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The role of the exocyst in renal ciliogenesis, cystogenesis, and tubulogenesis

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Primary cilia are centrally involved in the pathogenesis of polycystic kidney disease (PKD). In a series of papers, we previously showed in Madin Darby canine kidney (MDCK) cells that shRNA-induced knockdown of Exoc5, a central component of the highly-conserved eight protein exocyst complex, inhibited ciliogenesis, led to increased cell proliferation, and resulted in low intracellular calcium levels that did not increase in response to fluid flow. Cell polarity was not grossly affected. Exoc5 overexpression in MDCK cells resulted in longer cilia, with normal intracellular calcium levels that showed an enhanced response to fluid flow (Zuo et al, Mol. Biol. Cell, 2009). We next showed in zebrafish that exoc5 knockdown, with antisense morpholinos, resulted in altered ciliogenesis, nephrogenesis, and cardiac edema (Fogelgren et al, PLoS Genetics, 2011). We then generated $Exoc5^{fl/fl}$mice, and kidney-specific knockout of Exoc5 resulted in a nephronophthisis phenotype in mice surviving to 30 days (Fogelgren et al, PLoS One, 2015). We also showed that Cdc42 localizes the exocyst to the nascent cilium, and that Cdc42 and the exocyst genetically interact in zebrafish. We generated Cdc42 kidney-specific knockout mice, which also resulted in a nephronophthisis phenotype (Choi et al, J. Am. Soc. Nephrol., 2013). The exocyst is found in most cell types and has been linked by us and others to a wide variety of cellular processes, including: vesicular transport to the basolateral membrane (Lipschutz et al, Mol. Biol. Cell, 2000), primary ciliogenesis in the kidney and eye (Çhoi et al, Invest. Ophthalmol. Vis. Sci, 2015), protein synthesis in the endoplasmic reticulum (Lipschutz et al, J. Biol. Chem, 2003), and post-endocytic recycling. To investigate the exocyst functions, we recently exchanged proline for alanine in the highly conserved VxPx ciliary targeting motif of EXOC5 and generated stable EXOC5 ciliary targeting sequence-mutated MDCK cells. These experiments demonstrated that the exocyst, acting through the primary cilium, is necessary for renal ciliogenesis, cystogenesis, and tubulogenesis (Zuo et al, J. Biol. Chem., 2019).

Cardiac valve abnormalities, especially bicuspid aortic valve disease (BAV) which affects ~1% of humans, are associated with various forms of PKD, including ADPKD and Joubert syndrome. Our recent work showed that primary cilia are present on developing cardiac valves, but are reduced to absent in adulthood, and that normal valve development requires functional cilia (Toomer et al, Dev. Dynamics, 2017). Mutations in Exoc8 have been shown to result in Joubert syndrome, a nephronophthisis disorder. We performed a genome wide association (GWAS) and replication study using cohorts of 2,131 BAV and 2,728 control patients, respectively, which identified primary cilia genes, and especially the exocyst, as being associated with the BAV phenotype.We found that homozygous mutation of *exoc5* in zebrafish leads to a non-functional protein and reproduces the morphant phenotype, with defects that include cardiac edema and severe cardiac valvular stenosis. Rescue of *exoc5* mutants with human EXOC5 mRNA rescued the phenotype, while ciliary targeting sequence-mutated EXOC5 mRNA did not efficiently rescue the phenotype. In *exoc5* mutant zebrafish, the Hippo and MAPK pathways are turned on with significantly higher levels of active Mob1 and pERK. The Hippo and MAPK pathway have previously been linked, by us and others, to PKD. In mice, cardiac valve specific knockout of Exoc5, using both Tie2 and Nfatc1 driver lines, led to bicuspid aortic valve disease that is highly penetrant. Cardiac valvulogenesis, therefore, is dependent on normal ciliogenesis, and ciliogenesis in cardiac valves, in turn, is dependent on the exocyst. These data show that ciliogenic programs are conserved across organs and species, which helps explain the association of cardiac valve abnormalities and PKD. In addition, these animal models may be useful for testing therapeutic compounds.