**PO1231**

**Suppressed Autophagy Drives Increased Cellular Metabolic Activity in Human ADPKD Cells**

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**Background:** We published that the autophagy phenotype in Pkd1<sup>−/−</sup> mouse kidneys is characterized by decreases in crucial autophagy proteins (Cell Signal 2020). We attempt to determine the mechanistic role of suppressed autophagy as it relates to cell metabolic activity (viability and proliferation) in ADPKD cells.

**Methods:** Human primary immortalized cultured cells were used: normal renal cortical tubular epithelium (RCT, Pkd1<sup>+/+</sup> and ADPKD cyst-lining epithelium (9-12, Pkd1<sup>−/−</sup>). To measure autophagic flux, cells were treated with lysosomal inhibitor chloroquine (C) and Lc3 (immunoblot), a marker of autophagosomes or mCherry Lc3 (fluorescence) was monitored. ATTY assay was used to measure cellular metabolic activity (cell viability and proliferation). Relative densitometry units (RDU) were measured on immunoblots.

**Results:** There was an increase in MTT and a decrease in AnnV in 9-12 vs RCT cell. MTT OD was 0.36 in RCT vs 0.44 in 9-12 (p<0.01). Annexin A5 (AnnV) (% gated) marker of apoptosis, was 67 in RCT vs 18 in 9-12 (p<0.001). The increase in LC3-II in C with RCt was not seen in 9-12 indicating suppressed autophagy flux. LC3-II (RDU) beta C was 0.6 vs 1.4 in RCT (P<0.01) and 0.9 vs 1.1 (NS) in 9-12. mCherry (% gated) beta C was 6 vs 16 (p=0.05) in RCT and 6 vs 13 (NS) in 9-12 cells. p62 marker of autophagic cargo, was increased in 9-12 vs RCT. p62 (RDU) was 0.7 in RCT and 1.2 in 9-12 (p<0.05). Cells were treated with an ATG7 shRNA (SH) to inhibit a crucial autophagy protein. There was a 50% decrease in LC3-II and ATG7 protein in SH-treated 9-12 cells, SH resulted in an increase in MTT and a decrease in AnnV indicating that suppressed autophagy drives MTT and inhibits apoptosis. MTT OD was 0.7 with scrambled shRNA (SCR) and 0.9 with SH (p<0.05). Tat-Beclin peptide (TAT), a specific autophagy inducer, resulted in a decrease in MTT in 9-12 suggesting that autophagy decreases MTT. MTT OD was 0.95 with Veh vs 0.2 with TAT (p<0.001). TAT did not affect AnnV.

**Conclusions:** In ADPKD cyst lining epithelial cells there is increased MTT, suppressed autophagic flux, and decreased apoptosis. Autophagy inhibition increased MTT and suppressed proliferation. Suppressed autophagy driven increases in cellular metabolic activity in ADPKD cells. The effect of autophagy induction/inhibition on cyst growth in vivo merits further study.

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**Tsc2 Mutation Induces Renal Tubular Cell Nonautonomous Disease**

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**Background:** TSC renal cystic disease is poorly understood and has no approved treatment. In a new principal cell-targeted murine model of Tsc cystic disease, the renal epithelial cysticis is mostly composed of type A intercalated cells with an intact Tsc2 gene confirmed by sequencing; although, these cells exhibit a Tsc mutant disease phenotype.

**Methods:** We used a newly derived targeted murine model in lineage tracing and extracellular vesicle (EV) characterization experiments and a cell culture model in EV characterization and cellular induction experiments to understand TSC cystogenesis. For the lineage tracing experiments we used Aquaporin-2 Cre, Floxed Tsc2, and Confetti mice in breeding experiments. To characterize the EVs, we used tunable resistive pulse characterization and cellular induction experiments to understand TSC cystogenesis. For single cell RNA sequencing, parabiosis, and fate mapping on kidneys isolated for a single cell line can vary significantly. mTORC1 inhibition reduces EV production. mTORC1 pathway activity is independent form the EV production, and that the EV effects develop as increased secretory and proliferative pathway activity. We demonstrate that the mTORC1 pathway activity is independent form the EV production, and that the EV effects for a single cell line can vary significantly. mTORC1 inhibition reduces EV production.

**Conclusions:** TSC cystogenesis involves significant contribution from genetically intact cells conscripted to the mutant phenotype by mutant cell derived EVs.

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**PO1233**

**Exploring the Heterogeneity of Kidney Resident Macrophages Using Single-Cell RNA Sequencing**

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**Background:** Tissue resident macrophages are highly diverse, even when located within tissue similar functionality is thought to be driven by localization, tissue- and environment-specific cues. Importantly, these underlying factors likely influence resident macrophage phenotype and function during disease initiation and progression.

**Methods:** To understand the diversity of kidney resident macrophages (KRM), we performed single cell RNA sequencing, parabiosis, and fate mapping on kidneys isolated from wild type and transgenic knock-in reporter mice.

**Results:** Using single cell RNA sequencing, we identified three subpopulations of KRM including one with enriched expression of Ccr2. Using Ccr2-RFP knock-in reporter mice and Rosa Sca1<sup>+</sup> flow mice, we confirm that these resident macrophages were derived from monocyte precursors and preferentially localize to the renal cortex. Based on our single cell RNA sequencing data, we propose that monocytes undergo a series of differentiation steps upon entering the kidney in order to become Ccr2<sup>+</sup> KRM.

**Conclusions:** Collectively, our data indicate that monocytes undergo a series of differentiation steps upon entering the kidney and require Cx3crl1 for differentiation into pathogenic Ccr2<sup>+</sup> KRM.

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