Defining polycystin extracellular vesicle (EV) subtypes by single-EV molecular analysis in *Caenorhabditis elegans*

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Introduction: Urinary extracellular vesicles (EVs) are emerging biomarkers for autosomal polycystic kidney disease (ADPKD). EVs derived from ADPKD patients facilitate cystogenesis in 3D cell culture, yet the specific cargo and the contributions of these cargo to EV signaling and ADPKD progression are not understood. Here we use the *C. elegans* animal model to address critical questions in the ADPKD and EV fields: (i) the identification of proteins co-localizing with polycystins on EVs, and (ii) the roles played by the polycystin-associated proteome in ciliated cells and in bioactive EVs.

Methods: Here, we use proximity labeling, super-resolution microscopy, and genetic perturbations in *C. elegans* to define distinct EV subtypes. We identified and validated a conserved group of polycystin-associated EV cargo, including TRAFs (tumor necrosis factor TNF receptor-associated factors), transmembrane lectins, and putative cation channels.

Results: Our findings demonstrate that polycystins are crucial for selecting and targeting cargo to ciliary EVs. We discovered that TRAF signaling adaptors rely on polycystins for loading to ciliary EVs, but not for entering the cilium. Disruption of polycystin-1/LOV-1 leads to the ciliary release of polycystin-2/PKD-2 EVs that lack TRAFs, while polycystin-2/PKD-2 is essential for targeting of polycystin-1/LOV-1 and TRAFs to ciliary EVs. These findings suggest that the *C. elegans* polycystin pathway employs TRAFs for EV-based signaling, in line with clinical observations of aberrant TNF signaling and altered urinary EV proteome in ADPKD.

Conclusions: Our proximity labeling approach holds promise for systematically defining numerous EV subtypes. Use of the *C. elegans* model in ADPKD research enables identification of signaling modules assembled around polycystins on EVs. This study is the first to demonstrate the profound impact of a single genetic perturbation on the composition of a single EV subtype, highlighting the tremendous diagnostic potential of a single EV analysis for ADPKD diagnostics.

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