Increased Stat3 activation in pre-cystic kidneys of *Thm1* conditional knock-out mice

Luciane M. Silva[1], Sumedha S. Gunewardena[2], and Pamela V. Tran[1]

[1]Department of Anatomy and Cell Biology, Jared Grantham Kidney Institute, University of Kansas Medical Center, Kansas City, KS
[2]Department of Integrative and Molecular Physiology, University of Kansas Medical Center, Kansas City, KS

INTRODUCTION

Primary cilia are sensory organelles that mediate signaling pathways, and ciliary dysfunction leads to renal cystic disease. Multiple cellular and signaling aberrations contribute to renal cystogenesis, while initiating molecular events remain undefined. In mice, perinatal global deletion of ciliary gene, *Thm1*, causes renal cysts beginning at postnatal day (P) 15.

METHODS

To identify molecular events that initiate renal cystogenesis in *Thm1* conditional knockout (cko) mice, we performed RNA sequencing on kidney RNA lysates of pre-cystic P9 and cystic P42 *Thm1* cko mice and control littermates. We reasoned that genes with significantly altered expression at both P9 and P42 would represent early initiation events leading to cystogenesis.

RESULTS

We identified 10 genes significantly upregulated at P9 and further upregulated at P42. These included *endothelin 1* (*Edn1*), *fos*, *jun*, and *Stat3*, endothelial *Vcam1*, and immune genes, *Complement C3*, *Egr2* and *Adcy7*. Western blot analysis showed increased phospho-STAT3 at P10, and upregulation of multiple components of STAT and EDN1-MAPK signaling pathways at P42. Immunohistochemistry revealed more intense nuclear localization of phospho-STAT3 in epithelial cells of non-dilated and dilated tubules at P20 and in cyst-lining epithelial cells and interstitial cells at P42. To study the connection between primary ciliary dysfunction and upregulated STAT3 signaling, we have generated *Thm1* knock-down human renal 293T clonal cell lines that show shortened primary cilia with IFT81 accumulation in a bulb-like structure at the distal tip, indicative of a retrograde ciliary protein trafficking defect.

CONCLUSION

Our data reveal upregulated Stat3 activation in pre-cystic *Thm1* cko kidneys and suggest that simultaneous alteration of gene expression and signaling in renal epithelial, vascular and immune cells may potentiate renal cyst initiation. Efforts are underway to examine the mechanisms by which a *Thm1*-deficient ciliary defect causes increased Stat3 activation and to inhibit Stat3 signaling pharmacologically in *Thm1* cko mice. This may reveal initiating mechanisms underlying *Thm1*-deficient renal cystogenesis.