Título: A COMPREHENSIVE STUDY OF DYSKERATOSIS CONGENITA: HEMATOPOIETIC CHARACTERIZATION, DISEASE MODELLING AND INNOVATIVE THERAPIES

Nombre: Carrascoso Rubio, Carlos

Universidad: Universidad Autónoma de Madrid

Departamento: Bioquímica

Fecha de lectura: 17/12/2021

Programa de doctorado: Programa de Doctorado en Farmacología y Fisiología por la Universidad Autónoma de Madrid

Dirección:

> **Director:** ROSARIO PERONA ABELLON

> **Codirector:** GUILLERMO GÜENECHEA AMURRIO

Tribunal:

> **presidente:** JOSÉ CARLOS SEGOVIA SANZ

> **secretario:** ANTONIO PÉREZ MARTINEZ

> **vocal:** Jordi Barquinero Mañez

> **vocal:** Albert Catala Temprano

> **vocal:** MARÍA MOLINA MOLINA

Descriptores:

> FISIOLOGIA HUMANA

> VIROLOGIA

> HEMATOLOGIA

> BIOLOGIA CELULAR

El fichero de tesis ya ha sido incorporado al sistema

> http://hdl.handle.net/10486/700914

Localización: REPOSITORIO DE TESIS BIBLIOTECA UAM

Resumen: Dyskeratosis congenita (DC) is a low prevalence inherited bone marrow failure (BMF) syndrome caused by mutations in telomerase complex-related genes. Therefore, DC is also considered a telomere biology disorder. Pathogenic genetic variants have been discovered in 14 different genes and these mutations result in telomerase dysfunction leading to premature telomere shortening, which is the main molecular hallmark of DC. Clinically, DC is characterized by the mucocutaneous triad and systemic complications but BMF represents the cause of early mortality in more than 80% of the cases. Given the unawareness of the hematopoietic status of these patients, a deep characterization of peripheral blood (PB) and bone marrow (BM) samples has been conducted in this work. DC patients showed premature telomere shortening, severe pancytopenia and high

percentage of erythroid progenitors in PB. Moreover, reduced content in hematopoietic stem and progenitor cells (HSPC) was observed both in PB and BM in comparison to healthy donors (HD). Considering the low number of DC patients and their limited percentage of CD34+ cells, we decided to study two different models in which innovative therapies to treat the BMF could be screened. On one hand, we evaluated the hematopoietic status of an autosomal dominant DC mouse model. Defective in vitro growth was observed in bone marrow cells of mice with mutations in Terc and naturally shortened telomeres, suggesting an incipient BMF. On the other hand, a HSPC-based model of X-linked DC (X-DC) was established through the stable inhibition of DKC1 using shRNAs delivered by lentiviral vectors (LV). Although androgens have been proposed as a potential treatment for BMF, the only curative treatment nowadays is allogeneic HSPC transplantation. However, low availability of HLA-compatible donors and risks derived from conditioning regimes have led hematologists to ask for the development of alternative therapies to treat BMF in DC patients. To address this issue, we generated two LVs expressing GSE4 peptide and evaluated their safety and efficacy in HSPCs and lymphoblastic cell lines (LCL). While in vivo and in vitro properties were not altered in HD human HSPCs, we demonstrated that the ectopic expression of GSE4 recovered several phenotypic hallmarks in X-DC patient-derived LCLs and in DKC1-interfered CD34+ cells. Finally, we have also developed a knock-in targeted gene therapy strategy to treat BMF in DC patients with mutations in exons 2-15 of the DKC1 gene. Although confirmation of homology directed repair in HD human HSPCs is still a preliminary result, we consider this approach as a promising therapeutic alternative which could be also extended to other telomerase complex-related genes. Taken together, these results have improved the knowledge surrounding a complex disorder such as DC and constitute a significant step in the development of innovative treatments for the characteristic BMF of DC patients.