# mTOR inhibition attenuates cyst formation in *tmem67*-based cystogenesis in zebrafish

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INTRODUCTION

Polycystic kidney disease (PKD) is largely considered a ciliopathy because many PKD-causative genes are localized to the cilium and required for the formation and function of the cilium. TMEM67 is a ciliary transition zone protein and mutations in *TMEM67* lead to Meckel syndrome (MKS), a syndromic form of PKD. Depletion of TMEM67 results in various cilia phenotypes, however, the relationship between cilia defects and cystogenesis remains unclear.

METHODS

Using the transcription activator-like effector nucleases technology, we generated stable zebrafish mutants for *tmem67*. Because homozygous *tmem67* mutants survived to adulthood, we examined ciliogenesis and cystogenesis in both the zebrafish embryos and adult kidneys. We also assessed the role of mTOR signaling in the mutants via genetic manipulation and drug treatment.

RESULTS

While some homozygous *tmem67* mutants developed kidney cysts during embryogenesis, all mutants can survive to adulthood and developed renal cysts. Cyst in the adult fish kidney manifested features of the mammalian PKD, including progressive tubular dilation, switch of cyst origin from the proximal tubules to collecting ducts, and increased proliferation of the cyst-lining epithelial cells. In contrast to partially penetrant pronephric cysts, all mutant embryos exhibited cilia phenotypes - single cilia in the distal pronephros were shorter at first and then missing, and the number of multi-ciliated cells was increased. The similar cilia defects were also detected in the kidney of adult mutants. Importantly, the cilia abnormality precedes cystogenesis in both embryos and adults. Lastly, we found hyperactive mTOR signaling in the mutants, and that mTOR inhibition ameliorates renal cysts. Preliminary observation also noted attenuated cilia defects by mTOR inhibition.

CONCLUSIONS

We established *tmem67* zebrafish as the first PKD model in adult zebrafish. Prompted by our data, we propose that disrupted ciliogenesis is primary to later cystogenesis and that mTOR signaling could be a therapeutic target for *tmem67*-based cystogenesis.