Heijden, E. H., Eichhorn, D. S., Meijer, R., Lemmink, H. H., Scheffer, H., ... & Van den Akker, P. C. (2019). Natural exon skipping sets the stage for exon skipping

as therapy for dystrophic epidermolysis bullosa. Molecular Therapy-Nucleic Acids, 18, 465-475.

19) Noé, V., & Ciudad, C. J. (2021). Polypurine Reverse-Hoogsteen Hairpins as a Tool for Exon Skipping at the Genomic Level in Mammalian Cells. International journal of molecular sciences, 22(7), 3784. https://doi.org/10.3390/ijms22073784

20) Turczynski, S., Titeux, M., Tonasso, L., Décha, A., Ishida-Yamamoto, A., & Hovnanian, A. (2016). Targeted Exon Skipping Restores Type VII Collagen Expression and Anchoring Fibril Formation in an In Vivo RDEB Model. The Journal of investigative dermatology, 136(12), 2387–2395. https://doi.org/10.1016/j.jid.2016.07.029

21) Vahidnezhad, H., Youssefian, L., Zeinali, S., Saeidian, A. H., Sotoudeh, S., Mozafari, N., Abiri, M., Kajbafzadeh, A. M., Barzegar, M., Ertel, A., Fortina, P., & Uitto, J. (2017). Dystrophic Epidermolysis Bullosa: COL7A1 Mutation Landscape in a Multi-Ethnic Cohort of 152 Extended Families with High Degree of Customary Consanguineous Marriages. The Journal of investigative dermatology, 137(3), 660–669. https://doi.org/10.1016/j.jid.2016.10.023

22) Solé Ferré, A., Félix, A. J., & Noé Mata, V. (2017). Polypurine Reverse Hoogsteen Hairpins as a tool for gene repair and editing. Recent Advances in Pharmaceutical Sciences VII, 2017, Research Signpost. Editors: Diego Muñoz-Torrero, Montserrat Riu & Carles Feliu. ISBN: 978-81-308-0573-3. Chapter 4, p. 51-68.