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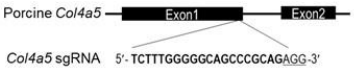
Proteomic analysis for urinary biomarkers in a novel Alport porcine model using CRISPR/Cas9

Gwangeon Sim[1], Hyunwoo Lee[1], Donghyun Kwon[1], Jaeheun Sin[1], Geumjoo Choi[1], Jineop Kim[1], Myeonkyu Cho[1], Jong-woo Yoon[1], Hyunho Kim[2], Hyunsuk Kim[1]
[1]Department of Internal Medicine-Nephrology, Chuncheon Sacred Heart Hospital, Korea, Republic of
[2]Department of Internal Medicine-Nephrology, Seoul National University Hospital, Korea, Republic of

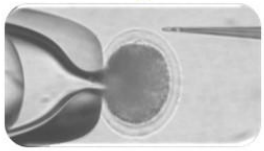
Objectives : Alport syndrome (AS) is an inherited genetic disorder of characterized with deafness and end-stage kidney disease. X-linked Alport syndrome (XLAS) reaches 80% of AS and XLAS is mainly caused by pathogenic variants in COL4A5. Several animal models of AS have been already developed, however, these models are limited to spontaneous AS in dogs and murine models. Pigs are highly similar with humans in size, anatomy and physiology.
Methods : We generated COL4A5 knockout pigs to serve as a model for XLAS research using CRISPR/Cas9 system. After confirming their modification in the COL4A5 gene by real-time PCR analysis, phenotype of the COL4A5 knockout pigs was characterized by the blood urea nitrogen (BUN) and creatinine (Cre) levels and glomerular structural abnormalities by electron microscopy. Additionally, proteomic analysis was performed using pig urine to find the urine biomarkers for Alport syndrome.
Results : COL4A5 mRNA of Alport syndrome porcine model was not observed in real-time PCR analysis. Alport syndrome porcine model showed proteinuria, high levels of BUN, interstitial fibrosis and then died at 26 to 28 months of age. In transmission electron microscope, the irregular thickening in glomerular basement membrane and the effacement of podocyte foot process were shown. To define the expression patterns of differentially expressed proteins (DEPs) in urine samples, hierarchical clustering was performed. In particular, a cluster of proteins that sequentially increased showed significantly enriched GO terms associated with proteolysis. In comparison, a cluster that sequentially decreased was significantly enriched in GO terms related to cell adhesion, locomotion and cell motility, suggesting that factors associated with cell-to-cell junctions were decreased concomitant with renal function deterioration.
Conclusions : These COL4A5 KO pigs can be a valuable model for mechanisms of disease and therapeutic development for XLAS and proteomic analysis using pig urine would help to identify the urine biomarkers.

슬라이드 1.JPG

CRISPR/Cas9로 체세포 유전체 편집



체세포 핵이식



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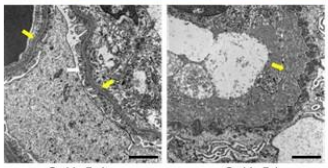

신장 사구체의 전자현미경 사진

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