Abstract

Kallikrein-related peptidases (KLKs) are a subgroup of serine proteases of undisputable importance for a variety of functions, whose dysregulation has been linked to several pathological phenotypes. Among those pathologies, the Netherton syndrome stands out, since it is one of the very few that has its mechanism directly linked to KLK proteases as the main culprit of the disease, namely KLK5, KLK7 and to a lesser degree, KLK14. In this case, a mutation in the *SPINK5* gene leads to uncontrolled hyperactivity of those proteases, which results in epidermal barrier breach due to excessive epidermal desquamation and severe inflammation of the skin. Inflammation mechanisms of NS are still relatively poorly understood, with important roles being attributed to the activities of KLKs in the processing of immune system molecules and also to the dysregulation of the cutaneous microbiome.

TNFα signalling plays a key role in the homeostasis and immune response in the skin. Chronic skin infections may lead to deleterious effects with strong participation of TNFα signalling. To address the degree of its effects on the pathogenesis of NS, we have created a mouse model where the TNFR1 is disrupted by knockout of the *Tnfr1* gene on the background of a previously established mouse model of the Netherton syndrome.

We have successfully created the *Tnfr1*$^{-/-}$ mouse model and subsequently produced the desired *Sp5*$^{-/-}$ *Klk5*$^{-/-}$ *Tnfr1*$^{-/-}$ mice. Surprisingly, subsequent analyses suggest that not only *Tnfr1* ablation does not alleviate cutaneous inflammation present in previously created mouse models, but further increases its severity.

Key words: KLK, protease, skin, epidermis, inflammation, mouse model, CRISPR