

brane but rather compromises AVP-dependent, but not basal, expression of water channels. This finding suggests that the point of convergence between the Ang II and AVP cascades is neither signal transduction nor AQP2 trafficking but rather the capacity to express the protein. More specifically, it suggests that Ang II enhances the ability of AVP to promote AQP2 expression at the genomic level, inferring that Ang II signaling either leads to *trans*-activation of the *AQP2* gene in the presence of AVP signaling or facilitates *trans*-activation in some manner by AVP signaling. The mechanism of this transcriptional control remains elusive. Other studies also have documented reduced levels of adenylate cyclases III and V/VI in the innermedullary collecting duct of AT1a receptor-null mice<sup>5</sup> that were not apparent in the study by Stegbauer and colleagues.<sup>2</sup> This discrepancy remains unresolved.

One of the more striking observations made investigating collecting-duct-specific AT1a receptor knockout mice is that the effect of AT1a receptor deletion on water transport in the distal nephron does not arise from changes in the osmotic draw for water reabsorption but rather is restricted to the route of reabsorption. Unlike the case in the proximal tubule, where AT1a receptor deletion disturbs renal sodium handling and pressure natriuresis,<sup>7</sup> deletion of the AT1a receptor in principal cells of the distal nephron does not produce measurable changes in sodium excretion and does not appear to affect the abundance of the epithelial Na<sup>+</sup> channel. The activity of this channel is limiting for sodium reabsorption in this portion of the nephron. However, additional research is called for here in this model because it was only touched upon in brief in this study. It is also interesting that collecting duct and principal cell-specific AT1a receptor-null mice do not exhibit the morphologic abnormalities apparent in global AT1a receptor-null mice that have modest atrophy of innermedullary collecting duct and papilla. This finding suggests that other Ang II receptors play an important role in development. The shortening of the nephron in global AT1a receptor-null mice likely accounts for these animals having concentrating problems under all conditions and differences in serum AVP levels. That collecting-duct-specific AT1a receptor-null mice have normal kidney anatomy and yet show resistance to AVP-dependent water reabsorption is consistent with this model providing a more precise understanding of the role of AT1a receptors in renal water metabolism.

This study by Stegbauer and colleagues<sup>2</sup> also offers additional appreciation from a broader physiologic perspective. It exemplifies that complex biologic functions often are modulated by coordinated but discrete input from converging signals, in this case Ang II and vasopressin, to achieve appropriate outcomes, namely water excretion and urinary concentrating ability. Although complex, the final result is a product of how individual contributions integrate. Thus, detailed understanding of discrete control systems, as provided by Stegbauer and colleagues for AT1a in the collecting duct, is fundamental to understanding physiology and treating disease.

## DISCLOSURES

None.

## REFERENCES

1. Bhavé G, Neilson EG: Body fluid dynamics: Back to the future. *J Am Soc Nephrol* 22: 2166–2181, 2011
2. Stegbauer J, Gurley SB, Sparks MA, Woznowski M, Kohan DE, Yan M, Lehrich RW, Coffman TM: AT<sub>1</sub> receptors in the collecting duct directly modulate the concentration of urine. *J Am Soc Nephrol* 22: 2237–2246, 2011
3. Zaman MA, Oparil S, Calhoun DA: Drugs targeting the renin-angiotensin-aldosterone system. *Nat Rev Drug Discov* 1: 621–636, 2002
4. Audoly LP, Oliverio MI, Coffman TM: Insights into the functions of type 1 (AT1) angiotensin II receptors provided by gene targeting. *Trends Endocrinol Metab* 11: 263–269, 2000
5. Oliverio MI, Delnomdedieu M, Best CF, Li P, Morris M, Callahan MF, Johnson GA, Smithies O, Coffman TM: Abnormal water metabolism in mice lacking the type 1A receptor for ANG II. *Am J Physiol* 278: F75–F82, 2000
6. Li XC, Shao Y, Zhuo JL: AT1a receptor knockout in mice impairs urine concentration by reducing basal vasopressin levels and its receptor signaling proteins in the inner medulla. *Kidney Int* 76: 169–177, 2009
7. Gurley SB, Riquier-Brison AD, Schnermann J, Sparks MA, Allen AM, Haase VH, Snouwaert JN, Le TH, McDonough AA, Koller BH, Coffman TM: AT1A angiotensin receptors in the renal proximal tubule regulate blood pressure. *Cell Metab* 13: 469–475, 2011

See related article, "AT<sub>1</sub> Receptors in the Collecting Duct Directly Modulate the Concentration of Urine," on pages 2237–2246.

## The Renal Papilla: An Enigma in Damage and Repair

Jessica Vanslambrouck, Joan Li, and Melissa H. Little  
Institute for Molecular Bioscience, The University of Queensland, St. Lucia, Australia

*J Am Soc Nephrol* 22: 2145–2147, 2011.  
doi: 10.1681/ASN.2011100984

It is now well established that organ-specific adult stem cells exist in a variety of tissues throughout the body where their survival, proliferation, and multipotency are regulated by the niche in which they reside. The controversy over whether such populations also exists in a relatively nonproliferative, nonregenerative organ such as the kidney has been the topic of debate for almost a decade.<sup>1</sup> A variety of interstitial and epithelial populations have been identified

Published online ahead of print. Publication date available at www.jasn.org.

**Correspondence:** Dr. Melissa Helen Little, Institute for Molecular Bioscience, The University of Queensland, St. Lucia, Queensland 4072, Australia. Phone: +617 3346 2054; Fax: +617 3346 2101; E-mail: M.Little@imb.uq.edu.au

Copyright © 2011 by the American Society of Nephrology

as potential renal stem cells based on location, stem cell marker expression, or functional properties such as Hoechst-dye efflux. Considerable focus has been directed toward the renal papilla as a putative stem cell niche based on the observation of long-term label retaining cells in the papilla after bromodeoxyuridine (BrdU) injection around birth in the mouse. However, the prospective isolation of this enigmatic papillary stem cell population has proven problematic, and their identity and *in vivo* properties remain elusive and controversial.

In this issue of *JASN*, Song *et al.* investigate mTert as a potential marker for this population.<sup>2</sup> Somewhat inadvertently, their findings lead us to revisit papillary development and response to injury, highlighting our lack of understanding of this unique cellular compartment. The papilla, or inner medulla, lies in the center of the adult kidney protruding into the pelvis. The collecting ducts pass through the papilla providing a conduit for the urinary filtrate to reach the ureter. The loops of Henle extend down into the papilla, and it is the length of these tubules that determines urinary concentrating capacity.

At birth, the papilla is very short and contained within the pelvis. During the immediate postnatal period, this region undergoes extensive elongation to form a final structure that protrudes out of the kidney along the existing ureter. Although an abundant literature exists pertaining to the out-budding and branching of the ureteric bud that forms the collecting ducts, the literature is relatively silent on the process of papillary elongation and maturation.

A number of studies implicate the papilla as a potential stem cell niche. Certainly, the environment around the cells of the papilla differs markedly from that of its surrounding tissue, being both hyperosmotic and hypoxic. This is in some ways reminiscent of niche microenvironments harboring stem cells in other organs, such as bone marrow and brain, where hypoxia is thought to play a role in stem cell maintenance and protection against DNA damage.<sup>3</sup> When cultured *in vitro*, a portion of papillary cells form spheres that coexpress mesenchymal and epithelial markers,<sup>4</sup> a behavior arguably reminiscent of neurosphere formation by neural stem cells. Furthermore, genetic labeling techniques (using BrdU and transgenic mice possessing doxycycline-inducible expression of green fluorescent protein [GFP] fused to histone 2B) indicate the presence of slow-cycling populations of label retaining cells (LRCs) in rodent kidney, predominately located in the papilla.<sup>4,5</sup> These papillary LRCs are found in the interstitium as well as incorporated into collecting ducts of the papilla and are proposed to be stem cells.<sup>5</sup> Based on the observation that LRCs from the papilla migrate toward the cortical region in response to injury, a role for these cells is predicted not only in homeostasis of the papilla, but also in renal repair.

A number of additional features lends further support to the concept that these cells are stem cells. Similar to other organ-specific stem cells, the interstitial LRCs are in close association with endothelial cells. When analyzed for marker expres-

sion, the LRCs express nestin, a marker of stem and progenitor cells first identified in neuroepithelial cells, and the somatic stem cell marker, prominin/CD133. Interestingly, similar CD133<sup>+</sup> nestin<sup>+</sup> cells have also been identified in the human papilla.<sup>6</sup> Here these cells are enriched in the loops of Henle of the renal medulla and papilla, coexpress embryonic and stem-related genes, and are capable of forming epithelial tubular structures *in vitro*.

Despite this substantial body of evidence in support of papillary stem cells, prospective isolation of this population has not been achieved. Hence, it has not been possible to validate clonogenicity or fully characterize the origin or potential of such cells. Consequently, there remains no proof that a stem cell population exists. In this issue, Song *et al.*<sup>2</sup> adopt a marker-based approach in their continued efforts to identify a papillary stem cell population. Founded on evidence that telomerase (mTert) marks stem cells of other embryonic and adult tissues,<sup>7</sup> their study seeks to characterize the telomerase (mTert)<sup>+</sup> population of the renal papilla. Song *et al.*<sup>2</sup> identify a subset of papillary epithelial cells that show strong expression of telomerase, approximately 5% of which are BrdU label-retaining cells. A more minor fraction of these cells is present in the papillary interstitium. However, fate tracing of telomerase-expressing cells postischemic injury using an *mTert*-GFP reporter mouse shows that these cells do not proliferate, migrate, or play any role in tubular repair.

The concept that cells from the papilla migrate into the cortex to contribute to tubular repair contrasts with the favored model that involves the proliferation of surviving terminally differentiated epithelial cells.<sup>8</sup> In agreement with the more traditional model, lineage-tracing studies show no evidence for the involvement of non-epithelial-derived cells in tubular repair,<sup>8</sup> casting doubt on contributions from migrating papillary cells. Subsequent fate-mapping studies confirms the presence of an LRC population in the papilla, but shows no evidence for migration to the site of injury.<sup>9</sup> Song *et al.*,<sup>2</sup> while investigating mTert as a possible marker of papillary stem cells, also show no evidence for migration of mTert<sup>+</sup> LRCs from the papilla in response to injury. However, not all papillary LRCs are mTert<sup>+</sup>, and so a distinct subset of papillary LRCs may have migratory capacity.

Other reports, utilizing alternative markers, have independently provided evidence for an interstitial migratory cell population during renal injury. In a model of fetal urinary obstruction in the primate, a CAII<sup>+</sup>  $\alpha$ SMA<sup>+</sup> population of cells was shown to arise from the collecting ducts and migrate into the interstitium of the obstructed kidney through epithelial-mesenchymal transition.<sup>10</sup> Whether these cells represent the migrating LRCs originally observed by Oliver *et al.*<sup>4,5</sup> is not known.

The initial observation of a LRC population in the kidney need not necessarily represent a stem cell population. It is also possible that LRCs within the papilla represent a population that enters quiescence close to birth. This concept

has been investigated through pulse labeling at different time points.<sup>11</sup> By embryonic day 17.5, little proliferation remains in the distal papilla (away from the medulla), whereas proliferation is more evident in other regions of the kidney.<sup>11</sup> By late gestation, epithelial proliferation appears restricted to the proximal papilla (closest to the medulla). Indeed, excessive collecting duct proliferation results in obstruction,<sup>12</sup> suggesting that cell cycle arrest is important for the development of normal physiological functions within the papilla. If proliferation within the papilla ends around birth, LRCs would not be detected in this region if the BrdU was administered after this point in time. This is evident when BrdU is delivered after birth, resulting in LRCs restricted to the proximal tubules and not the papillary interstitium.<sup>13</sup>

The data of Song *et al.* also provide little evidence for papillary proliferation,<sup>2</sup> again raising the question of how this region can undergo such extensive elongation in the immediate postnatal period and how it can respond to injury. Cell cycle arrest in the collecting duct epithelium has been proposed in response to the extremes of osmolarity and oxygen tension present in this region of the kidney. The majority of cells in the collecting duct arrest in G0/G1 and induce the expression of p53 and chaperones, including Hsp70, rendering these cells resistant to apoptosis.<sup>14,15</sup> As a result of these adaptations, cells of the papilla appear more resistant than the cortical epithelia to injury.

*mTert* null mice show an increased sensitivity to renal injury that had been attributed to critically shortened telomeres, decreased proliferation, increased p21 expression, and increased apoptosis throughout the kidney.<sup>16</sup> Although this may be the case, it is unlikely that this represents the response of the *mTert*<sup>+</sup> cells within the papilla, which already appear to be relatively nonproliferative. Song *et al.* do show that *mTert* expression is upregulated in response to injury, although this does not trigger cell division or migration.<sup>2</sup>

In conclusion, this most recent study rules out a role for *mTert*<sup>+</sup> cells in the papilla as stem cells responsible for injury-induced repair, leaving open the question of whether there is a stem cell in this region. Although consistent with the concept that there is no stem cell involved in renal tubular repair, this study fails to explain the apparent resistance of this compartment to injury. Given that all of the filtration units drain through the collecting duct epithelium of the papilla, loss or damage to this portion of the epithelial network would be catastrophic for renal function. Hence, this plumbing is likely to have evolved a robust response to a variety of damage signals. Understanding the mechanism of this response may prove invaluable in modulating responses to injury throughout this organ.

## DISCLOSURES

M. H. L. is a Principal Research Fellow of the National Health and Medical Research Council, Australia.

## REFERENCES

- Hopkins C, Li J, Rae F, Little MH: Stem cell options for kidney disease. *J Pathol* 217: 265–281, 2009
- Song J, Czerniak S, Wang T, Ying W, Carlone DL, Breault DT, Humphreys BD: Characterization and fate of telomerase-expressing epithelia during kidney repair. *J Am Soc Nephrol* 22: 2256–2265, 2011
- Morrison SJ, Csete M, Groves AK, Melega W, Wold B, Anderson DJ: Culture in reduced levels of oxygen promotes clonogenic sympathoadrenal differentiation by isolated neural crest stem cells. *J Neurosci* 20: 7370–7376, 2000
- Oliver JA, Maarouf O, Cheema FH, Martens TP, Al-Awqati Q: The renal papilla is a niche for adult kidney stem cells. *J Clin Invest* 114: 795–804, 2004
- Oliver JA, Klinakis A, Cheema FH, Friedlander J, Sampogna RV, Martens TP, Liu C, Efstratiadis A, Al-Awqati Q: Proliferation and migration of label-retaining cells of the kidney papilla. *J Am Soc Nephrol* 20: 2315–2327, 2009
- Ward HH, Romero E, Welford A, Pickett G, Bacallao R, Gattone VH, 2nd, Ness SA, Wandinger-Ness A, Roitbak T: Adult human CD133/1(+) kidney cells isolated from papilla integrate into developing kidney tubules. *Biochim Biophys Acta* 1812: 1344–1357, 2011
- Montgomery RK, Carlone DL, Richmond CA, Farilla L, Kranendonk ME, Henderson DE, Baffour-Awuah NY, Ambruzs DM, Fogli LK, Algra S, Breault DT: Mouse telomerase reverse transcriptase (*mTert*) expression marks slowly cycling intestinal stem cells. *Proc Natl Acad Sci USA* 108: 179–184, 2011
- Humphreys BD, Valerius MT, Kobayashi A, Mugford JW, Soeung S, Duffield JS, McMahon AP, Bonventre JV: Intrinsic epithelial cells repair the kidney after injury. *Cell Stem Cell* 2: 284–291, 2008
- Humphreys BD, Czerniak S, DiRocco DP, Hasnain W, Cheema R, Bonventre JV: Repair of injured proximal tubule does not involve specialized progenitors. *Proc Natl Acad Sci U S A* 108: 9226–9231, 2011
- Butt MJ, Tarantal AF, Jimenez DF, Matsell DG: Collecting duct epithelial-mesenchymal transition in fetal urinary tract obstruction. *Kidney Int* 72: 936–944, 2007
- Adams DC, Oxburgh L: The long-term label retaining population of the renal papilla arises through divergent regional growth of the kidney. *Am J Physiol Renal Physiol* 297: F809–F815, 2009
- Smeeton J, Zhang X, Bulus N, Mernaugh G, Lange A, Karner CM, Carroll TJ, Fassler R, Pozzi A, Rosenblum ND, Zent R: Integrin-linked kinase regulates p38 MAPK-dependent cell cycle arrest in ureteric bud development. *Development* 137: 3233–3243, 2010
- Maeshima A, Yamashita S, Nojima Y: Identification of renal progenitor-like tubular cells that participate in the regeneration processes of the kidney. *J Am Soc Nephrol* 14: 3138–3146, 2003
- Santos BC, Pullman JM, Chevaile A, Welch WJ, Gullans SR: Chronic hyperosmolarity mediates constitutive expression of molecular chaperones and resistance to injury. *Am J Physiol Renal Physiol* 284: F564–F574, 2003
- Dmitrieva N, Michea L, Burg M: p53 Protects renal inner medullary cells from hypertonic stress by restricting DNA replication. *Am J Physiol Renal Physiol* 281: F522–F530, 2001
- Westhoff JH, Schildhorn C, Jacobi C, Homme M, Hartner A, Braun H, Kryzer C, Wang C, von Zglinicki T, Kranzlin B, Gretz N, Melk A: Telomere shortening reduces regenerative capacity after acute kidney injury. *J Am Soc Nephrol* 21: 327–336, 2010

See related article, “Characterization and Fate of Telomerase-expressing Epithelia during Kidney Repair,” on pages 2256–2265.