KB104: an HSV-based gene therapy vector engineered to deliver functional SPINK5 for the treatment of Netherton Syndrome

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INTRODUCTION

Netherton Syndrome (NS) is a rare, debilitating autosomal recessive skin disorder that causes defective keratinization, severe skin barrier defects, and recurrent infections, affecting approximately 1:200,000 people worldwide. The disease arises due to mutations in the Serine Protease Inhibitor Kazal-type 5 (SPINK5) gene. In healthy individuals, SPINK5 is one of the serine protease inhibitors expressed in the outermost layers of the skin. In patients suffering from Netherton Syndrome, the suppressive effects of SPINK5 on these serine proteases is abolished due to the underlying genetic mutations in the *SPINK5* gene. Consequently, hyperactivated serine proteases in the skin cause uncontrolled desquamation, leading to a defective skin barrier. Clinically, NS is characterized by congenital ichthyosiform erythroderma, hair shaft defects, recurrent infections, and a defective skin barrier.

There are no approved therapies for Netherton Syndrome, and existing treatment options are limited to expensive and time-consuming palliative care. As such, novel targeted therapeutics are necessitated. To this end, we have developed KB104, a replication-defective HSV-1 gene therapy vector encoding human SPINK5 for direct topical application to the skin for the molecular correction of NS.

MATERIALS/METHODS

Test Article
KB104: Krystal Biotech, Inc.'s propriety replication-defective HSV-1 vector encoding optimized human *SPINK5*.

Table 1. Critical Reagents

Reagent Description:	Source:	Cat. No.:
SPINK5 ELISA Kit	LSBio	LS-F7754
Recombinant human KLK5	R&D Systems	1108-SE
KLK5 fluorogenic substrate Boc-VPR-AMC	R&D Systems	ES011
Rabbit anti-human SPINK5 antibody	R&D Systems	AF8515
Mouse anti-mouse Filaggrin antibody	Booster Biological	M01063
Alexa Fluor® 488-conjugated anti-rabbit antibody	Invitrogen	A11034
Alexa Fluor® 594-conjugated anti-mouse antibody	Abcam	Ab150120
Anti-rat IgG (H+L) cross-adsorbed, Cyanine5	ThermoFisher	A10525

RESULTS

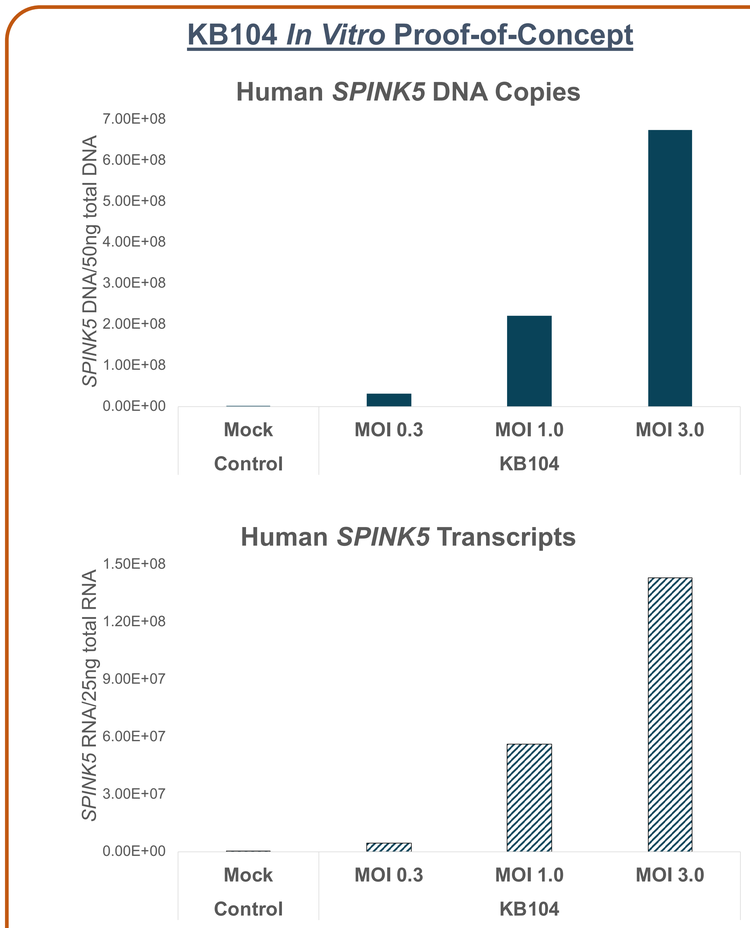

Figure 1. Dose-dependent increase in *SPINK5* DNA and transcript levels upon KB104 infection in immortalized normal human keratinocytes

RESULTS (CONTINUED)

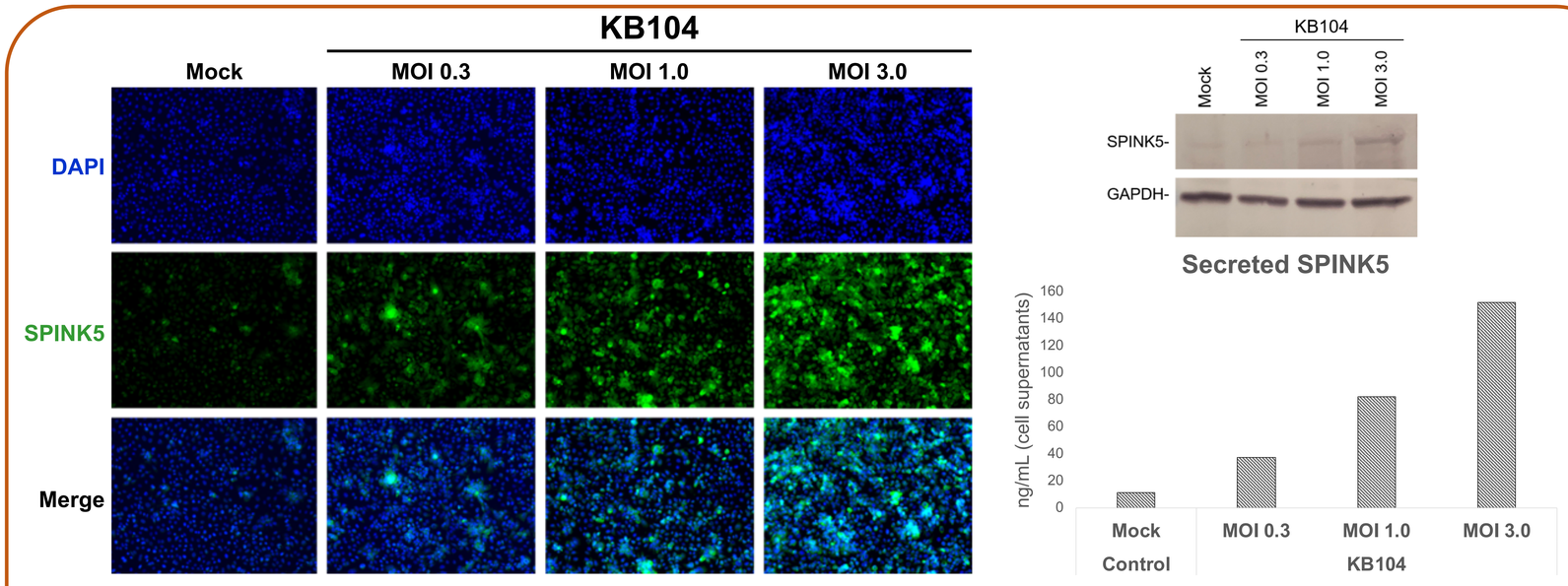

Figure 2. Dose-dependent increase in SPINK5 protein expression and secretion upon KB104 infection in immortalized normal human keratinocytes

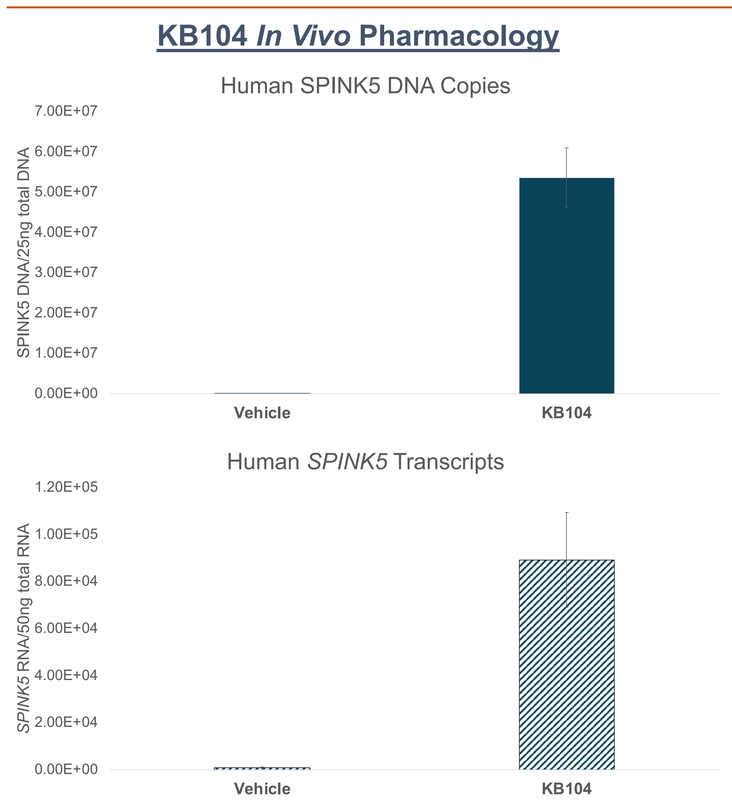

Figure 4. Robust expression of human *SPINK5* after topical delivery of KB104

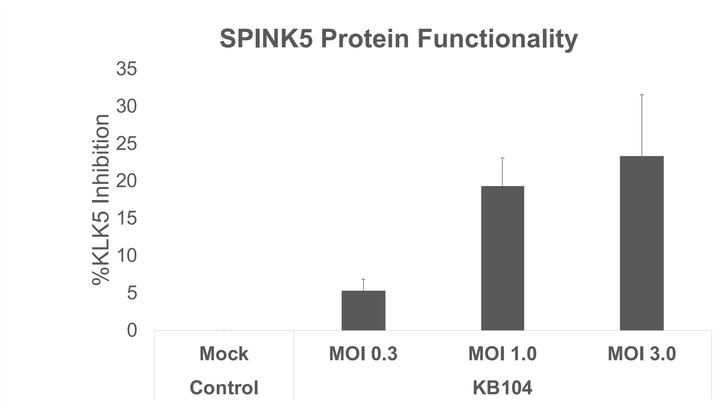

Figure 3. Dose-dependent increase in Kallikrein 5 (KLK) inhibition by SPINK5 secreted from KB104-infected immortalized normal human keratinocytes

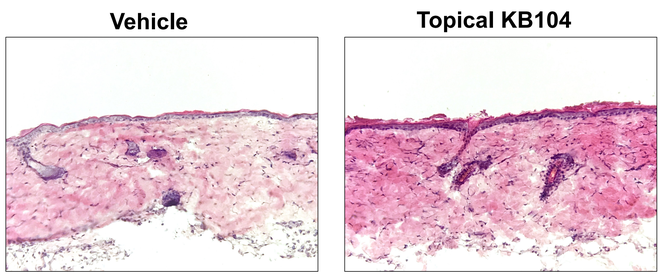

Figure 5. KB104-treated skin appears morphologically normal (comparable to vehicle-treated skin)

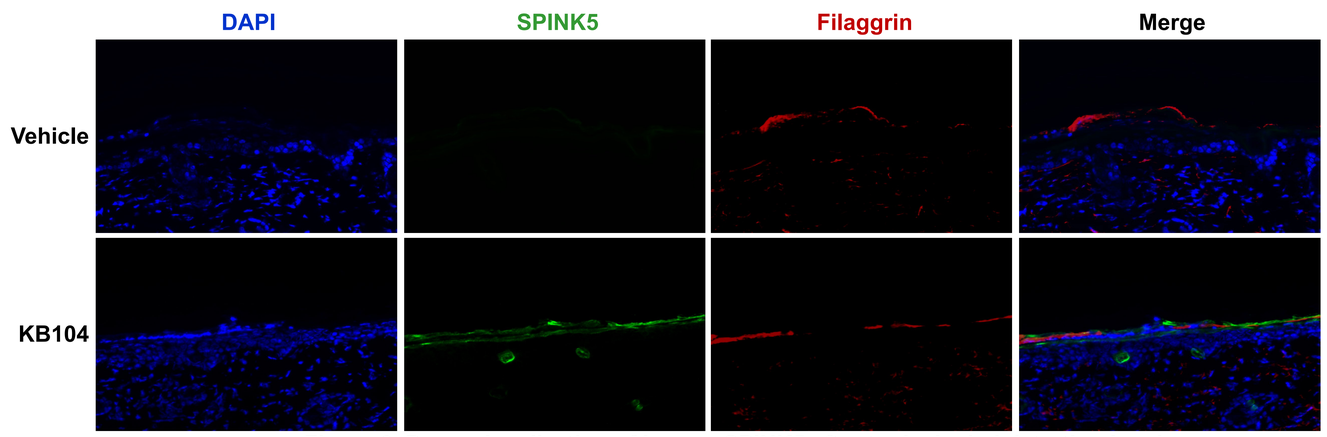

Figure 6. Proper localization of human SPINK5 after topical administration in mice

CONCLUSIONS

- Secreted KB104-expressed SPINK5 produced in human keratinocytes is functional, retaining its protease inhibitory activity
- Topical KB104 efficiently transduces mouse skin, is well tolerated, and KB104-expressed human SPINK5 is correctly localized to the epidermis
- These data support KB104 as a novel topical gene therapy for the treatment of Netherton Syndrome