Identification of novel polycystin-associated cargo of extracellular vesicles (EVs) in *Caenorhabditis elegans*

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Introduction: Urinary extracellular vesicles (EVs) are an emerging source of biomarkers for autosomal polycystic kidney disease (ADPKD). EVs isolated from ADPKD patients differ from EVs of unaffected patients and promote cystogenesis in 3D culture. The identity of cargo proteins on individual EV subtypes in biofluids such as urine is unknown, as is the role they play in EV biogenesis, signaling, and ADPKD progression. Here we use *C. elegans* animal model to answer fundamental questions in the field of ADPKD: (i) what proteins co-localize with polycystins on EVs, and (ii) what role the polycystin-associated proteome plays in the physiology of ciliated cells and in bioactive EVs.

Methods: Using proximity labeling methodology in *C. elegans*, we recently identified a conserved group of polycystin-associated EV cargo. For that, we engineered targeting of a proximity-labeling enzyme TurboID to the evolutionarily conserved polycystin PKD-2::GFP EVs using an anti-GFP nanobody domain. Targeted EVs were enriched using buoyant density centrifugation, followed by pulldown of biotinylated proteins. Mass spectrometry analysis revealed 15 candidate interactors of PKD-2, as opposed to 2,888 EV cargo of the whole EV proteome. For validation, we generated CRISPR knock-in fluorescent reporters for the top 8 hits and analyzed their trafficking to ciliary PKD-2 EVs.

Results: The novel polycystin-associated cargo included adhesion receptor proteins, suggesting a role in EV targeting, and soluble cargo, suggesting a role in EV signaling. We are currently dissecting genetic pathways that direct polycystins and their functional interactors to EVs at the single-cell/single EV subtype resolution using super-resolution *in vivo* imaging of the knock-in reporters in *C. elegans*.

Conclusions: Use of the *C. elegans* model in ADPKD research enables identification of signaling modules assembled around polycystins on EVs. This research has a potential to reveal novel biomarkers for the ADPKD diagnostics.

Funding Sources: NIH DK059418, DK116606, NS120745