Título: GENE EDITING-BASED PROTOCOLS FOR THE EX VIVO CORRECTION OF RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA

Nombre: BONAFONT ARAGO, JOSE

Universidad: Universidad Carlos III de Madrid

Departamento: Bioingeniería e ingeniería aeroespacial

Fecha de lectura: 28/09/2020

Programa de doctorado: Programa de Doctorado en Ciencia y Tecnología Biomédica por la Universidad Carlos III de Madrid

Dirección:

> **Director:** FERNANDO LARCHER LAGUZZI

> **Codirector:** RODOLFO MURILLAS ANGOITI

> **Codirector:** MARCELA DEL RIO NECHAEVSKY

Tribunal:

> **presidente:** Fulvio Mavilio

> **secretario:** Paula Río Galdo

> **vocal:** MARIA CARMEN AYUSO GARCIA

Descriptores:

> INGENIERIA GENETICA

> BIOLOGIA CELULAR

> GENETICA MOLECULAR

> BIOLOGIA MOLECULAR

El fichero de tesis ya ha sido incorporado al sistema

> http://hdl.handle.net/10016/31881

Localización: E-ARCHIVO, REPOSITORIO INSTITUCIONAL DE LA UNIVERSIDAD CARLOS III DE MADRID

Resumen: The skin is the largest organ of the human body, covering an area of approximately 2 square meters in average. This organ is made up of three main layers: epidermis, dermis and hypodermis. The dermal-epidermal junction, called Basement Membrane Zone (BMZ), allows the physical attachment of dermis and epidermis. Within this BMZ, anchoring fibrils are structures necessary for attachment of the dermis to the basement membrane and are mainly composed of Type VII collagen (C7). The loss of C7 causes fragility of the skin.

Epidermolysis Bullosa (EB) is a heterogeneous family of rare genetic skin disorders characterized by loss of dermal-epidermal adhesion, blistering of the skin, erosions and scar formation after minor trauma. Among the different subtypes described, the Recessive Dystrophic Epidermolysis Bullosa (RDEB) is the most severe subtype, with an increased risk of developing squamous cell carcinoma (SCC). Mutations causing RDEB have

been found throughout the COL7A1 gene. A frame-shift mutation (c.6527insC) in the exon 80 of COL7A1 gene is present in 46% of the Spanish population of patients with RDEB. This mutation results in a premature termination codon (PTC) and when it is present in both COL7A1 alleles, it leads to the absence of this protein. C7 is primarily provided by keratinocytes, making this cell type the target of choice for gene therapy approaches. Despite efforts, there is currently no curative treatment for EB. Different pharmacological, cellular and genetic approaches have been tried showing promising results, but, until now, treatment has focused primarily on symptomatic relief such as daily wound care or pain reduction. Recently, precise gene modification technologies based on genomic nucleases have been developed, such as TALENs and CRISPR/Cas9. These nucleases can create double strand breaks (DSBs) in the DNA sequence that can be repaired by two main mechanisms: Non-homologous end joining (NHEJ), which introduces indels to modify the DNA sequence, and Homology-directed repair (HDR), which uses a donor template for accurate genetic correction.

This thesis presents three different approaches based on genome editing with the aim of treating RDEB, focused on genetically corrected epidermal stem cells to achieve long-term healthy skin regeneration. The first strategy used TALENs and showed the feasibility of an exon deletion-based approach to correct RDEB epidermal stem cells as safe clonal therapy. Secondly, gene correction efficiency was improved by designing a dual-guide CRISPR/Cas9 system, capable of efficient deletion of the mutated exon, leading to a high proportion of cells expressing functional C7 (over 80%). Ex vivo gene-corrected bulk keratinocytes achieved normal human skin regeneration upon transplantation onto immunodeficient mice, opening up the way for a polyclonal therapy scenario. Finally, in the third chapter, an HDR-based approach offers the most accurate way to correct the disease-causing mutations, with gene correction efficiencies above the threshold (30%) needed to sustain the dermal-epidermal junction of the skin. This marker-free HDR-based approach is based on a CRISPR/Cas9 system in combination with a donor template-carrying AAV.

Taken together, the different approaches described show epidermal stem cells correction efficient enough to make skin equivalents with therapeutic potential for RDEB patients.