Deletion of SPINK7 by CRISPR/Cas9 Elicits Pro-Inflammatory and Impaired Epithelial Barrier Responses in Esophageal Epithelial Cells

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Introduction: Eosinophilic esophagitis (EoE) is an inflammatory disorder characterized by eosinophil infiltration and impaired epithelial barrier which is triggered by immune hypersensitivity to food antigens. Previous studies indicate that the serine peptidase inhibitor kazal type *(SPINK)7* is constitutively expressed during esophageal epithelial differentiation and is depleted in EoE.

Hypothesis: We aimed to test the consequences of CRISPR/Cas9-mediated knockout (KO) of SPINK7, focusing on the hypothesis that SPINK7 deficiency might recapitulate EoE inflammatory features, at least in part.

Methods: The *SPINK7* locus was targeted for double-strand break introduction by transfecting human esophageal epithelial cells with a construct expressing Cas9 nuclease and identified gRNA. Control and SPINK7 KO cells were cultured at the air-liquid interface (ALI) or in high calcium-high confluency conditions to promote squamous cell differentiation. SPINK7 protein expression was analyzed by western blot, barrier integrity examined by trans-epithelial electrical resistance measurements during differentiation, and mRNA expression of filaggrin analyzed by quantitative PCR. Trypsin-like proteolytic activity and urokinase-plasminogen activator (uPA) activity were determined through functional assays. Additionally, pro-inflammatory cytokine release (i.e. thymic stromal lymphopoietin (TSLP) and interleukin (IL)-8) was assessed by ELISA.

Results: CRISPR/Cas9-mediated genetic editing produced genetic knockout of SPINK7 in human esophageal epithelial cells, verified by undetectable SPINK7 protein expression in knockout cells as compared to control cells. Functionally, SPINK7 KO cells produced lower transepithelial resistance during differentiation and exhibited decreased expression of filaggrin, indicating impaired epithelial barrier function in comparison to control cells. Loss of SPINK7 unleashed proteolytic activity observed by enhanced trypsin-like and uPA activities in SPINK7 KO compared to control cells. Further, a pro-inflammatory response was triggered with increased release of cytokines IL-8 and TSLP in SPINK7 KO as compared to control cells.

Conclusions: SPINK7 loss induces an EoE-like phenotype with impaired epithelial barrier integrity and unhindered proteolytic activity, as well as a pro-inflammatory cytokine response. Thus, SPINK7 deficiency during differentiation may weaken esophageal barrier function and initiate the inflammatory cascade of an allergic response. Verification of preliminary studies of SPINK7 loss with CRISPR/Cas9-edited cells validates the utility of the knockout model for further investigation of SPINK7 deficiency and potential drug screening.

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