Slow-Cycling Cells in Renal Papilla: Stem Cells Awaken?

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J Am Soc Nephrol 20: 2277–2279, 2009.
doi: 10.1681/ASN.2009090911

A fundamental goal of kidney stem cell models in regenerative medicine is to harness the kidney's prodigious capacity for endogenous repair to develop new therapeutic strategies. Despite progress in understanding kidney injury and repair mechanisms, few regenerative therapies to treat human kidney injury are on the horizon. In part, this reflects the kidney's complex architecture and cellular heterogeneity, a shortage of validated stem cell markers, slow homeostatic cell turnover, and the lack of robust transplantation assays. These factors combine to slow our ability to decipher cellular hierarchies in adult tissue repair and complicate the search for adult stem and progenitor cells.

The renal papilla has attracted interest as a potential niche for kidney stem cell since Oliver *et al.*[1] originally identified this kidney region as a site of slowly cycling cells, defined by retention of the DNA analogue 5-bromo-2-deoxyuridine (BrdU) after a short postnatal pulse followed by a long chase. Because subsequent cell divisions in the absence of label dilute the incorporated BrdU, only cells with the lowest replication profile are detected (label-retaining cells [LRCs]), and this approach has been used in other organs to enrich for stem cells that typically divide slowly. Importantly, the specificity and sensitivity of BrdU label retention is undefined for solid tissues and, in fact, is remarkably low for hematopoietic stem cells, the best characterized adult stem cell pool. For example, <6% of hematopoietic stem cells retain BrdU and <0.5% of BrdU-retaining hematopoietic cells are hematopoietic stem cells.[2]

Other factors recommend the papilla as a potential kidney stem cell niche beyond the residence of LRCs. Papilla has low oxygen tension and hypoxia may protect stem cells against DNA damage. There is preferential expression of genes known to regulate other stem cells in the renal papilla, including Wnt and Hedgehog ligands.[3,4] In their previous study, the depletion of papillary LRCs after ischemia reperfusion injury and the ability of papillary cell populations enriched in LRCs to form "nephro-spheres" in culture provide additional, indirect evidence for the existence of stem or progenitor cells in renal papilla.[1]

In this issue of *JASN*, Oliver *et al.*[5] present a welcome follow-up to their original work describing LRCs in the papilla. They use a powerful genetic model with doxycycline-inducible expression of a histone 2B-GFP fusion protein (H2B-GFP) to identify and further characterize slow-cycling cells in renal papilla. Because the H2B-GFP protein is stable *in vivo*, long chase times are possible and this approach has distinct advantages over BrdU label retention. BrdU labeling requires that cells be in S phase at the time of the pulse, but the slow-cycling nature of stem cells prevents a majority from labeling during that pulse. The H2B-GFP approach labels all cells, not just the fraction in S phase, and the superiority of this approach is proved in hematopoietic stem cells, where approximately 20% of hematopoietic stem cells retain the H2B-GFP label after a 24-wk chase compared with approximately 2% of hematopoietic stem cells retain BrdU after a similar chase.[6] In addition, the H2B-GFP label is bright and does not require denaturing conditions for detection (like BrdU), allowing co-labeling and FACS sorting and potentially recovery of viable cells for functional testing.

Oliver *et al.*[5] observe that, when pulsed with doxycycline during embryogenesis, newborn mice bigenic for both the tetracycline transactivator and a tet-O-H2B-GFP transgene have robust and ubiquitous kidney expression of H2B-GFP, but in adult mice, the H2B-GFP label is found only in the renal papilla, confirming the group's previous findings. Co-staining experiments allowed the authors to determine that the ratio of interstitial LRCs to tubular LRCs is almost equal in the upper papilla, but many more interstitial LRCs were found in the tip of the papilla, compared with tubular LRCs. Interestingly, interstitial LRCs express the neural stem cell marker nestin, an intermediate filament that is also linked to interstitial cells recruited after ischemic injury,[7] a finding reminiscent of the ability of papillary cell clones to produce nestin-positive progeny.[1] The LRCs also express the stromal-derived factor 1 (SDF-1) receptor, CXCR7, which also expresses on renal progenitors isolated from Bowman's capsule,[8] and SDF-1 is a well-characterized chemokine for mesenchymal stromal cells.

To address the functional properties of papillary LRCs, Oliver *et al.*[5] examined whether these cells proliferate during homeostasis and after injury. During adult homeostasis, the authors found a small number of cells in the upper papilla, adjacent to the urinary space, that are positive for the proliferation marker Ki-67, and some of these Ki-67–positive cells also retain the H2B-GFP label. Moreover, just 12 h after ischemic injury and before outer medullary proliferation occurs,

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

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proliferation in this same upper papillary region increases and many proliferating cells are LRCs, suggesting selective recruitment soon after injury. Of note in some cells, the H2B-GFP label has a perinuclear localization compared with nuclear Ki-67 staining. Future analyses require quantification of the relative percentage of LRCs *versus* non-LRCs that proliferate after injury as well as high-resolution subcellular imaging to verify marker localization.

If, as Oliver *et al.*[5] suggest, a subgroup of LRCs in the upper renal papilla expand selectively after injury to replace damaged cells in the outer medulla, then these cells must migrate up to hundreds of cell diameters after injury and traverse the basement membrane to gain access to injured epithelia in the outer medulla. To investigate this hypothesis, Oliver *et al.* labeled endogenous papillary cells *in vivo* with a vital dye and examined other regions of the kidney for cells stained with the dye after injury. Three days after injury, cells containing dye were found in both medulla and cortex, some of them even within tubules, suggesting a migration of cells induced by injury from papilla toward other kidney regions. The proportion of papillary cells migrating during this time frame is unclear, but this result provides proof of concept validation for this idea.

A limitation of the experimental strategy in this study is that cell proliferation dilutes the H2B-GFP label, complicating the task of identifying LRC progeny, a requirement for the evaluation of the stemness of LRCs. This emphasizes the need for lineage tracing, as suggested by the authors, to define the *in vivo* capacities of kidney LRCs. Tracking the lineage of a particular cell in complex tissues is best accomplished *in vivo* by activation and persistent expression of a reporter gene in all of the cell's descendents. Cell-specific expression of Cre recombinase has proved to be a robust technique in mammalian cells, and great progress has been made using these approaches in defining cell hierarchies in renal development,[9] but less is known about adult homeostasis and repair. Toward this end, a nestin-Cre or CXCR7-Cre animal, for example, would no doubt be very helpful.

By providing a more detailed characterization of the cellular phenotype of papillary LRCs, this study raises several intriguing questions. What is the significance of cellular proliferation in the upper papilla after injury, and are there regionally distinct mechanisms of kidney repair? Recent findings suggesting parietal epithelium is a podocyte progenitor niche,[10,11] supporting the probable existence of multiple progenitor niches in the kidney. How can a quiescent differentiated cell that retains label, such as tubular LRCs identified in this study, be distinguished from LRCs with true stem or progenitor qualities? Can endogenous cells migrate from interstitium into tubules, as proposed here? Our previous analysis suggested that such an event must be rare if it occurs, because we could not detect any dilution of labeled epithelial cells by unlabeled interstitial cells during kidney repair.[12] The selective proliferation of interstitial LRCs remains of great interest, because these cells might contribute to regeneration of other critical cell types (vasculature or myofibroblasts, for example), and the ability of such cells to differentiate into epithelia, even if rare, would provide proof of principle for derivation of a new source of epithelial progenitors that could be useful in disease modeling and cell-based therapies. Another unresolved question is why so many papillary LRCs lose label after injury, whereas only a fraction of them proliferate after injury, given the absence of apoptosis seen in this compartment.[1]

A speculative hypothesis suggested indirectly by these results is that nestin-positive LRCs might identify a kidney-specific mesenchymal stromal cell population. The perivascular interstitial localization of these cells fits with their possible identity as a subset of pericytes or perivascular stromal cells, as does their expression of nestin.[13] Their expression of CXCR7, which may be involved in recognition of endothelial cells, supports this concept.[14]

While providing important new insights concerning papillary LRCs, the study by Oliver *et al.*[5] highlights our need for new experimental tools to understand fully the regional capacity and mechanisms for kidney regeneration. To generate new candidate stem cell markers, the transcriptional signature of various relevant cell types, such as the nestin-positive papillary LRCs described here, parietal cell podocyte precursors, dedifferentiated proximal tubule epithelia, and so forth, must be defined during homeostasis and repair. Laser capture microscopy has been successfully used for this purpose during nephrogenesis,[15] but its application to noncontiguous cell populations such as interstitial or individual glomerular cell types is questionable. To minimize artifact from cell isolation, particularly after injury, when tissues are fragile and inflamed, an ideal transcriptional profiling approach would also avoid enzymatic dissociation and FACS sorting entirely. Finally, as with other quiescent solid organs, the kidney regeneration field is limited by the absence of robust transplantation assays to evaluate the capacity of isolated stem cells to incorporate and function in kidney structures *in vivo*.

Surmounting the challenges facing kidney stem cell biologists will require development of new reagents and approaches to evaluate and characterize putative stem cells in adult homeostasis and repair. The results presented by Oliver *et al.*[5] represent an important step in this rapidly evolving field.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health (DK073628 and DK084316) and the Harvard Stem Cell Institute.

I thank the members of the Humphreys laboratory for helpful discussions and Ivica Grgic for critically reading the manuscript.

DISCLOSURES

None.

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See related article, "Proliferation and Migration of Label-Retaining Cells of the Kidney Papilla," on pages 2315–2327.

Cell Therapy for Alport Syndrome

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J Am Soc Nephrol 20: 2279–2281, 2009.
doi: 10.1681/ASN.2009090915

Cell therapy for the treatment of human disease holds much promise, but few to date are standard of care. For regenerative medicine to be practicable, it is essential to have easily accessible sources of stem cells with high therapeutic potential. Advanced stem cell therapeutics aim to correct tissue or organ defects in a targeted manner by supplying stem cells that differentiate into the required cells *in situ* or are predifferentiated *in vitro* before infusion. Furthermore, it is critical that the cell preparation be safe and the method of delivery and patient preparation free of complications. In this issue of *JASN*, LeBleu *et al.*[1] report on the use of different cell types for the treatment of Alport syndrome that are safe and efficacious.

Historically, many stem cell studies have shown efficacy in animals but used systems that were not conducive to clinical application. For example, introduction of genes into cells using viral vectors to trigger differentiation or to correct a mutation are successful in animal models, but their use in humans remains a source of concern.[2] Similarly, for human embryonic stem (ES) cells to be used in patients, hurdles such as tissue matching, sourcing of cells, and the risk for teratomas need to be overcome. Some of these hurdles are circumvented by the production of induced pluripotent stem cells using removable vectors[3] and the development of methods to prevent teratoma formation.[4] Although many cell therapy studies are not suitable for clinical use, they are important to our understanding of stem cells and their role in treating disease. To date, the only stem cells used therapeutically are hematopoietic stem cells for bone marrow transplantation.

Cell-based therapies for the treatment of kidney disease have not been explored extensively. The current treatment for most kidney disease is dialysis or allogeneic kidney transplantation, but dialysis is not a permanent solution and donor organs are in short supply. Using the keywords "stem cell" and "kidney" to search the National Institutes of Health clinical trials web site, 186 clinical trials display, 184 of which are adjunctive therapies for kidney cancer using hematopoietic stem cells to replace patient bone marrow after aggressive chemotherapy or radiation.[5] This brief

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

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