Is There Such a Thing as a Renal Stem Cell?

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ABSTRACT

Increasing interest in the potential of adult stem cells in regenerative medicine has led to numerous studies focused on the identification of endogenous renal stem cells within the mature mammalian kidney. A variety of approaches have been taken to identify such cells, including physical location, cell surface marker expression, and functional properties. Proof of clonogenicity or renal potential remains questionable, and few such populations have been characterized in humans; however, recent evidence that even podocytes, a cell type with limited proliferative capacity under normal conditions, are constantly regenerated from a population within the Bowman’s capsule has breathed new life into the quest for a renal stem cell. Here we examine whether current evidence is sufficient to conclude such a population does indeed exist or whether the jury is still out. We also ask which properties we would wish such a cell to possess to allow for repair of the diseased kidney.


There is considerable recent interest in potential stem cell–based therapies for renal disease. As for other damaged organs, cells from bone marrow are recruited to the injured kidney.1,2 Bone marrow–derived mesenchymal stem cells (MSCs) show considerable promise in ameliorating chronic inflammation when recruited or delivered to the kidney. This seems to result from a humoral response by the MSCs, including the production of growth factors such as IGF-1.3–7 Whereas some studies proposed these bone marrow–derived cells transdifferentiate into specific renal cell types, the evidence suggests this is a rare event. Hence, it has been argued these cells simply act on an existing endogenous cell population, potentially a renal stem cell population, to repair renal architecture and function.

The kidney is classically regarded as an organ incapable of true regeneration. Indeed—or so it seems—no new nephrons appear after 36 wk of gestation in humans as a result of the exhaustion of progenitor mesenchyme.8 This does not rule out the existence of a renal stem cell able to elicit repair through cellular replacement of postnatal renal cell types as opposed to formation of new nephrons. Should such a renal stem cell exist, autologous cellular therapies may be feasible. The search for endogenous stem cells in the adult kidney has identified a wide variety of potential populations through their phenotypic characteristics, cell surface marker expression, or localization within the kidney.9 The majority of these studies have been performed in rodents (predominantly mice), with only a few exceptions.10–12 Whether any of these proposed stem cells elicit repair in humans or even exist in patients with chronic renal disease is not known.

Assessing the validity of claims for the existence of renal stem cells requires the reader to revisit the definition of a stem cell. A stem cell is an uncommitted, uncommitted cell capable of indefinite division or self-renewal but also capable of giving rise to one or more specialized cell types. Even within this definition, there is considerable room for variation. The most highly characterized stem cell is the hematopoietic stem cell (HSC). Even here, some HSCs have a longer self-renewal and repopulating capacity than others.13 Variation in the degree of differentiation potential of a stem cell is extreme, ranging from pluripotentiality (able to give rise to any cell type) to unipotentiality (able to give rise to only one cell type). Delineating a progenitor cell from a stem cell, therefore, must rely on the ability of a cell to divide indefinitely—its clonogenicity or capacity for self-renewal.

Several groups use functional criteria, including cell turnover, to identify potential stem cell populations. The ability of a cell to retain bromodeoxyuridine (BrdU) over a long chase period has been used to identify stem cells in the skin, stomach, and other organs.14,15 Three groups have searched for BrdU label–retaining cells (LRCs) in murine kidney. Maeshima et al.16 initially located such cells to the epithelial tubule, but further examination revealed LRCs in the interstitium after ureteric obstruction.17 These cells demonstrate multipotentiality, tubule formation, and apparent inte-
migration into the developing kidney.\textsuperscript{18} Using a similar approach, another group identified LRCs in the papilla.\textsuperscript{19} These cells are predominantly interstitial and reenter the cell cycle in response to ischemia. Although this behavior is as one might expect for a stem cell, Vogtseder \textit{et al.}\textsuperscript{20} also observed morphologically fully differentiated epithelial cells that retain BrdU, suggesting these cells were merely quiescent differentiated cells.\textsuperscript{20} Another functional feature of some stem cells, including HSCs, is efflux of certain dyes, including Hoechst,\textsuperscript{21} a characteristic allowing FACS sorting. Several groups report such cell types in mouse\textsuperscript{22,23} and human\textsuperscript{12} kidney; however, such populations are clearly heterogeneous, and it has been difficult to show which subsets of cells are behaving as multipotent cells. These fractions do seem to improve renal function when injected into animal models, but this is from the production of growth factors and not through transdifferentiation.\textsuperscript{22–24} Finally, Gupta \textit{et al.}\textsuperscript{25} isolated multipotent renal progenitor cells using similar cell culture methods as those used to isolate multipotent adult progenitor cells from bone marrow. This involved extended passaging in culture to identify long-term self-renewal, after which these cells express the stem cell marker Oct4 and the renal transcription factor Pax2.

Another approach adopted to search for renal stem cells is the expression of previously proposed stem cell markers. Bussolati \textit{et al.}\textsuperscript{11} isolated potential progenitors from adult human kidney on the basis of expression of the stem cell marker CD133. These cells are multipotent and express markers of epithelium. Dekel \textit{et al.}\textsuperscript{26} used the stem cell marker Sca1 plus the absence of hematopoietic lineage markers to isolate a nontubular multipotent stem/progenitor cell population that displays a tubular phenotype after delivery to ischemic kidneys. Sagri

nally, Kitamura \textit{et al.}\textsuperscript{26} examined which portions of the rat kidney would give rise to clones with potential to form epithelium in response to Wnt4. Cells from the corticomedullary junction showed long-term self-renewal; expression of epithelial markers such as aquaporin 1, aquaporin 2, and chloride channel-K; and improved renal function after injury.

One of the real challenges to this field has been the relevance of the assays used to evaluate putative stem cell populations. Although few studies aside from that by Gupta \textit{et al.}\textsuperscript{25} have stringently assessed long-term self-renewal or clonogenicity, some reported the culture of spheres of cells. Sphere formation is an assay developed in the neural field in which single neural stem cells form a floating colony that can be repeatedly passaged to form new spheres.\textsuperscript{29} Stringent application of this approach has yet to be reported for the kidney. Added to this is the absence of a gold standard assay for renal potential.\textsuperscript{13} No one cell can repopulate an entire kidney as is the case for the HSC; however, a variety of approaches have been used to examine epithelial potential, although surprisingly few markers definitively demonstrate renal epithelium. A number of studies have investigated the ability of potential renal stem cells to form renal structures in developing kidney (metanephric) organ cultures\textsuperscript{23} or to transdifferentiate into renal cell types within a kidney in vivo.\textsuperscript{10,11,22,23,25–27}

There are a number of important caveats here. Some apparent transdifferentiation will result from cell fusion. This has rarely been eliminated. Second, it is difficult to prove the presence of an introduced cell in a renal structure is actually functioning appropriately unless the recipient animal cannot survive without the function of the introduced cell. In addition, quantification of cellular integration, regardless of whether it is functional, is lacking in most studies. Given the capacity for some cell types to elicit repair through the production of growth factors, it is possible this is frequently the mechanism of action, rather than true replacement of renal parenchyma. Whether this actually matters in the clinical setting, when the outcome is positive, remains unclear. Arguably, the most critical outcome, stem cell or not, is for the introduced cell type to elicit functional repair and for that effect to be long lasting and without adverse effects. Here, studies to date have also failed, because almost all animal models used represent acute injury rather than experimental or genetic models of chronic renal disease.

Do renal stem cells exist in vivo, and do they contribute to repair in situ? Only one study examined this genetically from the perspective of lineage. Humphreys \textit{et al.}\textsuperscript{30} used a transgenic mouse strain in which all cells that were involved in nephrogenesis were lineage tagged. This enabled the researchers to damage the kidney postnatally and ask whether any endogenous cell type entered the tubules to contribute to repair. Their data showed that no nontubular cells were evident in renal tubules before or after damage. Hence, no exogenous cell or nontubular renal stem cell plays a role in tubular repair in these experimental models. This finding leaves open the possibility of an epithelial stem cell within the tubules or simply the proliferation of existing differentiated cell types within the tubules. It also does not rule out the possibility that some specific forms of renal damage evoke a stem cell response whereas others do not.

The most compelling evidence to date that the kidney does have an ability to replace lost cell types, both as an ongoing process of homeostasis and in response to damage, was shown recently.\textsuperscript{31,32} Appel \textit{et al.},\textsuperscript{31} using careful immunohistochemistry and a transgenic animal model that differentially tagged the parietal epithelium from glomerular visceral epithelium, showed within the Bowman’s capsule exist podocyte progenitor cells that migrate over the basement membrane of the Bowman’s capsule to ensure a constant resupply of podocytes. The location of the these cells at the urinary pole of the Bowman’s capsule opens up the possibility that they also migrate down the proximal tubules to ensure turnover of tubular epithelium as well (Figure 1A). Indeed, Ronconi \textit{et al.}\textsuperscript{32} isolated a CD133\textsuperscript{+}, CD24\textsuperscript{−} population of
cells from the Bowman’s capsule of human kidneys and showed, upon reintroduction into an immunodeficient animal model of renal damage, these cells contribute both to podocytes and to tubular epithelium; however, there is no definitive proof in either of these studies that these cells represent stem cells. Neither is there proof that podocyte localization in recipient xenotransplants is not due to fusion, but the evidence that they are responsible for podocyte turnover is a revelation in nephrology.

If such a progenitor population exists in the Bowman’s capsule, then where else in the kidney might one expect to find a renal stem cell/progenitor? First, we suggest it is unlikely those compartments of the adult kidney that were derived from different embryonic sources emerge from a single adult stem cell. The ureteric epithelium gives rise to the collecting ducts and calycal system, whereas cells of the metanephric mesenchyme give rise to the various cell types of the nephron. It is unlikely, then, that a single stem cell in the adult kidney can give rise to all cells of the uriniferous tubule.

Given that the kidney receives approximately 20% of the cardiac output and plays critical roles in water and electrolyte balance, one might speculate that stem cells would be sensibly located close to the vasculature so that specific epithelial and/or interstitial cell populations could be regenerated locally. Experience from some other organs tells us that stem cells are often located at or close to the base of tubular structures—places such as the intestinal crypts or hair follicle. In this case, a location close to the bend of the loop of Henle or close to the bend of the vasa recta might be envisaged. Alternatively, the junction between the thin and thick limbs of the loop of Henle might be appropriate, given that progeny from stem cell division might differentiate into one or other epithelial cell or migrate in either of two directions. The functions of cells of the extraglomerular mesangium have also remained a mystery for decades. Given their proximity to the juxtaglomerular apparatus and renal corpuscle, many would suspect some of these cells have progenitor capacities perhaps for mesangium. These possibilities are depicted in Figure 1B.

We must also draw attention to the link between hypoxia and cell differentiation and proliferation. Results from several studies suggested oxygen levels can profoundly influence stem cell niches and promote the differentiation of certain types of stem or progenitor cells while inhibiting the differentiation of others. Some stem cell niches are hypoxic, whereas others are relatively well oxygenated and close to the microvasculature. It is widely known that oxygen levels vary throughout the normal kidney,33 with cortical Po2 generally higher than that of the medulla. Fine et al.34 proposed that chronic hypoxia plays an important role in the progression of chronic kidney disease, which perhaps involves a hypoxia-induced dysregulation of cell differentiation leading to a profibrotic phenotype. Interestingly, oxygen availability regulates Notch and Wnt signaling, both being required for maintenance of stem cell niches in other organs and for the normal formation of nephrons.

Do we really need a renal stem cell or just a progenitor? This reopens the question of what we really understand about normal renal repair processes. The kidney has an excellent capacity to repair after transient insult. In both human and animal settings of acute kidney injury such as ureteric obstruction, nephrotoxicity, and transient ischemia,35,36 renal repair and remodeling occur spontaneously. Vogtsseder et al.20 proposed that proximal tubule repair does not require a stem cell but involves the proliferative response of the existing differentiated tubular epithelium. They showed that proximal tubular cells are not quiescent but rest in G1 of the cell cycle, rendering them able to divide rapidly in response to injury. Regardless of whether this is the sole mechanism of repair or a stem/tubular progenitor cell population is also involved, these mechanisms are clearly overwhelmed in chronic kidney disease.

Is this a problem with the progenitors or their environment? Can we even generalize this much? Although the long-term outcome is similar, the cause of chronic renal disease is highly variable, making it possible, even likely, that different repair processes occur under dif-
fferent conditions of damage. The corollary is that different therapies may be required for different situations. We can learn here from the liver.

The liver is a highly regenerative organ in which substantial replacement of parenchyma occurs from the simple proliferation of hepatocytes; however, under conditions in which the hepatocytes themselves cannot respond, there is a facultative response in which stem cells—referred to as oval cells—arise from the periphery of the bile ducts. It is possible that under some circumstances, the kidney also has a facultative response to damage. There are reports of murine strain variations in response to damage. Indeed, there is simple strain variation and even gender variation in the response of the murine kidney to ischemia. What is missing, then, is an understanding not only of the progenitor populations of the kidney but also of the normal repair responses that counter different insults.

Let us return to the hypothesis that a renal stem cell does exist and such a cell type can develop into a cellular therapy for kidney disease. In attempting to develop a cellular therapy for any disease state/organ, key questions include which cell type is required and how it will be delivered. Treatment for insulin-dependent diabetes clearly requires replacement of glucose-responsive, insulin-secreting β islet cells. The question of which cell type is required is not a simple one to answer for an organ such as the kidney, and the answer may well vary depending on the renal disease.

The nexus between glomerular and tubular function and disease is a close one, and it has been debated as to whether nephron loss results from glomerular injury or from tubulo-interstitial responses. It has been argued that the survival of a nephron after damage depends on the ongoing health of the glomerulus, rather than the tubule itself. This observation is consistent with the proposed notion of Bowman’s capsule progenitor cells. What is clear is that, whether replacement of cells within existing nephrons or loss of those nephrons followed by interstitial fibrosis, no new nephrons arise. So do we need to engineer tubular cells or glomerular epithelial cells/podocytes? Do we need to introduce progenitors of these cell types and expect them to integrate into existing structures and mature in that location, or do we need to provide some other cell type that encourages the existing renal parenchyma to survive an insult and proliferate to repair? In the case of mesenchymal stem cells, these cells are not transdifferentiating but rather provide a reparative cytokine/chemokine environment. The longevity of these positive effects versus the potential for long-term damage resulting from the aberrant differentiation of MSCs remains unclear. If both of these concerns are allayed, then the delivery of such “support cells” may prove a viable treatment without the need for a renal stem cell.

If stem, renal, or nonrenal cells are to be delivered into this complex solid organ, then they will have to be injected into the parenchyma or bloodstream. Delivery of cells through the systemic circulation results in entrapment, particularly within the pulmonary microvasculature. An active homing mechanism may also be required for introduced cells to preferentially reach the kidney. Some studies showed cells introduced into the circulation do reach the kidney—either by homing or passive transfer—and integrate into the parenchyma. Where there has been selective pressure, as for example in collagen α3IV or α2I mutant mice, there is at least a transient recovery of the appropriate collagen within the glomerular basement membrane, suggesting a capacity for MSCs to cross the basement membrane.

Delivery of cells directly into the renal parenchyma would deliver cells only into restricted regions of the kidney. Even if these cells proliferated and integrated locally, global organ-wide integration is unlikely. With a greater understanding of normal cellular turnover within the kidney, whether involving resident progenitor/stem cells or an exogenous cell homing to the kidney, the more feasible scenario may be to re-stimulate this response in situ rather than have to deliver the cells themselves. This is becoming the approach of choice in organs such as the brain, where cell delivery is presenting similar hurdles.

Current evidence supports a greater capacity for the kidney to repair in response to damage than once appreciated, including evidence for progenitors of specific cell types; however, there is no evidence for one master stem cell in the kidney that can recapitulate development. All in all, the quest for a renal stem cell has taken this field around in a circle and returned it to experimental nephrology and renal microanatomy, where there remains a great deal to be learned about the normal processes of renal cell turnover and response to damage. Indeed, much of the groundwork on renal cell turnover and replacement is still necessary, in contrast to the comprehensive studies conducted over many years in the gut and in spermatogenesis. The opportunity for better kidney science is there, and future findings will certainly provide new insights into new renal therapies.

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DISCLOSURES

None.

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