Kallikrein-related peptidases (KLKs) constitute a family of closely related serine proteases encoded by genes clustered in one chromosomal locus. KLKs are widely expressed in a variety of tissues and numerous *in vitro* experiments suggest their important roles in many physiological and pathological processes. However, the biological roles of KLKs *in vivo* are often obscured mainly due to unavailability of suitable animal models. Although gene deficient mouse models were generated for several KLK genes, they had limited use for understanding the roles of individual proteases in the complex environment *in vivo*. One of the main obstacles which hampers *in vivo* analysis is partial functional overlap between some KLKs. This makes traditional single-gene deficient animal models an inadequate tool to address the biological impact of the gene deficiency as compensatory mechanisms often result in a lack of phenotype.

In this work, we used the transcription activator-like effector nuclease (TALEN) technology to generate several novel mutant mouse models to study the complex KLK proteolytic pathways and their roles in healthy organism and in disease. We prepared a novel mouse model for Netherton syndrome (NS), an autosomal recessive skin disorder caused by mutation in the gene *SPINK5*, which encodes the KLK-inhibitor LEKTI. NS is associated with hyperactivity of KLKs, which results in the chronic inflammation of skin, hair defects and a severe disruption of skin barrier. By mimicking a patient-derived mutation of *SPINK5* in mouse genome, we generated a mutant mouse that mimics the symptoms of NS patients and shows early post-natal lethality due to skin barrier failure. To address the roles of KLKs in the disease, we prepared a set of mutant mice individually or simultaneously deficient for KLK5 and KLK7 that were crossed with *Spink5* mutants. We showed that although single ablation of KLK5 or KLK7 is not sufficient to rescue the lethal effect of LEKTI-deficiency, simultaneous inactivation of both KLKs completely rescues the epidermal barrier and the postnatal lethality allowing mice to reach adulthood with fully functional skin and normal hair growth. We also showed that both proteases KLK5 and KLK7 play important roles in the inflammation and defective differentiation in NS and KLK7 activity is not solely dependent on activation by KLK5. Furthermore, detailed analysis of KLK5/KLK7 double-deficient mice revealed prominent epidermal hyperkeratosis, which is the first *in vivo* evidence that both proteases are involved in the physiological process of shedding the epidermal cells from the skin surface.

We also demonstrated that TALEN technology can be efficiently used to produce unconventional animal models in which only a portion of cells is characterized by ablation of targeted gene (genetic mosaics). Mosaic inactivation of *Spink5* gene in mice generates a viable model for NS, which is characterized by patches of lesional skin that reproduces NS symptoms such as keratinocyte hyperproliferation, defective differentiation and alopecia. In contrast to traditional *Spink5*-deficient animals, *Spink5*-mosaics survive the neonatal period, which allows long term experiments, such as evaluation of therapeutic compounds.

In summary, we have used the technology of programmable nucleases to generate several novel mouse models deficient for KLK proteases or their inhibitor LEKTI. Detailed analysis of these mice brought novel insights into the molecular pathogenesis of Netherton syndrome and also into the roles of KLK5 and KLK7 in skin homeostasis. We believe that these animal models will become useful tools for development of therapeutic compounds for NS treatment and for further characterization of KLK proteolytic pathways *in vivo*.