EB2017-5th World Conference of EB Research

Abstract summarizing the lecture during the EB symposium

Gene editing in iPSC for DEB

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Induced pluripotent stem cells (iPSCs) hold great promise for modeling and recapitulation of human biological processes. Coupled with the advent of genome engineering tools, specifically the CRISPR-Cas9 systems, iPSCs have opened new possibilities to study human biology and creating novel cellular based therapies. Dystrophic epidermolysis bullosa (DEB) is a severe inherited skin disorder caused by mutations in the *COL7A1* gene encoding type VII collagen (C7), which is the major constituent of anchoring fibrils (AFs) at the basement membrane zone. In patients with the most severe form of recessive DEB (RDEB), both *COL7A1* mutations cause premature termination codons as a result of nonsense mutations, small insertions or deletions, splice site mutations resulting in frame shift of translation, or residual expression of truncated C7 polypeptides that are degraded within the cell. As a result, the AFs are usually missing in the skin which severely impair dermal-epidermal stability, leading to blister formation. In contrast, the dominant form of DEB (DDEB) results from glycine substitution mutations replacing one of the glycine in the Gly-X-Y repeat sequence with in-frame deletion or insertion mutations, which interfere in a dominant negative manner with C7 synthesis. Therefore, therapeutic approaches for DEB involved homology-directed repair (HDR) of mutant allele/s or knock down/out of harmful mutated *COL7A1* gene.

Recently, our lab and others have determined the molecular basis of DEB, providing the foundation for development of gene-and stem cell-based therapies. We generated DEB patient iPSCs and developed a protocol for their differentiation into keratinocytes (KC) and fibroblasts (FB). Our future goal is to establish a therapy approach with curative intent for patients with DEB using gene editing and patient specific iPSCs.

Several approaches for correcting mutations have been reported using sequence-specific nucleases, which allow for efficient genetic modifications at targeted sites of interest. However, the low efficiency and drug selection leads to concerns in moving toward clinical applications. We previously designed CRISPR-Cas9 and TALENs constructs to specifically target and knockout the c.8068del17insGA mutated allele in *COL7A1* that causes DDEB. Recently, we successfully corrected a recurrent hotspot mutation in exon 19 (c.2470insG) in the *COL7A1* gene using HDR with CRISPR Cas9-gRNA in iPSCs derived from a patient with RDEB who was homozygous for this mutation. We utilized single-strand oligodeoxynuleotides as the donor template, together with a high-fidelity CRISPR/Cas9 nuclease and an mCherry reporter gene to achieve both biallelic and monoallelic correction of *COL7A1* mutations, which allowed us to sort

the targeted cells by FACS without drug selection. This strategy resulted in an efficiency of 10% for biallelic correction (WT/WT) and 40% for monoallelic correction (WT/mut). Moreover, we recently demonstrated gene editing of a recurrent glycine substitution mutation in exon 73 (c.G2043R) in the *COL7A1* gene using Cas9-gRNA ribonucleoproteins (RNPs) which uses transfections of Cas9 proteins and synthesized RNA guides, instead of plasmids. We designed a gRNA adjacent to the patient mutation and utilized single-strand oligodeoxynuleotides as the donor template to stimulate the replacement of the mutation by HDR. Taken together, we have demonstrated the feasibility of permanent correction the *COL7A1* mutations in iPSCs with high efficiency, which is a crucial step for clinical applications to develop innovative stem cell therapies for DEB. Currently, we are developing clinical protocols for reprogramming of different somatic cells such as blood cells and fibroblasts into iPSCs and protocols for differentiation of keratinocytes and fibroblasts to construct human skin. These findings open new options for autologous cell sourcing in iPSC/CRISPR gene editing approaches aimed at developing innovative stem cell therapies for DEB and other skin diseases.