


## Renal cystic disease in tuberous sclerosis complex

Prashant Kumar<sup>1,2,\*</sup>, Fahad Zadjali<sup>1,2,3,\*</sup>, Ying Yao<sup>1,2</sup> and John J Bissler<sup>1,2,4</sup> 

<sup>1</sup>Department of Pediatrics, University of Tennessee Health Science Center and Le Bonheur Children's Hospital, Memphis, TN 38105, USA; <sup>2</sup>Children's Foundation Research Institute (CFRI), Le Bonheur Children's Hospital, Memphis, TN 38105, USA; <sup>3</sup>Department of Clinical Biochemistry, College of Medicine & Health Sciences, Sultan Qaboos University, Muscat, PC 123, Oman; <sup>4</sup>Pediatric Medicine Department, St. Jude Children's Research Hospital, Memphis, TN 38105, USA

Corresponding author: John J Bissler. Email: jbissler@uthsc.edu

\*These authors contributed equally to this work.

### Impact statement

Tuberous sclerosis complex renal disease has a complex mechanisms of expression. Recent findings suggest that the mechanism for tuberous sclerosis complex-associated renal cystic disease is somewhat different from that reported for associated angiomyolipomata. Tuberous sclerosis complex renal cystic disease is a cell nonautonomous trait mediated by extracellular vesicles that conscript genetically normal cells to participate to a large extent in the disease phenotype. These findings offer new insights and inroads to therapies for this rare disease.

### Abstract

Tuberous sclerosis complex (TSC) is associated with *TSC1* or *TSC2* gene mutations resulting in hyperactivation of the mTORC1 pathway. This mTORC1 activation is associated with abnormal tissue development and proliferation such that in the kidney there are both solid tumors and cystic lesions. This review summarizes recent advances in tuberous sclerosis complex nephrology and focuses on the genetics and cell biology of tuberous sclerosis complex renal disease, highlighting a role of extracellular vesicles and the innate immune system in disease pathogenesis.

**Keywords:** Tuberous sclerosis complex, renal cysts, extracellular vesicles, cell nonautonomous trait

**Experimental Biology and Medicine 2021; 246: 2111–2117. DOI: 10.1177/15353702211038378**

### Introduction

Tuberous sclerosis complex (TSC)-associated renal phenotypes vary considerably. These differences complicate the accurate diagnosis and thus proper treatment. Eighty percent of affected children,<sup>1</sup> and all adult postmortem samples<sup>2</sup> have renal disease, most commonly renal angiomyolipomata and cysts. Compared to the general population, 40% of TSC patients have decreased renal function even without angiomyolipomata hemorrhage or surgical interventions.<sup>3</sup> TSC-associated renal disease has been reported to be the most common cause of death in adults with TSC.<sup>4,5</sup> The exact number of TSC patients that go on to dialysis is unclear, in part because TSC is a rare disease and not always available as a diagnosis code for end-stage renal disease (ESRD) databases.

### Genetics of TSC

TSC, designated as an autosomal dominant disorder, can impact human development and postnatally growth leading

to lesions throughout the body. The birth incidence is ~1:5800 and results in over one million affected worldwide.<sup>6,7</sup> The condition may be missed if one limits the diagnostic criteria to facial angiofibromas, developmental delay, and intractable epilepsy, as fewer than 40% of patients have these features.<sup>8</sup> Approximately half of TSC patients experience behavioral disorders like autism or cognitive impairment.<sup>8</sup>

There are two gene loci associated with TSC. The *TSC1* gene is on chromosome 9 and the *TSC2* gene is on chromosome 16. Identification of the *TSC2* gene locus was facilitated by a family with who suffered with autosomal dominant polycystic kidney disease (ADPKD) due to a translocation involving the *PKD1* gene. The *TSC2* gene was discovered the proband in this family had both ADPKD and TSC as they had inherited an unbalanced translocation.<sup>9</sup> Although both the TSC loci gene products function together to regulate the mTORC1 pathway activity, the renal disease is more severe when associated with the *TSC2* gene.

## Cell biology of TSC renal angiomyolipomata

Significant progress has been made focusing on the genetic mechanisms at play in TSC-associated angiomyolipomata. Angiomyolipomata belong to the PEComa family of tumors and exhibit both smooth muscle and melanocytic differentiation characteristics.<sup>10,11</sup> Histologically, angiomyolipomata contain abnormal blood vessel wall architecture surrounded by muscle-like perivascular cells and adipocyte-like cells. The interaction of the mTORC1 pathway with the microphthalmia-associated transcription factor (MITF) family helps to explain why angiomyolipomata express the melanotic marker PMEL (premelanosome protein, also known as SILV, gp100), the antigenic target for the diagnostic human melanoma black (HMB)-45 antibody.<sup>12</sup> This associated MITF/transcription factor E (TFE) transcription factor activity has confounded the diagnostic boundary between TSC-associated renal lesions and translocation renal cell carcinomas and PEComas.<sup>13</sup>

Neural crest cells have been posited to give to angiomyolipomata because of the co-expression of melanocytic and smooth cell markers.<sup>14–16</sup> This possibility has been challenged by *TSC2* re-expression experiments in an angiomyolipoma cell line because this introduction changed the expression profile to resemble lymphatic endothelium rather than neural crest derived tissues.<sup>17</sup> A dual knock-out of *Tsc2* and *Cdkn2a* in murine epithelial cells altered the cell expression to be somewhat similar to angiomyolipoma when injected into immunocompromised mice.<sup>18</sup> However, *Cdkn2a* mutations are found in melanoma, raising concern over the interpretation of the HMB-45 staining. Mesenchymal stem cell-like cells also have been identified in angiomyolipomata.<sup>19</sup> Pericytes may give to angiomyolipoma<sup>20</sup> and this may explain the association of TSC with vascular aneurysms that arise in angiomyolipomata,<sup>21</sup> aorta,<sup>4</sup> and brain.<sup>22</sup>

While there are still many questions about the TSC-associated angiomyolipoma, the genetic mechanism of disease is less of an enigma. TSC-associated angiomyolipoma is thought to be phenotypically expressed by a second somatic mutation or epigenetic silencing event of the TSC non-mutant allele. A similar mechanism has been forwarded for the cystic disease in both TSC and ADPKD, though the supporting evidence for this mechanism is less robust.<sup>23,24</sup> One locus for both TSC and ADPKD is located on chromosome 16, specifically the *PKD1* and the *TSC2* loci. These loci account for about 70–80% of patients with ADPKD or TSC, respectively, and the disease associated with these genes is more severe than that associated with *PKD2* or *TSC1*. The *PKD1* and *TSC2* genes are adjacent in a tail-to-tail orientation. This configuration is important because intron 21 of the *PKD1* gene contains a polypurine-polypyrimidine tract that can adopt alternative DNA conformations.<sup>25</sup> This odd tract interferes with DNA replication in a polar fashion causing DNA double-strand breaks, facilitating mutagenesis.<sup>26,27</sup> Synergizing with this DNA damage, the renal osmolality inhibits DNA damage recognition,<sup>28–30</sup> creating the “perfect storm” for potential somatic mutation.

## Cell biology of TSC renal cystic disease

Many inherited renal cystic diseases are associated with the primary cilia, an organelle thought to be involved in sensing the external environment.<sup>31</sup> Likewise, perturbations in the mTORC1 pathway are associated with renal cystic disease,<sup>32,33</sup> and deflection of primary cilia has been shown to regulate the mTORC1 pathway.<sup>34,35</sup> Unilateral nephrectomy in transgenic mice that have undergone an “adult-induced” cilia disruption exhibited significantly higher levels of mTORC1 activity with increased cyst severity in the remaining kidney compared to the cilia-intact controls.<sup>36</sup>

The mechanism of disease is more nuanced because although the polycystic disease-related proteins and primary cilia are mechanistically involved in polycystic kidney disease, they have a complex interaction. The loss of a polycystin protein *or* the primary cilia results in similar renal cystic disease phenotypes, while simultaneous inactivation of both the polycystin gene *and* cilia expression cause a decrease in renal cystic disease severity compared with polycystin-only knockout mice.<sup>37</sup> The polycystins therefore appear to modulate an unidentified signaling pathway that requires intact cilia to function, possibly implicating extracellular vesicles (EVs). EVs carry cargo including modulators of polycystic disease signaling, such as Hedgehog<sup>38</sup> and Wnt.<sup>39</sup>

*Tsc* murine renal models exhibit elevated mTORC1 activity (phospho-S6 expression) in the cystic epithelium, but with a much lower frequency of cells that have lost *Tsc* gene expression.<sup>40–42</sup> The second hit mechanism for TSC angiomyolipoma alone is incongruent with this failure to identify significant loss of heterozygosity (LOH) in the TSC cystic epithelium.<sup>41,43</sup> Cystic epithelium from patients also continue to express both tuberlin and hamartin.<sup>44</sup> In contrast, angiomyolipomata clearly exhibit LOH and an absence of tuberlin staining.<sup>44</sup> This low frequency of LOH is also seen in *PKD1*-associated ADPKD, indicating that cystic disease in general may exhibit a more complex disease mechanism.<sup>24,45</sup>

## EVs and TSC renal cystic disease

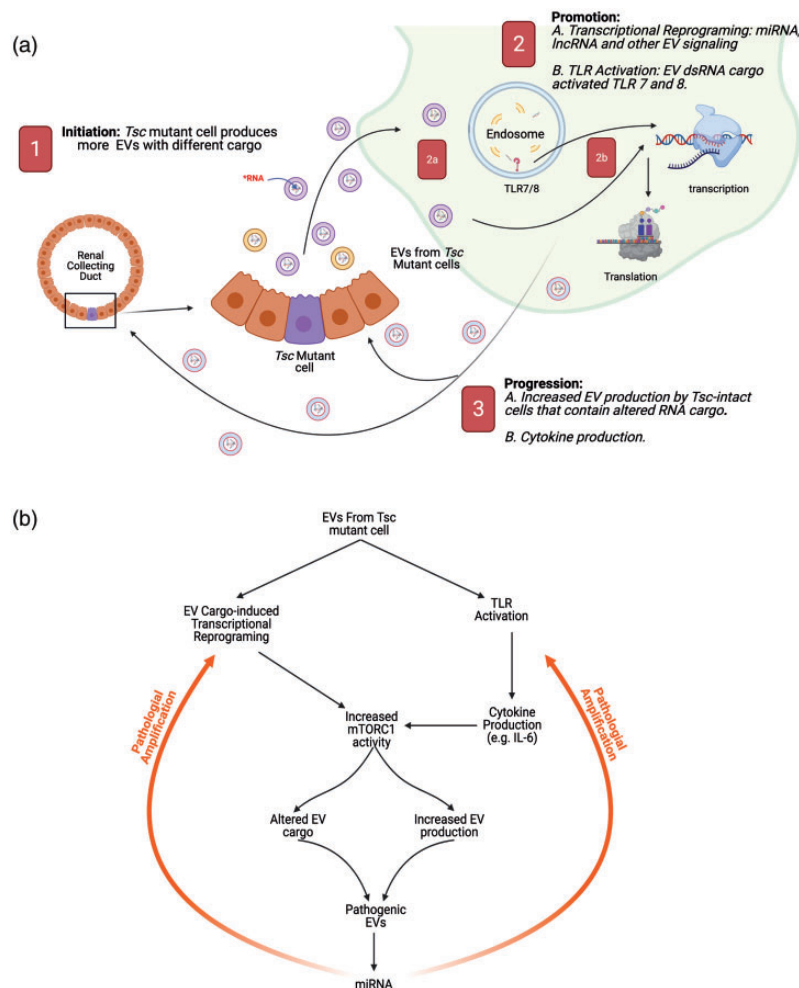
To understand the cystic disease in TSC better, a novel mouse model that inactivated the *Tsc2* gene specifically in principal cells was generated by breeding a *Tsc2<sup>fl/fl</sup>* mouse to one containing Cre-recombinase driven by the aquaporin-2 promoter. The resulting mice do not have a renal phenotype at birth but acquire significant cystic disease by 19 weeks of age.<sup>46</sup> Curiously, although the *Tsc2* gene was deleted in principal cells, the cysts were comprised of type A intercalated cells that contained an intact *Tsc2* gene.<sup>46</sup> This is also true if the *Tsc1* gene is disrupted using a similar approach.<sup>47</sup> Type A intercalated cells were also identified to be involved in human TSC-related cystic disease.<sup>46,47</sup>

Murine lineage tracing experiments using this principal cell-targeted model of *Tsc* cystic disease revealed that principal cells undergo limited clonal expansion, but ultimately can provide relatively few cells to the overall cyst (Figure 1

(a).<sup>48</sup> The cystic kidneys contained many more interstitial EVs than non-cystic kidneys but cystic mice excreted fewer EVs in the urine than did non-affected mice. The use of mTORC1 inhibitors reduces the EV production back toward normal kidney production.<sup>48</sup> Cystic fluid was also rich in EVs. The loss of the *Tsc2* gene caused the EV-generating cells to produce EVs that altered their effect on the transcriptome of normal renal tubular epithelium compared to EVs generated from isogenic cells with an intact *Tsc* gene.<sup>48</sup> A similar effect has been seen in an neuronal *in vivo* assay, where EVs produced from *Tsc1*-deleted cells transformed the neighboring wild-type cells so that they became similar to *Tsc1* mutant cells.<sup>49</sup>

The role of the *Tsc2* locus governing EV production is recapitulated in cell culture. *Tsc2* gene deletion in mouse inner medullary collecting duct (mIMCD3) cells resulted in a more than two-fold increase in EV production compared

to isogenic cells with a non-mutant *Tsc* gene.<sup>50</sup> Proteomic analysis of EVs from isogenic cell lines except for *Tsc2*-intact or *Tsc2*-deleted status revealed significant protein cargo differences.<sup>51</sup> This raises the possibility that renal EVs that participate in tissue homeostasis may induce disease by changing the concentration of EVs or by changing their cargo. Perhaps renal compensatory hypertrophy is mediated, at least in part, by EVs. In the cystic disease state, the EVs produced by renal cyst epithelia may drive growth contributing to disease. Such EV reprogramming of adjacent genetical intact cells involve miRNA EV cargo, because in cell culture experiments, quantitative polymerase chain reaction (PCR) analysis revealed the downregulation of miR-212a-3p and miR-99a-5p in EVs derived from *Tsc2* mutant cells compared to EVs purified from *Tsc1* mutant isogenic cells. These specific EV-derived miRNAs are expected to impact the mTORC1 activity in the recipient



**Figure 1.** Role of EVs in renal cystogenesis. (a) Role of EVs in the initiation, promotion, and progression of TSC renal cystic disease. The cystogenic process is initiated (labeled with the white “1” in the red background) by the loss of a *Tsc* locus in the tubule. This genetic change results in increased EV production with different cargo. These EVs are taken up by renal tubule cells with intact *TSC* genes or by kidney resident macrophages. These EVs cause an increase in the mTORC1 axis and promote cystogenesis (labeled as “2”). The increase in the recipient cell mTORC1 axis is through two mechanisms. The first one (labeled as 2a) is mediated by the EV RNA cargo (miRNA and lncRNA) altering transcription in genetically intact cells, and the other mechanism involves mTORC1 activation down-stream from toll-like receptors 7 and 8 activation by the double-stranded miRNA (2b). The lesion undergoes progression (labeled as “3”) by similar mechanisms because this genetically intact cell now (labeled 3A) increases production of EVs similar to cells that have lost their *TSC* gene function and have an increased mTORC1 activity. This TLR activation also increases cytokine production (e.g. IL-6) which induces down-stream mTORC1 activation (labeled 3B) in adjacent cells. (b) Flow diagram of the signaling amplification network. The EVs from the mutant cells initiate a potentially pathological amplification of mTORC1 activity that drives additional miRNA and EV production that further feedback to activate the system through reprogramming non-mutant cells and activating proliferation signals through TLR7 and 8 and signaling through cytokine receptors to also drive mTORC1 activity.

cell by altering the transcriptome and impacting the proliferative capacity.<sup>52</sup>

## Physiological and pathological roles of EVs in the kidney

The intercellular communication of EVs has a larger role in renal homeostasis and there also may be a role in the collecting duct plasticity. Embryologically, collecting duct epithelium arises from the ureteric bud and differentiates into principal and intercalated cells.<sup>53</sup> These epithelial cells display significant adaptability, and intercalated cells can participate with the principal cells in regulating the intravascular volume by way of tubular NaCl resorption.<sup>54</sup> In addition, type A and type B intercalated cells can interconvert between the two subtypes to help control systemic acid–base balance.<sup>55,56</sup> Both intercalated cell subtypes use carbonic anhydrase II, and knocking out the CA2 gene results in intercalated cell population depletion with a proportional increase in the principal cell population.<sup>57</sup> Cortical collecting duct cell plasticity also includes the relative ratio of intercalated cells to principal cells.<sup>58,59</sup> For example, potassium homeostasis is facilitated by principal cell secretion and intercalated cell absorption. Cell lineage tracing experiments in the context of potassium depletion demonstrated intercalated–principal cell interconversion in the medullary collecting duct.<sup>60</sup> Likewise, lithium can increase the percentage of intercalated cells through Notch and Foxi1 pathway activity, helping to explain lithium-associated diabetes insipidus.<sup>58,61–63</sup> Beyond plasticity, EVs also may help explain the disparate finding of collecting duct epithelial cell renin in the context of little or absent corresponding mRNA.<sup>64,65</sup> Such protein could be EV cargo from the vascular compartment or more upstream tubular epithelial cells.

EVs can mediate cellular proliferation and altered cellular function for normal organ homeostasis, but perhaps they are even more critical in response to disease states. When cells are stressed, they most often increase their EV production in addition to altering the EV cargo. This is true in human disease. Diabetic vascular dysfunction is mediated by transcriptionally modifying RNAs,<sup>66</sup> and more importantly to EV RNA cargo.<sup>67</sup> During normal physiological homeostasis, cells synchronize their metabolic activity, gene expression, and basic cellular processes. When cells are challenged or stressed, for example by a reduction of nutrients, they communicate to adapt their metabolism to the new condition. This same EV communication system can effectively conscript genetically normal cells to adopt a TSC disease phenotype<sup>48</sup> (Figure 1(a)). Once taken up, the EV cargo RNA can epigenetically alter recipient cell activity in two ways. First, the miRNA and lncRNA can alter cellular transcriptional activity and effectively reprogram the cellular activity. The second mechanism actually may activate an immune/inflammatory pathway.<sup>68,69</sup>

Dendritic cells help regulate innate and adaptive immunity in nonlymphoid tissues, and tissue-resident dendritic cells participate in an extensive, contiguous network in the kidney and appear to be positioned to respond to environment stimuli.<sup>70</sup> This immune cell network also functions as

important facilitators in renal cystogenesis. Renal resident macrophages phenotypically change from R2b to R2a during postnatal maturation, coincident with the timing when cyst formation slows considerably in genetic models of renal cystogenesis. Renal injury increases the number of juvenile-like resident macrophages in cilia mutant mice and accelerates cyst growth. Using a CSF1R kinase inhibitor in these animals to alter the resident macrophage activity assuaged cystogenesis.<sup>71</sup> There is innate immune functional overlap between renal tubular cells and the resident macrophage population that may contribute to this process in cystogenesis (Figure 1(a)).<sup>72</sup>

Environmental factors can induce EVs that impact immune cell fate decisions, activation, polarization, and memory cell development important to immune homeostasis.<sup>73,74</sup> This effect involves pattern recognition receptors (PRR), such as the toll-like receptors (TLR), that recognize pathogen characteristics to induce an immune response including cytokine and reactive oxygen species secretion.<sup>75</sup> Under normal circumstances, such PRR activation initiate immune cell recruitment and tissue repair. Pathological conditions of sustained PRR-signaling can result in autoimmunity or lethality.<sup>76,77</sup> This miRNA activation of the renal tubular epithelium, with or without the tissue resident mononuclear cell involvement, may further activate the mTORC1 signaling pathway through the TLR7 and 8 (Figure 1(b)). Such pathological amplification of mTORC1 activation through both mechanisms could go a long way to help explain the “phenotypic spreading” described in *Tsc* mutant cell systems.<sup>49</sup>

This sort of cell nonautonomous disease trait is not new, and a role for EVs in cellular reprogramming has been observed in a targeted mouse model that inactivated *Brcal*. Chodankar *et al.* use a targeted *Brcal* inactivation in mouse ovarian granulosa cells. The inactivation of the *Brcal* gene in granulosa cells induced cystic tumors with genetically intact *Brcal* genes in the ovaries and uterine horns. The authors hypothesized that the loss of *Brcal* by granulosa cells caused them to secrete something that caused the tumor to arise in a different cell type<sup>77</sup>, and we posit the mutant granulosa cells secreted EVs that lead to the tumors to arise in the uterine horns.

## Key questions and future directions

There are several initial key questions that will direct future investigations. The first question deals with the role of EVs in coordinating growth in developing tissue, and healing in existing, but damaged renal tissue. The renal cystic disease finding may have broader biological impact in general, and these findings then may offer a new insight into other proliferative diseases. Another key question focuses on how this signaling is directed and whether there is an addressing or targeting system involved in EV trafficking. If such an EV targeting system is at play, then this may offer a way to alter the EV signaling and possibly offer insights into therapy. Likewise, another key question focuses on the role of the cellular response to the double-stranded RNA found in the EV cargo. Separate from the noncoding RNA effects on altering transcription, this effect, for example

through the PRR, would also provide additional therapeutic avenues. Taken together, these questions and directions have great potential for leveraging the biology to reduce the disease burden.

## Conclusions

While TSC renal disease is a leading cause of morbidity and mortality, new insights into the disease pathogenesis also offers new therapeutic avenues. Understanding the detailed contributions may offer additional pathways to modulate the signaling pathway that can be leveraged to improve patient outcome. Beyond TSC, EVs and the innate immune system may be at play in other renal proliferative diseases such as other forms of renal cystic disease and malignancy. While further research is required to better illuminate the disease mechanisms and therapeutic potential, there is great potential for benefit.

## AUTHORS' CONTRIBUTIONS

Conceptualization: FZ, JJB, PK, and YY; Resources: JJB; Writing—original draft preparation: FZ, JJB, PK, and YY; Writing—review and editing: FZ, JJB, and PK; Supervision: JJB; Funding acquisition: JJB.

## ACKNOWLEDGEMENTS

I (the corresponding author) am grateful for this opportunity to publish a paper in remembrance of Peter Stambrook, PhD. Having the privilege of his friendship was one of the best things I carry with me from my time in Cincinnati. The figure was created with BioRender.com.

## DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the DoD grant W81XWH-14-1-0343 (JJB), Federal Express Chair of Excellence (JJB), and Children's Foundation Research Institute (JJB).

## ORCID ID

John J Bissler  <https://orcid.org/0000-0001-9685-9183>

## REFERENCES

- Ewalt DH, Sheffield E, Sparagana SP, Delgado MR, Roach ES. Renal lesion growth in children with tuberous sclerosis complex. *J Urol* 1998;**160**:141–5
- Stillwell TJ, Gomez MR, Kelalis PP. Renal lesions in tuberous sclerosis. *J Urol* 1987;**138**:477–81
- Bissler JJ, Kingswood J. Optimal treatment of tuberous sclerosis complex associated renal angiomyolipomata: a systematic review. *Ther Adv Urol* 2016;**8**:1–12
- Shepherd CW, Gomez MR, Lie JT, Crowson CS. Causes of death in patients with tuberous sclerosis. *Mayo Clin Proc* 1991;**66**:792–6
- Lux A, Calder N, Laugharne M, Osborne J, O'callaghan F. Causes of mortality in individuals with tuberous sclerosis complex. *Dev Med Child Neurol* 2017;**59**:612–7
- Crino PB, Nathanson KL, Henske EP. The tuberous sclerosis complex. *N Engl J Med* 2006;**355**:1345–56
- Yates JR. Tuberous sclerosis. *Eur J Hum Genet* 2006;**14**:1065–73
- Kingswood JC, Belousova E, Benedik MP, Carter T, Cottin V, Curatolo P, Dahlin M, D'Amato L, d'Auge' res G, de Vries PJ, Ferreira JC, Feucht M, Fladrowski C, Hertzberg C, Jozwiak S, Lawson JA, Macaya A, Marques R, Nababout R, O'Callaghan F, Qin J, Sander V, Sauter M, Shah S, Takahashi Y, Touraine R, Yourouko S, Zonnenberg B and Jansen AC, on behalf of the TOSCA Consortium and TOSCA Investigators. Renal angiomyolipoma in patients with tuberous sclerosis complex: findings from the Tuberous Sclerosis registry to increase disease awareness. *Nephrol Dial Transplant* 2019;**34**:502–8
- European Chromosome 16 Tuberous Sclerosis Consortium. Identification and characterization of the tuberous sclerosis gene on chromosome 16. *Cell* 1993;**75**:1305–15
- Ohta S, Imaizumi Y, Okada Y, Okano H, Kawakami Y. Generation of human melanocytes from induced pluripotent stem cells. *PLoS One* 2011;**6**:10
- Wang A, Tang Z, Li X, Tsou DA, Li S. Derivation of smooth muscle cells with neural crest origin from human induced pluripotent stem cells. *Cells Tissues Organs* 2011;**195**:5–14
- Yang M, Liu E, Tang L, Lei Y, Sun X, Hu J, Dong H, Yang SM, Gao M, Tang B. Emerging roles and regulation of MiT/TFE transcriptional factors. *Cell Commun Signal* 2018;**16**:11
- Argani P, Aulmann S, Illei PB, Netto GJ, Ro J, Cho HY, Dogan S, Ladanyi M, Martignoni G, Goldblum JR, Weiss SW. A distinctive subset of PEComas harbors TFE3 gene fusions. *Am J Surg Pathol* 2010;**34**:1395–406
- Henske EP. Metastasis of benign tumor cells in tuberous sclerosis complex. *Genes Chromosomes Cancer* 2003;**38**:376–81
- Crooks DM, Pacheco-Rodriguez G, DeCastro RM, McCoy Jr JP, Wang J, Kumaki F, Darling T, Moss J. Molecular and genetic analysis of disseminated neoplastic cells in lymphangiomyomatosis. *Proc Natl Acad Sci U S A* 2004;**101**:17462–7
- Goncharova EA, Goncharov DA, Chisolm A, Spaitis MS, Lim PN, Cesarone G, Khavin I, Tliba O, Amrani Y, Panettieri Jr RA, Krymskaya VP. Interferon  $\beta$  augments tuberous sclerosis complex 2 (TSC2)-dependent inhibition of TSC2-null ELT3 and human lymphangiomyomatosis-derived cell proliferation. *Mol Pharmacol* 2008;**73**:778–88
- Yue M, Pacheco G, Cheng T, Li J, Wang Y, Henske EP, Schuger L. Evidence supporting a lymphatic endothelium origin for angiomyolipoma, a TSC2 L tumor related to lymphangiomyomatosis. *Am J Pathol* 2016;**186**:1825–36
- Filipa Goncalves A, Adlesic M, Brandt S, Hejhal T, Harlander S, Sommer L, Shakhova O, Wild PJ, Frew JJ. Evidence of renal angiomyolipoma neoplastic stem cells arising from renal epithelial cells. *Nat Commun* 2017;**8**:1466
- Yan X, Shi L, Chen G, Zhang X, Liu B, Yue W, Pei X, Sun S. Mesenchymal stem cell-like cells in classic renal angiomyolipoma. *Oncol Lett* 2012;**4**:398–402
- Siroky BJ, Yin H, Dixon BP, Reichert RJ, Hellmann AR, Ramkumar T, Tsuchihashi Z, Bunni M, Dillon J, Bell PD, Sampson JR, Bissler JJ. Evidence for pericyte origin of TSC-associated renal angiomyolipomas and implications for angiotensin receptor inhibition therapy. *Am J Physiol Renal Physiol* 2014;**307**:F560–70
- Yamakado K, Tanaka N, Nakagawa T, Kobayashi S, Yanagawa M, Takeda K. Renal angiomyolipoma: relationships between tumor size, aneurysm formation, and rupture. *Radiology* 2002;**225**:78–82
- Beltramello A, Puppini G, Bricolo A, Andreis IA, el-Dalati G, Longa L, Polidoro S, Zavarise G, Marradi P. Does the tuberous sclerosis complex include intracranial aneurysms? A case report with a review of the literature. *Pediatr Radiol* 1999;**29**:206–11
- Henske EP, Scheithauer BW, Short MP, Wollmann R, Nahmias J, Hornigold N, van Slegtenhorst M, Welsh CT, Kwiatkowski DJ. Allelic

- loss is frequent in tuberous sclerosis kidney lesions but rare in brain lesions. *Am J Hum Genet* 1996;**59**:400–6
24. Brasier JL, Henske EP. Loss of the polycystic kidney disease (PKD1) region of chromosome 16p13 in renal cyst cells supports a loss-of-function model for cyst pathogenesis. *J Clin Invest* 1997;**99**:194–9
  25. Blaszak RT, Potaman V, Sinden RR, et al. DNA structural transitions within the PKD1 gene. *Nucleic Acids Res* 1999;**27**:2610–7
  26. Liu G, Bissler JJ, Sinden RR, et al. Unstable spinocerebellar ataxia type 10 (ATTCT)-(AGAAAT) repeats are associated with aberrant replication at the ATX10 locus and replication origin-dependent expansion at an ectopic site in human cells. *Mol Cell Biol* 2007;**27**. DOI: 10.1128/MCB.01276-07
  27. Patel HP, Lu L, Blaszak RT, et al. PKD1 intron 21: triplex DNA formation and effect on replication. *Nucleic Acids Res* 2004;**32**:1460–8
  28. Dixon B, Lu LL, Chu A, et al. RecQ and RecG helicases have distinct roles in maintaining the stability of polypurine-polypyrimidine sequences. *Mutat Res – Res* 2008;**643**:20–8
  29. Dixon BP, Chu A, Henry J, et al. Increased cancer risk of augmentation cystoplasty: possible role for hyperosmolar microenvironment on DNA damage recognition. *Mutat Res – Res* 2009;**670**:88–95
  30. Dixon BP, Henry J, Siroky BJ, Chu A, Groen PA, Bissler JJ. Cell cycle control and DNA damage response of conditionally immortalized urothelial cells. *PLoS One* 2020;**77**:1–10
  31. McConnachie DJ, Stow JL, Mallett AJ. Ciliopathies and the kidney: a review. *Am J Kidney Dis* 2020;**1**–10
  32. Tao Y, Kim J, Schrier RW, Edelstein CL. Rapamycin markedly slows disease progression in a rat model of polycystic kidney disease. *J Am Soc Nephrol* 2005;**16**:46–51
  33. Weimbs T. Regulation of mTOR by polycystin-1: is polycystic kidney disease a case of futile repair? *Cell Cycle* 2006;**5**:2425–9
  34. Boehlke C, Kotsis F, Patel V, Braeg S, Voelker H, Bredt S, Beyer T, Janusch H, Hamann C, Gödel M, Müller K, Herbst M, Hornung M, Doerken M, Köttgen M, Nitschke R, Igarashi P, Walz G, Kuehn EW. Primary cilia regulate mTORC1 activity and cell size through Lkb1. *Nat Cell Biol* 2010;**12**:1115–22
  35. Lai Y, Jiang Y. Reciprocal regulation between primary cilia and mTORC1. *Genes (Basel)* 2020;**11**:711–26
  36. Bell PD, Fitzgibbon W, Sas K, Stenbit AE, Amria M, Houston A, Reichert R, Gilley S, Siegal GP, Bissler J, Bilgen M, Chou PC, Guay-Woodford L, Yoder B, Haycraft CJ, Siroky B. Loss of primary cilia upregulates renal hypertrophic signaling and promotes cystogenesis. *J Am Soc Nephrol* 2011;**22**:839–48
  37. Ma M, Tian X, Igarashi P, Pazour GJ, Somlo S. Loss of cilia suppresses cyst growth in genetic models of autosomal dominant polycystic kidney disease. *Nat Genet* 2013;**45**:1004–12
  38. Silva LM, Jacobs DT, Allard BA, Fields TA, Sharma M, Wallace DP, Tran PV. Inhibition of hedgehog signaling suppresses proliferation and microcyst formation of human autosomal dominant polycystic kidney disease cells. *Sci Rep* 2018;**8**:12
  39. Sedgwick AE, D'Souza-Schorey C. Wnt signaling in cell motility and invasion: drawing parallels between development and cancer. *Cancers (Basel)* 2016;**8**:1–15
  40. Armour EA, Carson RP, Ess KC. Cystogenesis and elongated primary cilia in Tsc1-deficient distal convoluted tubules. *Am J Physiol Renal Physiol* 2012;**303**:F584–92
  41. Onda H, Lueck A, Marks PW, Warren HB, Kwiatkowski DJ. Tsc2(+/-) mice develop tumors in multiple sites that express gelsolin and are influenced by genetic background. *J Clin Invest* 1999;**104**:687–95
  42. Zhou J, Brugarolas J, Parada LF. Loss of Tsc1, but not Pten, in renal tubular cells causes polycystic kidney disease by activating mTORC1. *Hum Mol Genet* 2009;**18**:4428–41
  43. Wilson C, Bonnet C, Guy C, Idziaszczyk S, Colley J, Humphreys V, Maynard J, Sampson JR, Cheadle JP. Tsc1 haploinsufficiency without mammalian target of rapamycin activation is sufficient for renal cyst formation in Tsc1+/- mice. *Cancer Res* 2006;**66**:7934–8
  44. Moksib SM, Boils C, Gokden N, Grignon D, Gu X, Higgins JP, Leroy X, McKenney JK, Nasr SH, Phillips C, Sangoi AR, Wilson J, Zhang PL. Tuberous sclerosis complex: hamartin and tuberin expression in renal cysts and its discordant expression in renal neoplasms. *Pathol Res Pract* 2016;**212**:972–9
  45. Badenas C, Torra R, Pérez-Oller L, Mallolas J, Talbot-Wright R, Torregrosa V, Darnell A. Loss of heterozygosity in renal and hepatic epithelial cystic cells from ADPKD1 patients. *Eur J Hum Genet* 2000;**8**:487–92
  46. Bissler JJ, Zadjali F, Bridges D, Astrinidis A, Barone S, Yao Y, Redd JR, Siroky BJ, Wang Y, Finley JT, Rusiniak ME, Baumann H, Zahedi K, Gross KW, Soleimani M. Tuberous sclerosis complex exhibits a new renal cystogenic mechanism. *Physiol Rep* 2019;**7**:e13983
  47. Barone S, Zahedi K, Brooks M, Henske EP, Yang Y, Zhang E, Bissler JJ, Yu JJ, Soleimani M. Kidney intercalated cells and the transcription factor FOXI1 drive cystogenesis in tuberous sclerosis complex. *Proc Natl Acad Sci U S A* 2021;**118**:e2020190118
  48. Kumar P, Zadjali F, Yao Y, Johnson D, Siroky B, Astrinidis A, Vogel P, Gross KW, Bissler JJ. Tsc2 mutation induces renal tubular cell nonautonomous disease. *Genes Dis* (in press). <https://doi.org/10.1016/j.gendis.2021.03.010>
  49. Patel B, Patel J, Cho J-H, Manne S, Bonala S, Henske E, Roegiers F, Markiewski M. Exosomes mediate the acquisition of the disease phenotypes by cells with normal genome in tuberous sclerosis complex. *Oncogene* 2015;**35**:1–10
  50. Zadjali F, Kumar P, Yao Y, Johnson D, Astrinidis A, Vogel P, Gross KW, Bissler JJ. Tuberous sclerosis complex axis controls renal extracellular vesicle production and protein content. *Int J Mol Sci* 2020;**21**:1729–44
  51. Kumar P, Zadjali F, Yao Y, et al. Tsc gene locus disruption and differences in renal epithelial extracellular vesicles. *Front Physiol* 2021;**12**:630933. doi: 10.3389/fphys.2021.630933.
  52. Little M, Georgas K, Pennisi D, et al. Kidney development: two tales of tubulogenesis. *Curr Top Dev Biol* 2010;**90**:193–229
  53. Eladari D, Chambrey R, Peti-Peterdi J. A new look at electrolyte transport in the distal tubule. *Annu Rev Physiol* 2012;**74**:325–49
  54. Wall SM. Recent advances in our understanding of intercalated cells. *Curr Opin Nephrol Hypertens* 2005;**14**:480–4
  55. Al-Awqati Q, Gao XB. Differentiation of intercalated cells in the kidney. *Physiology (Bethesda)* 2011;**26**:266–72
  56. Breton S, Alper SL, Gluck SL, Sly WS, Barker JE, Brown D. Depletion of intercalated cells from collecting ducts of carbonic anhydrase II-deficient (CAR2 null) mice. *Am J Physiol – Ren Fluid Electrolyte Physiol* 1995;**269**. DOI: 10.1152/ajprenal.1995.269.6.f761
  57. Jeong H, Jeon US, Koo B, Kim WY, Im SK, Shin J, Cho Y, Kim J, Kong YY. Inactivation of notch signaling in the renal collecting duct causes nephrogenic diabetes insipidus in mice. *J Clin Invest* 2009;**119**:3290–300
  58. Christensen BM, Marples D, Kim YH, Wang W, Frøkiaer J, Nielsen S. Changes in cellular composition of kidney collecting duct cells in rats with lithium-induced NDI. *Am J Physiol – Cell Physiol* 2004;**286**:952–64
  59. Kim W-YY, Nam SA, Choi A, Kim YM, Park SH, Kim YK, Kim J. Aquaporin 2-labeled cells differentiate to intercalated cells in response to potassium depletion. *Histochem Cell Biol* 2016;**145**:17–24
  60. Ma M, Jiang YJ. Jagged2a-notch signaling mediates cell fate choice in the zebrafish pronephric duct. *PLoS Genet* 2007;**3**:0133–45
  61. Blomqvist SR, Vidarsson H, Fitzgerald S, Johansson BR, Ollerstam A, Brown R, Persson AE, Bergström G Gö, Enerbäck S. Distal renal tubular acidosis in mice that lack the forkhead transcription factor Foxi1. *Differences* 2004;**113**. DOI: 10.1172/JCI200420665.1560
  62. Guo Q, Wang Y, Tripathi P, Manda KR, Mukherjee M, Chaklader M, Austin PF, Surendran K, Chen F. Adam10 mediates the choice between principal cells and intercalated cells in the kidney. *J Am Soc Nephrol* 2015;**26**:149–59
  63. Sequeira-Lopez MLS, Nagalakshmi VK, Li M, Sigmund CD, Gomez RA. Vascular versus tubular renin: role in kidney development. *Am J Physiol Regul Integr Comp Physiol* 2015;**309**:R650–7
  64. Chen L, Chou C-L, Knepper MA. A comprehensive map of mRNAs and their isoforms across all 14 renal tubule segments of mouse. *J Am Soc Nephrol* 2021;**32**:897–912
  65. Zhang H-Na, Xu Q-Q, Thakur A, Alfred MO, Chakraborty M, Ghosh A, Yu XB. Endothelial dysfunction in diabetes and hypertension: role of microRNAs and long non-coding RNAs. *Life Sci* 2018;**213**:258–68

66. Zhang C, Zhang K, Huang F, Feng W, Chen J, Zhang H, Wang J, Luo P, Huang H. Exosomes, the message transporters in vascular calcification. *J Cell Mol Med* 2018;**22**:4024–33
67. Surace AEA, Hedrich CM. The role of epigenetics in autoimmune/inflammatory disease. *Front Immunol* 2019;**10**:1–16
68. David T, Ling SF, Barton A. Genetics of immune-mediated inflammatory diseases. *Clin Exp Immunol* 2018;**193**:3–12
69. Soos TJ, Sims TN, Barisoni L, Lin K, Littman DR, Dustin ML, Nelson PJ. CX3CR1+ interstitial dendritic cells form a contiguous network throughout the entire kidney. *Kidney Int* 2006;**70**:591–6
70. Zimmerman KA, Song CJ, Li Z, Lever JM, Crossman DK, Rains A, Aloria EJ, Gonzalez NM, Bassler JR, Zhou J, Crowley MR, Revell DZ, Yan Z, Shan D, Benveniste EN, George JF, Mrug M, Yoder BK. Tissue-resident macrophages promote renal cystic disease. *J Am Soc Nephrol* 2019;**30**:1841–56
71. Hato T, El-Achkar TM, Dagher PC. Sisters in arms: myeloid and tubular epithelial cells shape renal innate immunity. *Am J Physiol Renal Physiol* 2013;**304**:F1243–51
72. Curtale G, Rubino M, Locati M. MicroRNAs as molecular switches in macrophage activation. *Front Immunol* 2019;**10**:1–13
73. Mehta A, Baltimore D. MicroRNAs as regulatory elements in immune system logic. *Nat Rev Immunol* 2016;**16**:279–94
74. Medzhitov R, Preston-Hurlburt P, Janeway CA. A human homologue of the drosophila toll protein signals activation of adaptive immunity. *Nature* 1997;**388**:394–7
75. Yu L, Wang L, Chen S. Endogenous toll-like receptor ligands and their biological significance. *J Cell Mol Med* 2010;**14**:2592–603
76. Bosch S, Young NA, Mignot G, Bach JM. Epigenetic mechanisms in immune disease: the significance of toll-like receptor-binding extracellular vesicle-encapsulated microRNA. *Front Genet* 2020;**11**:578335
77. Chodankar R, Kwang S, Sangiorgi F, et al. Cell-nonautonomous induction of ovarian and uterine serous cystadenomas in mice lacking a functional Brca1 in ovarian granulosa cells. *Curr Biol* 2005;**15**:561–5