KLK5 and KLK7 ablation fully rescues lethality of Netherton syndrome-Like Phenotype

programmable nucleases help to reveal functional networks

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Kallikrein-related peptidases (KLKs) in epidermis

Expressed in SC
Degradation of corneodesmosomal proteins (in vitro)
PAR-2 activation

Expressed in SC
Degradation of corneodesmosomal proteins (in vitro)
What is the role of individual kallikreins in the epidermal-kallikrein network? Cooperation, redundancy?
Generation of Klk-deficient models

Klk5 -/-  

Klk7 -/-  

Klk5/7 -/-
KLK5 and KLK7 single–deficient mice do not show any obvious skin phenotype

Compansatory, redundancy, cooperation effects between KLK5 and KLK7?

=> generation of Klk5/Klk7 DKO mice
Klk5 deficient mice do not show any obvious phenotype
Organization of Klk locus

**Human**

- ACPT
- LOC546967
- SIGLEC-9

**Mouse**

- 1
- 15
- 2-ps
- Siglec-l1

**Klk1-related genes and pseudogenes**

- KLK gene
- pseudogene
- non-KLK gene
Generation of Klk5/Klk7 dKO mice using TALEN technology
KLK-inhibitor network:
generation of Klk x Spink5 double and triple deficient mice

Spink5 -/- × KLk5 -/- → Spink5 -/-

KLk5 -/- × KLk7 -/- → Spink5 -/-

KLk5/7 -/- × KLk5 -/- → Spink5 -/-

KLk5 -/- × KLk7 -/- → Spink5 -/-

KLk5/7 -/- × KLk7 -/- → Spink5 -/-

Spink5 -/-

Klk5 -/-

Klk7 -/-

Klk5/7 -/-
Netherton syndrome

- Autosomal recessive genetic disorder
- 1 in 200,000 newborn children
- Red, scaly, exfoliating epidermis
- Chronic skin inflammation
- Growth retardation
- Specific hair shaft defects (bamboo hair)
- Caused by mutation in Spink5 gene
Generation of a mouse model for Netherton syndrome

human wt: 5´CTG TGT GCT GAG AAT GCG 3´
human 398delTG: 5´CTG TGC TGA 3´

murine wt: 5´CTG TGT GCT GAG AAT GCG 3´
murine 402delTG: 5´CTG TGC TGA 3´

Raghunath et al., 2004
Generation of a mouse model for Netherton syndrome

**Abnormal differentiation of epidermis:**
- Acanthosis
- Parakeratosis

**Images:**
- wt
- Spink5^{A135X/A135X}

**TALEN mutagenesis**

**Lethal**
Generation of Klk x Spink5 double and triple deficient mice

Spink5 -/-  x  Klk5 -/-  x  Klk7 -/-  x  Klk5/7 -/-
Simultaneous inactivation of KLK5 and KLK7 rescues lethal phenotype of NS mouse model


Klk5−/−

Klk7−/−

Klk5−/−Klk7−/−

day 0  day 5  day 0
Both, KLK5 and KLK7 contribute to skin barrier defects

- Unregulated activity of KLK5 causes severe postnatal dehydration at P0
- KLK7 causes damage of epidermal barrier independently of KLK5 activation
Abnormal differentiation of epidermis in Spink5-/- pups is rescued upon ablation of KLK5/KLK7

Abnormal processing of profilaggrin is associated with KLK5 activity and may contribute to impaired water retention of Spink5-/- and Spink5-/-KLK7-/- epidermis.
Epidermal barrier disruption in NS mouse model is rescued by inactivation of KLKs 5 and 7

Kasparek et al., PLOS Genetics, 2017
Unregulated activity of KLK7 causes severe epidermal barrier damage in time dependent manner

Spink5−/−
Klk5−/−

P0

P5

wt
Spink5−/− Klk5−/−
Spink5−/− Klk5−/− Klk7−/−
Unregulated activity of KLK7 causes severe epidermal barrier damage in time dependent manner.
Unregulated activity of KLK7 causes severe damage of epidermis in time dependent manner.
Bamboo hair in Spink5\(^{-/-}\)-Klk5\(^{-/-}\)-Klk7\(^{-/-}\) mice are not found after 3 weeks of age.
Adult Spink5⁻/⁻Klk5⁻/⁻Klk7⁻/⁻ mice do not show any major skin defects.
Summary

• Novel mouse model for NS was generated by mimicking the mutation from NS patients

• KLK5 and KLK7 are responsible for skin barrier disruption of Spink5\(^{-/-}\) skin

• KLK7 causes severe skin-barrier defects in the proximity of hair follicles independently of KLK5 activation

• Bamboo hair defect does not depend on KLK5 or KLK7

• KLK5 and KLK7 together are required for inflammation and differentiation of Spink5 deficient epidermis

• Only simultaneous inactivation of KLK5 and KLK7 fully rescues lethality of NS-mouse model
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