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Promoting Sustainable Kidney Health: The Asia-Pacific and Beyond

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Proteomic analysis of urinary extracellular vesicles in patients of diabetic kidney disease

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Objectives : Diabetic kidney disease (DKD) is a devastating complication of diabetes, and 40% of patients are known to progress to ESKD. However, clinical indicators, such as eGFR or albuminuria, are insufficient to monitor the accurate kidney status. Recently, urinary extracellular vesicles (uEVs) are considered as attractive biomarker source, because uEVs equipped with parental biological components are secreted directly from nephron into urine, reflecting kidney function and histological changes. Therefore, we investigated the uEVs to identify new biomarkers exhibiting the pathophysiological status of DKD kidneys.
Methods : EVs were isolated from urine of DKD patients and normal control participants (NC) by a combined method of concentration, size exclusion chromatography, and ultracentrifugation. Size and concentration of uEVs were measured by nanoparticle tracking analysis. Confident isolation of uEVs was confirmed by Western blotting and TEM. Proteome of uEVs were identified by OrbiTrap Tribrid mass spectrometer coupled with liquid chromatography. MS files were analyzed by MaxQuant and Scaffold software (FDR<1%). Gene Ontology analyses of proteomes were conducted by DAVID database. Candidate proteins were validated by ELISA.
Results : From label-free quantitative proteomic analyses, significantly (p-value < 0.05) regulated proteins were identified between NC and DKD. Most of them were associated with pathological changes in the kidney resulting from the development of DKD. Especially, proteins involved in epithelial-to-mesenchymal transition, mTORC1 signaling, complement pathway, and immune response were highly enriched in DKD-uEVs. ELISA of uEVs against complement C3 and ceruloplasmin showed the significant overexpression in DKD with significant sensitivity and specificity, suggesting the potent biomarker to effectively diagnose the DKD.
Conclusions : In this study, we identified the uEV proteome affected by DKD status. In particular, complement C3 and ceruloplasmin are the most prominent biomarker to detect DKD. Therefore, this study suggests the feasibility of uEVs as a sensitive biomarker in liquid biopsy to monitor the pathophysiological status of kidney in DKD patients.

그림 1.jpg

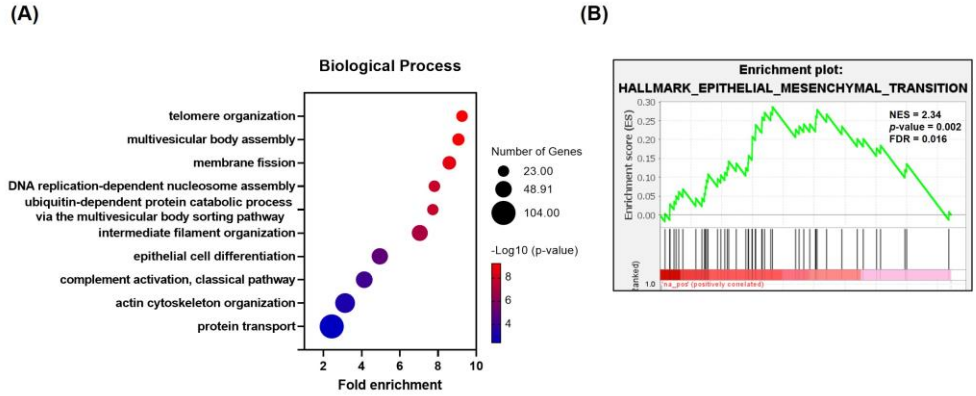
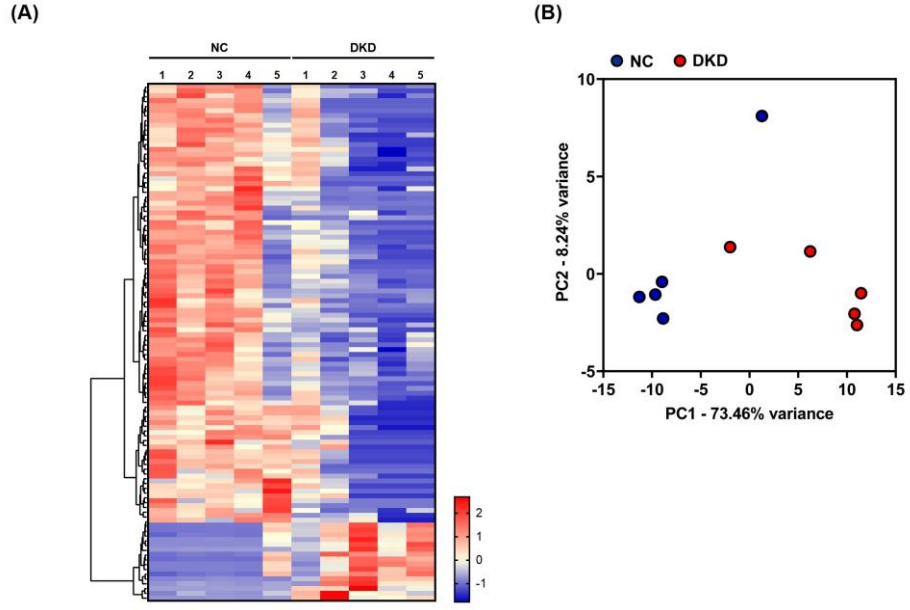

(A)
(B)
Biological Process
telomere organization
multivesicular body assembly
membrane fission
DNA replication-dependent nucleosome assembly
ubiquitin-dependent protein catabolic process via the multivesicular body sorting pathway
intermediate filament organization
epithelial cell differentiation
complement activation, classical pathway
actin cytoskeleton organization
protein transport
Number of Genes
23.00
48.91
104.00
-Log10 (p-value)
Fold enrichment
Enrichment plot:
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
NES = 2.34
p-value = 0.002
FDR = 0.016
Enrichment score (ES)

그림 1.jpg


(A)
(B)
NC
DKD
NC
DKD
PC2 - 8.24% variance
PC1 - 73.46% variance