Do Stem Cells Exist in the Adult Kidney?

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Key Words

Acute renal failure • Oct-4 stem cell marker • Progenitor cells • Sca-1 stem cell marker • Stem cells, regeneration

Abstract

Adult stem cells exist in many organs and play a critical role in normal cell turnover and the response to injury. The existence of adult stem cells in the mammalian kidney remains controversial. Kidney stem cells have been isolated and characterized by many groups, often with discrepant results. This article will review the current state of knowledge regarding adult kidney stem cells and discuss future directions for kidney stem cell research.

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Introduction

Stem cells are specialized cells that have the capacity to renew themselves and differentiate into specialized cell types. Adult stem cells are found in differentiated tissues and can give rise to specialized cells of that tissue. A progenitor cell is generally considered a more intermediate cell between a stem cell and a differentiated cell. This partially specialized cell can divide and generate new progenitor cells, but is usually viewed as generating differentiated cells. Growing attention has focused on organ-specific adult stem cells both to better understand how injured organs regenerate and as a therapeutic target for disease modulation. Adult stem cells and their niches have been well characterized in such sites as the bone marrow, intestine, skin, and brain [1–3]. There is ongoing

debate and controversy on whether stem cells exist in the adult kidney, and if they do, what role these cells play in the pathophysiology of kidney disease. Much of this interest in adult kidney stem cells has derived from studies of the regenerative response following acute kidney injury (AKI).

Kidney Regeneration following AKI

Toxic and ischemic insults to the kidney lead to AKI, most often manifest as acute tubular necrosis. Loss of injured or dead cells leads to denudation of tubular basement membrane, with sloughed cells and cellular debris filling tubular lumens. Following injury, the kidney undergoes a robust regenerative response, leading to recovery of kidney function. New cells are required to replace damaged cells. Three possible sources of new tubular cells are: adjacent less damaged tubular cells, extrarenal cells presumably of bone marrow origin that home to the injured kidney, or resident kidney stem cells.

There is evidence that bone marrow-derived cells can migrate to the kidney and form tubular epithelial cells following AKI [4–8]. However, the *in vivo* contribution of extrarenal cells to kidney regeneration is minimal [9–11]. Arguments for less damaged tubular cells as being the source of regenerating cells come from studies of gene expression following AKI [12]. Recapitulating developmental paradigms, these cells dedifferentiate, proliferate, and eventually reline denuded tubules restoring the structural and functional integrity of the kidney [12–17]. Molecular events defining this regenerative response have been characterized and strategies to accelerate the

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Table 1. A summary of studies isolating kidney stem cells

Authors	Species	Isolation method	Stem cell markers	Other markers	Location	Differentiate into kidney tubules	Differentiate into other lineages
Oliver et al. [36]	rat	label retaining			papilla	yes	neuron
Maeshima et al. [38]	rat	label retaining			proximal tubule	yes	not tested
Iwatani et al. [39]	rat	side population	Sca-1	C-kit, CD45	proximal tubule	no	heme, muscle, liver
Hishikawa et al. [40]	mouse	side population	Sca-1	Musculin/MyoR	interstitium	not tested	not tested
Challen et al. [41]	mouse	side population	Sca-1, CD24	endoglin/CD105	tubule	yes	osteocyte, adipocyte
Bussolati et al. [42]	human	marker	CD133	Pax-2, CD44	interstitium	yes	endothelium
Sagrinati et al. [43]	human	marker	Oct-4, CD24, CD133, Bml1		glomerular parietal epithelium	yes	osteocyte, adipocyte, neuron
Dekel et al. [44]	mouse	marker	Sca-1		interstitium of papilla	not tested	osteocyte, adipocyte, neuron
Kitamura et al. [45]	rat	culture	Sca-1, Musashi-1	Pax-2, Wt-1, Wnt-4	proximal tubule	yes	not tested
Gupta et al. [46]	rat	culture	Oct-4, Rex-1	Pax-2, CD44	proximal tubule	yes	neuron, liver endothelium

repair process tested in both experimental models and in humans [12–19]. The rapidity and extent of DNA synthesis and cell proliferation that follow kidney injury have been used to support a role for remaining epithelial cells as the major source of regenerating cells. It has been argued that the response is too large to be accomplished by a small number of putative adult stem cells residing in the kidney. However, this argument does not take into account the potential presence of transit amplifying cells that may be present in the kidney. Transit amplifying cells are rapidly cycling, but can acquire differentiated function and eventually undergo terminal differentiation [20]. Moreover, the cell cycle dynamics of stem cells is far from being understood.

Other arguments favoring less damaged cells include cell lineage tracking studies demonstrating that differentiated tubular epithelial cells can proliferate and repopulate damaged tubules following AKI [21, 22]. However, these studies do not rule out the possibility that kidney stem cells can express epithelial markers, nor do they exclude a role for kidney stem cells as contributing to the regenerative response.

Outcomes following an episode of AKI are poor, with an in-hospital mortality >50% in individuals who develop postoperative AKI requiring dialysis [23]. Even after multivariate adjustment, AKI continues to remain an independent risk factor for mortality [24]. Animal models have demonstrated permanent damage to peritubular capillaries, interstitial fibrosis, and altered gene expression following an episode of AKI [25]. These observations suggest that AKI is not always a fully reversible condition

and can lead to permanent changes in the kidney. This poor outcome may be due to an inadequate regenerative response, perhaps related to the availability of kidney stem cells. Also, consumption of kidney stem cells following AKI may explain why AKI often accelerates the progression of underlying chronic kidney disease. A potential role for stem cells in other kidney disease states is less well defined. Stem cell depletion could be a pathophysiological factor in many chronic progressive kidney diseases.

Lessons from Development and Lower Organisms

Stem cells exist in the metanephric mesenchyme, which is the tissue of origin for most of the structures of the mature kidney, except for the collecting duct, interstitium, and vasculature [26, 27]. Multipotent stem cells have been localized to the lower tubule and ureter of the *Drosophila* Malpighian tubules [28]. These cells are present in abundant numbers, undergo self-renewal, and can give rise to all cell types of the adult Malpighian tubule. In contrast to other stem cells that are regulated in fixed niches through specific signaling between them and adjacent supporting cells, these cells autoregulate their self-renewal and differentiation behavior through the JAK-STAT signaling pathway. Kidney stem cells persist in the adult kidneys of other organisms, such as the skate and the freshwater teleost. These cells can participate in new nephron formation following partial nephrectomy [29–31].

Knowledge derived from kidney development and lower organisms has contributed to the study of adult kidney stem cells. The molecular signature of developing kidney cells has been used to define stem cell markers in order to identify and track adult stem cells. For example, CD24 and cadherin-11 are two cell surface proteins that are strongly expressed by uninduced metanephric mesenchyme [32]. However, this approach has been difficult given the complex nature of kidney development, with the metanephric kidney being preceded by the pronephros and mesonephros, and the fact that mesenchymal-to-epithelial transformation must occur during development. Nonetheless, pathways involved in cell lineage progression in the metanephric kidney have provided important clues to manipulate embryonic and adult stem cells to undergo kidney cell lineage progression. Metanephric organ culture has been used as a readout to define the differentiation potential of injected stem cells. Finally, both the metanephric mesenchyme and the intact metanephric kidneys are being used in experimental studies for the treatment of adult kidney diseases [33, 34].

Isolation of Adult Kidney Stem Cells

The lack of a definitive marker for kidney stem cells makes it difficult to isolate adult kidney stem cells, to define a kidney stem cell niche, or to follow cell lineage progression in the normal or injured kidney. The kidney is a particularly challenging organ to work with, given its complex architecture comprised of at least 26 different cell types. Candidate adult kidney stem cells have been isolated using four different selection strategies that have been used to successfully isolate stem cells from other organs. The first method takes advantage of the fact that stem cells are slow-cycling cells. Therefore, when the DNA of the cells is labeled with a marker such as bromodeoxyuridine, the cells retain the label for a long period of time. This label retention can be used to identify and isolate putative stem cells. The second method of isolating side-population (SP) cells takes advantage of the fact that SP cells extrude Hoechst dye through the activity of multidrug resistance proteins that are part of the ATP-binding cassette transporter superfamily. SP cells (Hoechst low cells) isolated from many different organs contain multipotent stem cells [35]. The third method used to identify and isolate kidney stem cells takes advantage of specific cell surface markers that have been used to identify stem cells in other organs or the metanephric kidney. The markers used to isolate kidney stem cells in-

clude CD133, stem cell antigen-1 (Sca-1), and CD24. The fourth method has been to use culture conditions that have been successful in selecting stem cells in other organ systems.

Label-Retaining Cells

Oliver et al. [36] have isolated cells from the kidney papilla of young mice and rats that are slow-cycling cells and have characteristics of kidney stem cells. When grown in culture, these papillary cells express epithelial and mesenchymal markers, form cellular spheres, and display some evidence of plasticity with differentiation into neurons under appropriate culture conditions. These cells proliferate and migrate following in vivo ischemic kidney injury.

Maeshima et al. [37] identified a population of cells in the adult rat kidney scattered among kidney tubular cells. These cells were identified as label-retaining cells and were found predominantly in proximal tubules. Following kidney ischemia, these label-retaining cells undergo proliferation, and progeny of these cells initially express vimentin, a mesenchymal cell marker, and later become positive for E-cadherin, an epithelial cell marker. The cells that have been subsequently isolated demonstrate plasticity and can be integrated into the developing kidney [38].

SP Cells

Three different groups isolated and characterized kidney SP cells. Iwatani et al. [39] isolated SP cells from adult rat kidney that comprised 0.03–0.1% of the cells of the digested kidney. These cells do not participate in kidney regeneration following experimental glomerulonephritis or tubular injury induced by gentamicin. Hishikawa et al. [40] isolated SP cells from adult mouse kidneys that express *Musculin/MyoR*, a transcription factor found in skeletal muscle precursors. These cells localize to the kidney interstitium. The number of cells decreases following AKI, and infusion of the cells is associated with the expression of kidney-protective factors and improved kidney function in an acute model of kidney injury, but not in chronic kidney disease. Challen et al. [41] isolated SP cells from adult mouse kidney, where they comprise 0.14% of the kidney cells. These cells do not express *Musculin/MyoR*, have multilineage differentiation potential, and are heterogeneous, including the presence of a monocytic component. Interestingly, these cells express genes involved in Notch signaling. Kidney-protective, paracrine effects are observed following infusion of these cells in AKI.

Candidate Markers Used to Isolate Kidney Stem Cells

Bussolati et al. [42] isolated and cultured a population of cells from adult human kidneys using CD133 as a selection marker. These cells can be differentiated in vitro and in vivo into epithelial and endothelial cells, can form tubules and vessels, and express early and late nephron markers. Injection of these cells 3 days following myoglobinuric AKI resulted in some incorporation of these human cells into mouse tubules. These cells have limited self-renewal properties and express HLA class I antigens.

Sagrinati et al. [43] used CD24 and CD133 to select a subset of parietal glomerular epithelial cells that have a high self-renewal potential and can be differentiated into kidney tubular cells, as well as osteocytes and adipocytes. Injection of these cells into SCID mice with myoglobinuric AKI resulted in incorporation of injected cells into regenerating tubules and improved kidney function.

Dekel et al. [44] used Sca-1 magnetic cell sorting to isolate nontubular Sca-1-positive cells from mouse kidneys. The cells are present in the interstitium of the kidney and can differentiate into myogenic, osteogenic, adipogenic, and neural lineages. When injected into the kidney parenchyma in a model of AKI, the cells adopt a kidney phenotype.

Selective Culture Conditions

Kitamura et al. [45] isolated a population of rapidly proliferating cells from microdissected proximal tubules that express the stem cell markers Sca-1 and Musahi-1 as well as early nephron markers. These cells can differentiate into mature tubular cells in culture. These cells have a triploid karyotype, although they do not undergo tumor formation in nude mice.

Gupta et al. [46] isolated a unique population of cells from rat kidneys that were called multipotent renal progenitor cells. Features of these cells include: spindle-shaped morphology, self-renewal for over 200 population doublings without evidence for senescence, normal karyotype and DNA, expression of vimentin, CD90 (Thy1.1), Pax-2, and Oct-4, but not cytokeratin, MHC class I or II, or other markers of more differentiated cells. Multipotent renal progenitor cells exhibit plasticity demonstrated by the ability of the cells to be induced to express endothelial, hepatocyte, and neural markers by quantitative RT-PCR and immunohistochemistry. The cells can differentiate into kidney tubules when injected under the capsule of an uninjured kidney or intra-arterially following kidney ischemia-reperfusion injury.

Summary

A summary of studies isolating kidney stem cells is given in table 1. Differences in the cells isolated in these studies may be due to different selection markers, species of origin, age of the kidneys, and culture conditions. The different cells have been localized to multiple sites in the kidney, including tubular and interstitial cells of the kidney papilla, the proximal tubule, the cortical interstitium, and the parietal epithelial cells of the glomerulus. Different markers have been used to confirm these localizations, although in many cases it remains a challenge to confirm whether the cells isolated are the same as the in vivo cells. Despite this, the proximal tubule was a site for the stem cells in many of the studies. Interestingly, the proximal tubule is the site of greatest injury in AKI. Hence, it is logical to house stem cells at the site of maximal demand.

The isolated cells express a number of stem cell markers including Sca-1, Oct-4, Rex-1, and CD24 and CD133. The marker expressed by many of the cells was Sca-1. Sca-1 is a member of the Ly-6 family and is one of the earliest cell surface markers of hematopoietic stem cells, but has since been found to be expressed in bone marrow-derived multipotential cells and skeletal muscle satellite cells and is also present on mature cells. Oct-4 (also referred to as POU5F1) is a POU (Pit-Oct-Unc)-domain transcription factor expressed in embryonic stem cells, as well as in primordial germ cells and adult gonads [47–50]. Oct-4 plays a critical role in maintaining pluripotency of embryonic stem cells and the viability of primordial germ cells [48, 50–52]. Differentiation of embryonic stem cells is associated with downregulation of Oct-4 [53, 54]. Oct-4 expression has been demonstrated in stem cells isolated from umbilical cord blood, bone marrow, hair follicles, muscle, skin, breast, pancreas, liver, amniotic fluid, as well as endothelial progenitor cells and neural stem cells [47, 55–68]. These markers will be useful to localize a kidney stem cell niche and can be used to study cell lineage progression in the normal and injured kidney.

Future Directions

Despite significant advances in the field of kidney stem cells, significant major issues remain unresolved. The fundamental question of whether stem cells exist in the adult kidney remains controversial. Many groups have isolated cells from the kidney with properties of kidney progenitor cells, although the phenotype of many of these cells may be influenced by in vitro culture condi-

tions. Defining kidney stem cell markers is critical for the field to move forward. Such markers will allow for the characterization of the kidney stem cell niche and the signaling pathways that control stem cell behavior in the normal and injured kidney. The presence of stem cells in the adult kidney has important implications for our understanding of normal cell turnover in the kidney and the source of regenerating cells following AKI. Identification of kidney stem cell markers will set the stage for transgenic strategies to define such kidney cell lineage progression.

The in vitro differentiation of kidney stem cells can provide an important model system to study the cell biology involved in nephron formation and to dissect the role of specific factors in kidney cell lineage progression. Understanding the process of mesenchymal-epithelial transformation may also lead to strategies to limit or reverse kidney interstitial fibrosis. During the process of fibrosis, epithelial cells undergo epithelial-mesenchymal transformation, leading to migration of cells from the tubule to the interstitial space and subsequent transformation into fibroblasts [69–72]. The potential to reverse this process through mesenchymal-epithelial transformation has exciting therapeutic potential for the treatment of chronic kidney disease. In vitro generation of kidney tubular epithelial cells from stem cells would also be critical for developing a supply of cells to be used for cellular therapy, or a source of cells for kidney tubular assist devices.

AKI is associated with a poor short-term prognosis and adverse long-term effects on kidney function. The

lack of specific beneficial therapies has led investigators to explore strategies both to protect against kidney injury and to accelerate the repair process [73–77]. In most cases, strategies targeting kidney protection are dependent on knowing ahead of time when the injury event will occur. Therapeutic strategies directed at enhancing kidney repair have the potential to limit the duration of AKI and to better preserve long-term kidney function. Pharmacologic therapies directed at enhancing kidney regeneration are limited in their scope and have been unsuccessful when applied to the treatment of human AKI [78, 79]. On the other hand, cellular therapies using bone marrow- or kidney-derived stem cells can affect a number of different mechanisms, both autocrine and paracrine, and, therefore, have greater potential to enhance repair. In addition, stem cells could be used to deliver therapeutic genes or proteins to the injured and regenerating kidney.

Stem cell depletion may be a pathophysiological factor in many chronic progressive kidney diseases. The ability to identify and quantify stem cell numbers is essential to the study of this ‘stem cell depletion hypothesis’ and is dependent on the identification of stem cell markers. The availability of these tools along with a greater understanding of the in vivo control of stem cell behavior will enable studies to increase stem cell numbers and/or to accelerate the replacement of injured or dying cells through genetic and pharmacologic strategies.

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