DEVELOPMENT AND CHARACTERIZATION OF A KLK5 OVEREXPRESSING SKIN HUMANIZED MOUSE MODEL RESEMBLING NETHERTON SYNDROME

Victoria Gálvez Cortés^{1,2}, Marta Carretero^{1,2}, Blanca Duarte^{1,2}, Wei-Li Di³, Fernando Larcher^{1,2,4}

1. CIEMAT -Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER-U714), Madrid 2. Instituto de Investigación Sanitaria de la Fundación Jiménez Díaz (IISFJD) 3. Institute of Child Health, University College London, London 4. Bioengineering Department, Universidad Carlos III de Madrid (UC3M), Madrid.

Abstract

Netherton Syndrome (NS) is an autosomal recessive form of ichthyosis regarded as one of the most severe skin hereditary disorders affecting newborns, and patients present both cutaneous and immunological abnormalities. NS is caused by loss-of-function mutations in the *SPINK5* gene, encoding the serine-protease inhibitor LEKTI, whose deficiency results in unrestricted activity of kallikreins (e.g. KLK5 and KLK7) that leads to degradation of corneodesmosomal proteins, thus producing the detachment of the stratum corneum and disruption of the epidermal barrier. Due to the limited availability of NS skin biopsies and ethical constraints of experimentation on human subjects we sought to develop a skin humanized mouse model for the disease based on the grafting of transgenic bioengineered human skin onto immunodeficient mice. In order to recapitulate the histopathological hallmarks of the condition we first considered an approach consisting in the overexpression of the KLK5 gene in human keratinocytes by lentiviral transduction. We have performed an initial characterization of the model through the analysis of histopathological markers associated to NS as well as KLK5 expression and activity. The study confirmed the alterations in the KLK5 pathway, which results in clear epidermal proliferation and differentiation alterations, similar to those observed in skin biopsies of NS patients. In a preliminary experiment, a topical formulation of a KLK5 inhibitory peptide was applied to KLK5 overexpressing grafts. The analysis of the treated grafts showed a lower activity of KLK5 and a certain degree of reversion of the main NS phenotypic markers.

Background: Netherton Syndrome

Among the responsible proteases for skin desquamation are KLKs. The KLK5, 7 and 14 contribute to desquamation by the degradation of desmosomal cadherins: desmoglein1 (DSG-1) and desmocollin1 (DSC-1). KLK5 and 14 are also responsible for pro-inflammatory and pro-allergic signals by activation of the active proteinase receptor PAR2, which promotes the expression of pro-inflammatory molecules (TNF- α , ICAM-1, IL8) and pro-allergic molecules (TSLP, cytokine pro-Th2). These alterations lead to congenital ichthyosiform erythroderma, trichorrhexis invaginata and allergic manifestations such as severe atopic dermatitis and a high level of IgE in serum, associated with chronic inflammation of the skin.

Results II: KLK5 model analysis

Conclusions

- Over expression of KLK5 in normal keratinocytes can be considered an appropriate approach to reproduce some of the main pathological features of NS including enhanced KLK5, PAR2 and TSLP expression and alteration of the DSG-1 pattern.
- A topical formulation of a KLK5 inhibitory peptide (SFTI) was capable to partially decrease the proteolytic activity of the transgenic KLK5 and to restore the normal phenotype in the skin.
- Transgenic KLK5 is a good target to screen for inhibitory peptide-based therapies.
- Establishing a reliable skin humanized mouse model for hyperkeratotic genodermatoses such as NS would enable to understand the underlying pathogenic mechanisms as well as developing novel therapeutic strategies to treat these genetic skin disorders.

Results I: Animal model production

Results III: Effect of a KLK5 inhibitory peptide