mTORC1 signaling is involved in nephron size control in the metanephric kidney

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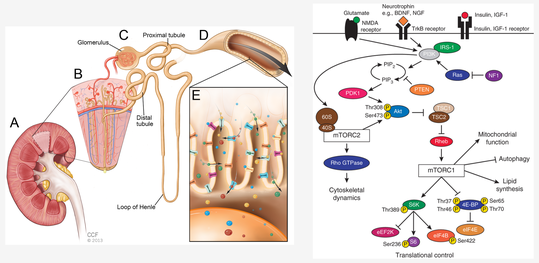
ABSTRACT

The nephron is the functional unit of the kidney that uses channels and transporters to regulate water concentration and recover essential biomolecules. Previous studies in mouse have shown that the proximal tubules, which are primarily responsible for biomolecule recovery, undergo a rapid burst of cell proliferation. Other studies suggest that the development and growth of proximal tubules in *Xenopus* is regulated by the developmental signaling pathway mTORC1. Here we now identified that various mTORC1 signaling molecules are expressed in mouse kidneys and that they follow a pattern that mimics the previously identified growth cycle. Additionally, comparing mouse kidneys lacking mTORC1 signaling to those with an intact pathway demonstrates, size variations in the kidney structures. Together these data suggest that the mTORC1 pathway is crucial in the development of mouse kidneys and follows a similar expression pattern in the proximal tubules as the *Xenopus* model.

INTRODUCTION



The kidney is responsible for filtering blood and recovering necessary molecules such as ions, glucose, and amino acids from the primary filtrate. Postnatally, the proximal tubule of the nephron expands its surface area in several ways, one of which is through tightly regulated cell proliferation[1]. The current mechanisms involved in this regulation are still being identified. One candidate for this regulation is mTORC1 signaling, a pathway, which is involved in regulating growth and development through mechanisms including stimulation of protein, lipid, and nucleic acid biosynthesis, promoting ATP production, and inhibiting frequent autophagy and lipid degradation[2]. Studies in *Xenopus laevis*, a species of aquatic frogs, showed that normal proximal tubule development is characterized by a rapid cellular proliferation phase. Additionally, blocking the mTORC1 signaling pathway with pharmaceutical inhibitors reduces proximal tubule growth[3]. The pronephric kidney of *Xenopus* larvae consists of only one nephron, while humans and mice have a more complex metanephric kidney comprised of many more nephrons. The goal of this study was to identify the role of the mTORC1 pathway in metanephric kidney development and illuminate how this impacts kidney size regulation.

METHODS

- Immunochemistry on postnatal kidney sections using antibodies and staining with LTA and DBA
- Mouse breeding technique using Wnt4-Cre recombinase promoter to excise the RAPTOR gene from nephrons
- In situ hybridization of postnatal kidney sections using RNA antisense probes
- Image acquisition using confocal microscopy

RESULTS

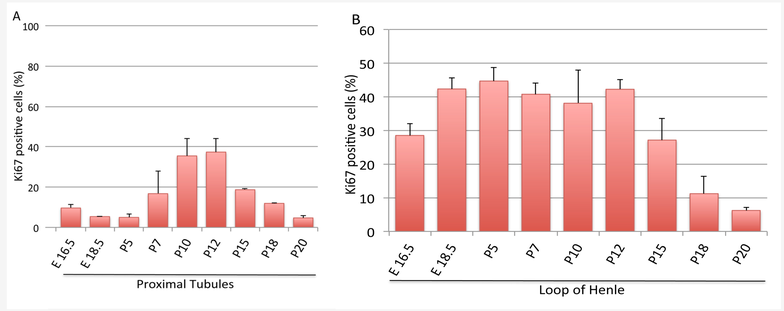


Figure 1. Analysis of embryonic and postnatal growth of proximal tubules reveals a burst of proliferation not present in other nephron segments. Proliferating cell number was determined by counting the percentage of Ki67-positive cells compared to the total number of DAPI-positive cells.

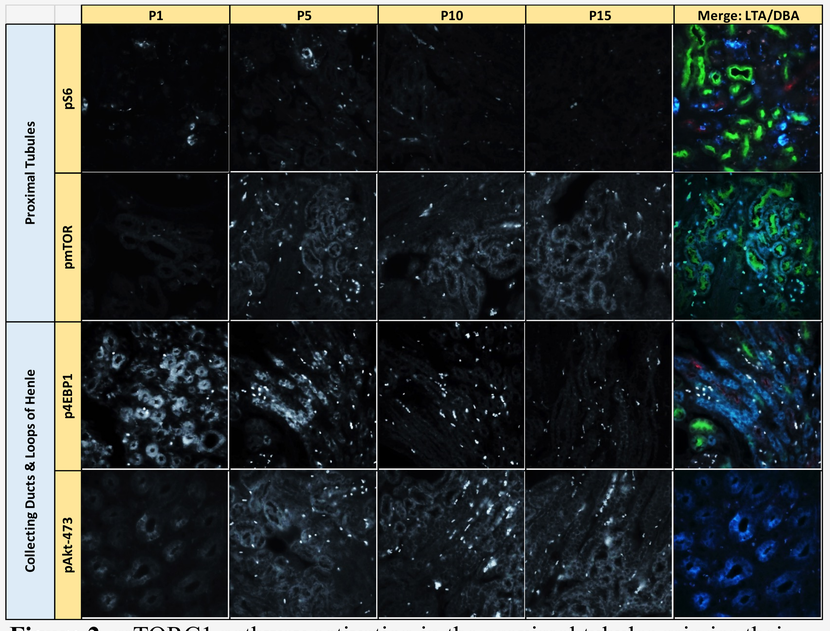


Figure 2. mTORC1 pathway activation in the proximal tubules mimics their proliferation pattern. LTA (proximal tubules, green) and DBA (collecting ducts, red), stains were performed in conjunction with antibodies assessing mTORC1 signaling (aqua) to identify their localization in the kidney.

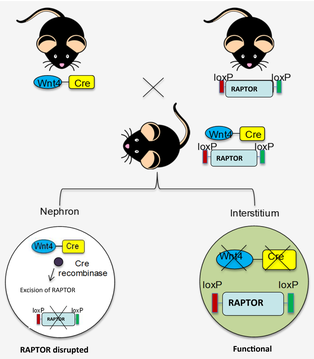


Figure 3. Breeding strategy using a Wnt4-Cre recombinase mouse strain and conditional allele of RAPTOR to excise the RAPTOR gene from kidney nephrons, while preserving its expression in the interstitium.

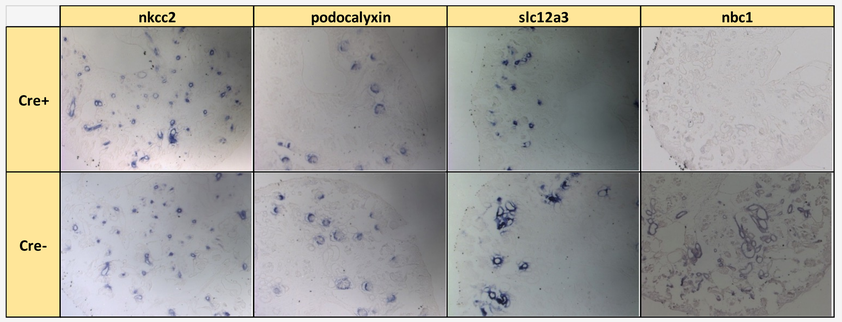


Figure 4. In situ hybridization using RNA antisense probes detecting Nkcc2 (thick ascending loop of Henle), Podocalyxin (glomerulus/podocytes), Slc12a3 (distal convoluted tubule), and Nbc1 (proximal tubules). Note that postnatal kidneys lacking the nephronic RAPTOR gene exhibit smaller distal convoluted tubules and negligible proximal tubules, while the size of other nephron segments remains unaffected.

CONCLUSIONS

The mTORC1 pathway is involved in the development of the metanephric kidney. It is activated postnatally in a manner that mimics the burst of proliferation seen in *Xenopus laevis* as well as previous mouse studies. Additionally, the expression of RAPTOR plays a role in the size control of proximal tubules during development.

FUTURE DIRECTIONS

- Quantify protein levels of the mTORC1 pathway at each stage
- Identify mechanisms for RAPTOR's control of proximal tubule size
- Explore a link between RAPTOR and size control of other nephron segments

REFERENCES

1. The bigger the better: determining nephron size in kidney
2. Regulation of mTORC1 by PI3K signaling
3. microRNAs are critical regulators of tuberous sclerosis complex and mTORC1 activity in the size control of the *Xenopus* kidney

ACKNOWLEDGEMENTS

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