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P463 -Exploratory proteomics of urinary vesicles in chronic kidney disease progression

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Introduction

Urine offers a direct readout from the diseased kidney, and as such is an excellent bio-fluid for chronic kidney disease (CKD) biomarkers. Despite this, studies analysing urine have only been partially successful due to a predominance of abundant plasma proteins, masking those originating from the cells lining the kidney tubules, which may better mirror disease progression. However, urine also contains nanosized vesicles (exosomes and ectosomes) released directly from kidney cells, which contain many low-abundance proteins that may be suitable CKD progression biomarkers. In a pilot study we aimed to determine the feasibility of using urinary extracellular vesicles (uEV) proteomics to monitor CKD progression.

Methods

Exosomes and ectosomes were isolated from pools of urine samples from 23 patients with stable CKD (< 1.78 mL/min eGFR loss per year; average eGFR loss = - 0.17 mL/min per year) and 18 patients with progressive CKD (> 2.52 mL/min eGFR loss per year; average eGFR loss = 6.94 mL/min per year), along with 10 control subjects with no evidence of CKD, as we previously described[1]. Stable and progressive CKD patients were stratified from cohort of patients with membranous nephropathy, IgAn nephropathy and autosomal polycystic kidney disease, sourced from the CKD-Urine Biorepository based at the Sheffield Kidney Institute (SKI), and at the University Hospital Patras, Greece. Tryptic peptides from uEV preparations were subjected to reverse-phase high-pressure liquid chromatography electrospray ionization tandem mass spectrometry (RP-HPLC-ESI-MS/MS), using a SCIEX TripleTOF 5600 mass spectrometer in data-dependent acquisition mode.

Results

In the uEV of endosomal origin (exosomes), 486 proteins were isolated from the pool of stable CKD patients, and 581 from the pool of progressive CKD patients, of which 52% proteins overlapped. Approximately 30% (213) proteins were uniquely found in the progressive CKD patients' exosomes. Likewise, in the uEV of plasma-membrane origin (ectosomes), 254 proteins were found in the pool of stable patients and 473 proteins were found in the ectosomes of the pool of progressive CKD patients, of which 35% overlapped, with 45% (266) proteins uniquely found in the progressive CKD patients' ectosomes. uEV-free urines displayed 393 proteins in stable and 326 proteins in progressive CKD patients, but notably only 12% (82) were unique in the progressive phenotype, indicating that exosomes and ectosomes offer a larger fingerprint of disease progression. A qualitative comparison to identify enriched biological functions in uEV from the patients' pools, using the Database for Annotation, Visualization, and Integrated Discovery (DAVID), showed several over-represented pathways in progressive uEV, of which the most significant ($p<0.05$) consisted of small GTPase mediated signal transduction in both exosomes and ectosomes. Furthermore, exosomes uniquely featured a pathway related to matrix remodelling (metallopeptidase Inhibitor 1 and matrix metalloproteinases) which is relevant to fibrosis progression underlying all CKD forms.

Conclusions

Collectively these data from patients' pools with either stable or progressive CKD suggest that uEV derived from the kidney offer the potential for novel non-invasive biomarkers of CKD progression, and show that urinary exosomes and ectosomes display an exclusive fingerprint which may represent an alternative platform to monitor CKD progression.