MEDICAL THERAPIES AND PHARMACOLOGY

CRISPR/CAS9-HOMOLOGOUS RECOMBINATION MEDIATED CORRECTION OF EPIDERMOLYSIS BULLOSA SIMPLEX MUTATIONS

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Introduction: Epidermolysis bullosa Simplex (EBS) is a rare autosomal dominant skin disease. It is caused by different mutations of the KRT5 and KRT14 genes resulting in non-scarring blisters and erosions caused by minor mechanical trauma. Even though several potential attempts to cure EBS were initiated and are under development, treatment for EBS is still largely limited and the care is primarily palliative. This highlights a real need for the development of better treatments addressing the underlying genetic defects. The clustered regularly interspaced palindromic repeats CRISPR-Cas9 (CRISPR/Cas9) genome editing technology seems to be an attractive method for the treatment of EBS.

Objective: We aim to use a specific gene editing approach based on the CRISPR/Cas9 technology to restore two dominant point mutations within KRT5 and KRT14 genes respectively. The two target mutations are responsible for the generalized severe epidermolysis bullosa simplex phenotype (EBS-gen sev).

Materials and Methods: The therapeutic method is based on a chosen single guide RNA that introduces DNA cleavage in patient's primary cells close to the mutation site and a donor DNA template that replace the mutant allele with a wild type sequence by directed homologous recombination (HDR).

Results: We observed successful cleavage of DNA by the Cas9 endonuclease and higher donor DNA incorporation by HDR in HEK293T cells. In patient's cells, the percentage of mutation correction by HDR was ranging from 1.5% to 7%.

Conclusions: The current study describes a protocol for a simple model for point mutation repair directed by CRISPR/Cas9 system and HDR. Moreover it provides a proof-of principle that gene editing has clinical therapeutic prospects and could be used to cure EBS and other monogenic diseases in a heritable and precise manner.