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FOCUS ON

The contribution of adult stem cells to renal repair ☆

Implication des cellules souches adultes dans les processus de réparation après lésions rénales

Benjamin D. Humphreys*, Joseph V. Bonventre

Renal Division, Brigham and Women's Hospital, Department of Medicine, Harvard Medical School, Harvard Stem Cell Institute and Harvard-MIT Division of Health Sciences and Technology, Harvard Institutes of Medicine Room 550, 4 Blackfan Circle, Boston, MA 02115, USA

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Abstract The kidney undergoes continuous, slow cellular turnover for tissue maintenance and rapid cell replacement after injury. The cellular origin of newly differentiated tubular epithelium remains controversial. In some non-renal organs, adult stem cells are recognized as the cell of origin for tissue replacement, such as the hematopoietic system, intestine and skin. These findings have prompted intense investigation for evidence of renal stem cells because of the great need for new therapeutic approaches to treat acute kidney injury and chronic kidney disease. Early excitement at reports that bone marrow-derived cells transdifferentiate into renal epithelial cells has been tempered by findings that show such events to be rare or potentially explained by cell fusion. More recent studies have focused on the possibility that renal progenitors exist within the kidney. In this review we compare data supporting the existence of adult renal stem cells with the body of evidence indicating that the kidney regenerates by self-duplication of differentiated cells. The identification of adult renal epithelial progenitor cells will ultimately determine the future direction of renal regenerative medicine.

MOTS CLÉS
Cellules souches ;
Lésions de reperfusion ;
Régénération ;
Moëlle osseuse ;
Progéniteurs

Résumé Le rein est le siège d'un turnover cellulaire permanent, lent lors du renouvellement tissulaire physiologique et rapide lorsque les cellules doivent se différencier et proliférer après un processus lésionnel. L'origine cellulaire de l'épithélium tubulaire nouvellement formé reste controversée. Dans certains organes, les cellules souches adultes ont été identifiées comme étant à l'origine de la régénération tissulaire, notamment au niveau du tissu hématopoïétique, du tube digestif ou de la peau. Ces données ont conduit à d'intenses recherches, dans le but d'identifier les cellules souches rénales afin de développer de nouvelles approches thérapeutiques dans l'espoir de traiter l'insuffisance rénale aiguë ou les maladies rénales chroniques. Les premières données encourageantes, concernant le rôle des cellules

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* Corresponding author.
E-mail address: bhumphreys@partners.org (B.D. Humphreys).

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dérivées de la moelle osseuse comme pouvant se transdifférencier en cellules épithéliales rénales, ont été tempérées par la mise en évidence que ces évènements étaient rares et qu'ils pouvaient être expliqués par la fusion des cellules in situ. Les études plus récentes se sont focalisées sur l'existence possible de progéniteurs d'origine rénale existant au sein même de l'organe. Dans cette revue, nous avons essayé de comparer les données qui concluent à l'existence de cellules souches d'origine rénale adultes à celles qui indiquent que la réparation rénale se fait par la multiplication des cellules déjà différenciées. L'identification de cellules progénitrices adultes d'origine épithéliale au niveau du rein déterminera finalement le futur de cette approche thérapeutique.

Points forts

- Les cellules souches adultes ou cellules souches tissus-spécifiques ont deux propriétés : elles sont capables de proliférer spontanément et de se différencier en un nombre limité de cellules spécifiques d'organes ;
- le rein a une grande capacité de régénération lorsqu'il est lésé ;
- l'implication des cellules souches rénales dans le processus de réparation est discutée. Le modèle le mieux documenté est celui de la dédifférenciation des cellules in situ, de leur prolifération et de leur redifférenciation avant migration et réparation tissulaire ;
- les cellules qui permettent la reconstitution d'un épithélium tubulaire après nécrose tubulaire aiguë peuvent dériver soit de l'épithélium tubulaire lui-même, soit de cellules résidantes intrarénales ou de cellules souches extrarénales. L'absence de marqueur spécifique rend pour l'instant ces trois hypothèses toujours d'actualité ;
- la meilleure preuve de l'implication des cellules souches dans le processus de réparation tissulaire vient des expériences de transplantation de moelle dont le donneur est un mâle et le receveur une femelle. Après transplantation, on observe non seulement une reconstitution du système hématopoïétique mais également la présence de cellules ayant un chromosome Y dans le foie, le tube digestif et les poumons des animaux femelles.

Organ-specific adult stem cells

The defining characteristics of stem cells are self-renewal and the ability to differentiate into mature progeny. During embryonic development, for example, a single totipotent cell (the zygote) gives rise to the more than 250 terminally differentiated cell types making up the human body. In the adult, stem cells with these two properties (self-renewal and differentiation ability) also exist. These are called tissue-specific or adult stem cells, and they are multipotent rather than totipotent, i.e. able to generate a limited number of progeny found in the organ in which they reside, but not cells of other organs. The hematopoietic stem cell (HSC) remains the best characterized example of an adult multipotent stem cell population and it has long been recognized that a single HSC can reconstitute all hematopoietic cell lineages [1,2].

Strong evidence supports the existence of tissue-specific stem cells in skin and intestine and it is likely that such cells exist in muscle and nervous system [3–6]. A summary of some adult organs for which reasonable evidence supports the existence of tissue-specific stem cells is shown in Table 1. Multipotent cutaneous stem cells, for example, are localized to a specialized niche within the hair follicle known as the bulge. These are slow-cycling cells with high, clonogenic proliferative capacity which can give rise to all cell types in the epidermis [7]. The identification of markers for bulge stem cells such as the cell surface marker CD34 and keratinocyte 15 promoter activity have enabled purification and lineage tracing of these cells [8,9]. In contrast, the lack of stem cell markers in other organs like kidney has hampered progress in identifying stem cells, emphasizing the importance of such markers for the characterization of tissue-specific progenitor populations.

Renal stem cells and therapeutics

The possible existence of renal stem cells has generated excitement because they may ultimately have important therapeutic applications [10–14]. The well-documented burden of acute and chronic kidney disease is costly in both human and financial terms, and is growing worldwide

Table 1 Tissue-specific stem cell populations and selected markers in adult organs

Adult organ	Tissue-specific stem cell	Stem cell markers
Hematopoietic system	HSC	$Lin^{-}Sca^{+}c\text{-}kit^{+}$ [56]
Skin	Bulge cells	CD34, K5 [7]
Intestine	Crypt stem cells	Msi1 [57]
Muscle	Satellite cells	Pax3, Pax7, CD34 [58]
Nervous system	Neural progenitor cells	CD133, GFAP, nestin [6]
Liver	Oval cells	None [59]
Lung	Bronchoalveolar stem cells	CCA, SP-C [32]
Kidney	?	None

[15]. Successes in renal transplantation are balanced by long waiting lists and a scarcity of donor kidneys [16]. The clinical need has driven rapid expansion of the field of renal stem cell research. A variety of applications for these cells can be envisioned. Renal stem cells could represent a cellular source for the bioartificial kidney, a hemofiltration system containing live human tubular epithelial cells that just completed testing in a phase-II clinical trial [17,18]. In chronic kidney disease, by contrast, the therapeutic emphasis has been on preventing renal cell loss and scarring but impaired renal regeneration from stem cell depletion may be equally important. Renal progenitor cells could be a therapeutic target, either by promoting their activation or preventing their depletion, to modulate tissue remodeling in chronic nephropathies. In acute tubular injury, knowledge of the signaling pathways regulating renal stem cells will set a baseline from which to attempt to enhance the regenerative capacity of these progenitors. The ultimate goal of reconstituting an entire kidney in vitro may be impossible due to the complexity of the tissue. Yet the combination of advances in our understanding of nephrogenesis, progress in the directed differentiation of embryonic stem cells and tissue-engineering techniques are providing the tools for future cell therapy-based solutions to kidney disease [19,20].

Renal repair after injury

It has long been appreciated that the kidney has a great capacity for regeneration after injury, and over the last 20 years many of the molecular details of this process have been elucidated [21,22]. After an ischemic insult there is cell death and loss of tubular epithelium through apoptosis and necrosis with sloughing of dead cells into the tubular lumen. The surviving cells dedifferentiate, expressing such mesenchymal proteins as vimentin, neural cell adhesion molecule (NCAM), Pax-2 and basic fibroblast growth factor [23,24]. Recently Lin and colleagues genetically tagged renal epithelial cells with green fluorescent protein (GFP). After ischemic injury, they found cells coexpressing GFP and vimentin, supporting previous observations that surviving epithelial cells undergo dedifferentiation [25]. Dedifferentiation after epithelial injury suggests that renal regeneration recapitulates some aspects of renal development, since many of the proteins induced in postischemic kidney play important regulatory roles during nephrogenesis.

The classical model for renal regeneration also includes spreading and migration of surviving cells to cover areas of denuded basement membrane followed by redifferentiation into an epithelial phenotype [21,26]. Although the normal proliferative rate in kidney is very low, we have shown a dramatic increase in tubular epithelial proliferation after injury, as assessed by Ki67 and proliferating cell nuclear antigen (PCNA) immunostaining, as well as 5-bromo 2′-deoxyuridine (BrdU) labeling studies [23,27]. At 48 hours after ischemia, $4.5 \pm 0.4\%$ of all outer medullary proximal tubule cells are in mitosis and $63.7 \pm 4.7\%$ of these cells express PCNA [28]. A variety of growth factors, matrix elements and inflammatory mediators regulate the various components of this repair process [29,30]. We have interpreted the high proliferative capacity of injured kidney to reflect an intrinsic ability of surviving epithelial cells to adapt to the loss of neighboring cells by dedifferentiating and proliferating and ultimately replacing the cells that have died as a result of the insult.

The concept that renal epithelia have the ability to divide by self-duplication is supported by observations in uninjured, healthy rat kidney. Vogetseder et al. found a very low basal rate of cell division, as assessed by BrdU incorporation and PCNA immunostaining, in healthy rat kidney. Rare tubular cells undergoing mitosis expressed markers of terminal differentiation such as basolateral Na-K-ATPase, NaPi-IIa and PMP70. Vimentin was not detected in dividing tubular in these uninjured kidneys. The authors concluded that replenishment of tubular epithelial cells in healthy kidney comes from cycling, differentiated cells rather than less differentiated progenitor cells [31]. In contrast to the postischemic kidney, dedifferentiation did not accompany cell proliferation (at least as defined by expression of vimentin). Another major difference between the injured and healthy kidney is the rarity of cell division in healthy kidney. While the mechanisms underlying tissue maintenance in healthy kidney and regeneration after injury may be quite different, these experiments support the concept that differentiated renal epithelia possess an intrinsic capacity to divide.

Sources of renal stem cells

That most evidence to date supports a model of dedifferentiation, proliferation and redifferentiation after injury does not rule out a contribution of renal stem cells to renal repair. A weakness to the accumulated data are that they have relied on histologic snapshots from immunostaining to draw conclusions about cell lineage. Only genetic lineage tracing will permit unambiguous determination of cell fate in renal repair because of variability in antigen expression patterns after cellular injury. Until such experiments are completed there will be continued uncertainty about the cellular origin of the new differentiated epithelia after injury. The sources of cells that could be involved with repair of damaged epithelium are depicted in Fig. 1. The cells that repopulate the tubule after injury may derive either from within the tubule (intra-tubular) or outside of the tubule (extra-tubular) either from intrarenal or extrarenal sources. As discussed above, our data indicates that there is a high degree of proliferation within the tubule 24–48 hours after ischemic injury, and one interpretation of this observation is that every tubular cell shares the capability to dedifferentiate and proliferate after injury (as long as it survives the initial insult). Alternatively, a subset of tubular epithelial stem/progenitor cells may be responsible for proliferation after ischemic injury. These cells may be localized to a discrete tubular region, be more resistant to injury and have a very high proliferative capacity. Without markers to identify such cells this hypothesis will be difficult to prove, yet it is an attractive theory by extension from what has been found in lung [32]. A third possibility is that extra-tubular stem cells contribute to the regenerating tubule. Such cells, whether derived from the interstitium or from bone marrow, would need to migrate to the site of

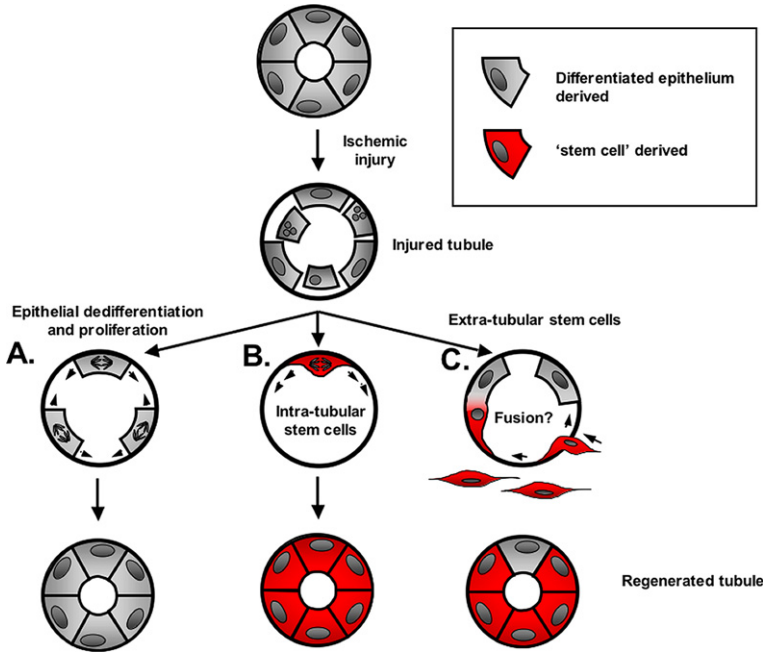

Figure 1 The origin of epithelial progenitors in acute kidney injury. After an ischemic renal injury, tubular epithelia undergo both apoptosis and necrosis, with decreased cell–cell contacts and sloughing of dead cells into the tubular lumen. Repopulation of the denuded basement membrane may occur by dedifferentiation of surviving epithelial cells with subsequent proliferation of any cells that survive the original injury (**A**). Alternatively, there may be a subset of intra-tubular stem cells (**B**) that are resistant to injury, and selectively respond to injury by proliferating. Finally, regeneration may be accomplished by migration of extra-tubular stem cells (e.g. bone marrow-derived or renal papilla resident stem cells) that cross the basement membrane into the tubule. Once within they tubule, they may contribute to renal regeneration either by fusion with surviving, dedifferentiated epithelial cells or by subsequent proliferation and differentiation into mature renal epithelium (**C**).

injury, cross the basement membrane and subsequently either proliferate and differentiate into the mature nephron or fuse with and reprogram surviving epithelial cells.

Bone marrow cells in renal regeneration

Experiments in the last 5 years have suggested that cells from bone marrow possess a surprising degree of plasticity. Transplantation of a single male bone marrow-derived cell into a lethally irradiated female mouse, in addition to reconstituting the hematopoietic system, resulted in Y-chromosome positive cells in other organs such as liver, intestine and lungs [33]. Similar results were obtained by a number of groups [34,35]. Based on these and other observations the model of tubular regeneration from surviving epithelial cells was questioned, with arguments presented for an alternative model where bone marrow-derived stem cells (BMSCs) contribute to new epithelial cells in functionally important numbers after kidney injury [36,37]. Subsequent work by several groups has shown that tubular cell replacement by BMSC is much lower than originally reported, calling into question the concept that BMSC could find a therapeutic application in kidney injury.

Lin et al. [38] purified BMSC ($Lin^{-}Sca\text{-}1^{+}ckit^{+}$) from male Rosa26 reporter mice and injected them intravenously into female wild-type recipients subjected to IRI. LacZ positive cells were seen in tubules of postischemic kidney both by X-gal stain and LacZ immunostaining. Y-chromosome positive cells were also seen in these tubules. In a follow up study, the same group performed ischemic renal injury after bone marrow transplantation and examined kidneys 28 days after injury. They found a majority of bone marrow-derived cells were present in interstitium (81%) and much fewer in glomerulus (10.6%) or tubule (8.4%) [25]. Importantly, there was no difference in degree of renal damage or rapidity of recovery in transplanted animals when compared to non-transplanted controls. These authors concluded that the main source of cells in regenerating kidney derive from within the kidney not from marrow.

In contrast, Kale et al. [37] injected purified LacZ BMSCs ($Lin^{-}Sca\text{-}1^{+}$) 12 hours prior to bilateral IRI, and observed significant protection from decline in renal function as well as an increase in the number of tubular cells that expressed ß-galactosidase activity. These experiments appeared to indicate that BMSC could be rapidly recruited to postischemic kidney, transdifferentiate into tubular cells and ameliorate renal damage. The absence of marrow-derived LacZ+ interstitial cells in these mice, however, prompted a reanalysis. Using sex-mismatched, lineage-negative bone marrow cells injected prior to bilateral renal IRI, the authors could find only very rare Y-chromosome cells within tubules that coexpressed proximal tubule markers [10]. The explanation for the presence of intra-tubular ß-galactosidase activity seen in the previous experiments may be either endogenous ß-galactosidase upregulated in postischemic kidney or uptake of the enzyme by injured tubular epithelial cells.

Our group generated three models in which transplantation of tagged bone marrow into a lethally irradiated, non-tagged recipient allowed lineage tracing of marrow-derived cells in the injured kidney [28]. Six weeks after transplantation of sex-mismatched marrow or transgenic marrow with constitutive expression of GFP or LacZ into wild-type recipients, mice underwent unilateral ischemia–reperfusion injury (IRI) and kidneys were assessed at days 2 and 7 post-IRI for replacement of epithelial cells by cells of bone marrow origin. Although there was a marked increase in BM-derived cells in the interstitium, tubular cells with Y-chromosome, GFP or LacZ in either postischemic or contralateral kidneys could not be identified. Rare BM-derived cells that appeared tubular were in fact interstitial after deconvolution microscopy [39], potentially explaining the discordance with previously published results.

It has recently become clear that BMSCs may fuse with differentiated cells in various adult organs, further complicating the interpretation of marrow transplantation studies. Grompe and coworkers have shown that BMSC fuse with both intestinal stem cells and hepatocytes [40,41]. These carefully performed studies involved a dual marker system for donor and recipient cells with Y-chromosome analysis in sex-mismatched transplants to assess cell fusion. Remarkably, cell fusion in these two instances did not appear to impair cell division, or confer genetic instability at least during the time course of the study. In a recent paper from the same group, cell fusion could be induced between bone marrow-derived cells and renal tubular cells under conditions of chronic renal damage and continuous genetic selection [42]. The model, combined fumarylacetoacetate and homogentisic acid dehydrogenase deficiency, causes chronic renal damage by renal production of toxic fumarylacetoacetate. Bone marrow transplantation with wild-type bone marrow resulted in variable degrees of fusion (from minimal to 50%) between bone marrow cells and renal epithelial cells 6–9 months after transplant. The fused cells had a growth advantage because they were able to metabolize the fumarylacetoacetate. Thus it appears that under these artificial conditions a high degree of bone marrow fusion with renal epithelia can be observed. Of note, the authors observed only very rare bone marrow-derived cells in tubular epithelia after IRI alone, confirming that bone marrow repopulation of renal epithelia does not represent a physiologic mechanism for renal repair.

Taken together, these experiments provide strong evidence against a physiologic role for BMSC-derived cells in regeneration of postischemic tubule by direct replacement of epithelial cells. There is a role for interstitial bone marrow-derived cells in the production of protective paracrine factors that may facilitate repair of the epithelium. The injection of cell populations prior to or just after renal injury may prevent renal damage by stem cell independent mechanisms. We and others have found that injection of 0.5×10^6 mesenchymal stem cells (MSCs) is protective for renal injury as assessed by serum creatinine measured 24 hours after ischemia [28]. Intriguingly, injection of embryonic fibroblasts was also protective and an independent group has confirmed that MSCs protect against ischemic renal injury by a differentiation-independent mechanism [43]. In the adriamycin-nephropathy model, injection of the side-population (SP) is also protective in the absence of tubular integration [44]. The mechanism of such protection may be through immune modulation, since injected cells may be rapidly ingested by immune cells in the spleen, liver and lungs. This area warrants further investigation.

Intrarenal stem cells

Work characterizing intrarenal stem cells remains at a preliminary stage. Due to the lack of any markers for intrarenal stem cells in the adult kidney, even basic questions such as the precise location, size of the pool or cellular morphology are either unknown or controversial. It is encouraging that several groups have been able to culture cells with stem characteristics such as BrdU retention or spherical growth in suspension culture. These initial efforts should facilitate gene profiling of putative renal progenitor populations by microarray analysis, as well as the identification of culture conditions allowing expansion of renal progenitors in tissue culture.

Oliver et al. have used a BrdU labeling approach to identify putative adult renal stem cells after kidney injury. By pulsing 3-day-old rat pups with BrdU followed by a long chase of at least 2 months, a population of label-retaining cells were identified in the renal papilla [45]. These cells were primarily interstitial, although a small fraction were intra-tubular and coexpressed renal epithelial markers. After transient renal ischemia, BrdU label was lost in the absence of apoptosis, suggesting that these putative stem cells proliferated in response to injury. Intriguingly, Oliver et al. identified conditions supporting the growth of cell spheres from isolated papilla that were positive for the intermediate filament nestin, a marker for neural progenitor cells. These cellular spheres grew best in serum-free culture conditions and many floated, both properties of neural progenitor cells grown in culture [6].

BrdU labeling has proven to be a powerful technique in stem cell biology, but its limitations should be kept in mind. BrdU label may be released by dying cells and taken up by adjacent, dividing cells [46]. Such BrdU release might occur in injured kidney, where considerable cell death occurs by either apoptosis or necrosis. A separate concern with BrdU is that after ischemic injury, some cells may synthesize DNA without dividing, further complicating interpretation of injury models in the kidney [47]. Ultimately a genetic approach may prove to be the most robust means to identify the label-retaining population in kidney. Tumbar et al. [8] have used one such approach to identify and isolate cutaneous stem cells. These authors created a transgenic mouse to express histone H2B-GFP controlled by a tetracycline-responsive regulatory element. They crossed this mouse with a second transgenic mouse expressing the tet repressor protein (VP16) under control of a skin-specific promoter (keratin-5). Progeny exhibited doxycycline-repressible nuclear histone H2B-GFP expression in skin. By feeding 4-week-old mice doxycycline for 4 months (the “chase” period), they were able to identify and purify cells that still expressed histone H2B-GFP as a slowly dividing cell population present in hair follicles. A drawback to this technique is that it requires that tet repressor expres-

sion be driven by a promoter that is active in the adult stem cell population to be studied, and which promoter would be suitable in kidney remains unclear.

Whether the putative renal stem cells identified by Oliver et al. [45] are the only source of new epithelial cells in regenerating kidney remains to be determined, given the rapid epithelial proliferation seen in postischemic kidney 24–48 hours after injury. The migration of these progenitors from the papilla to the S3 segment of the proximal tubule, where most of the injury occurs, would seem to require significantly longer than 24–48 hours. Despite these concerns, this intriguing study provides evidence that adult kidney may possess an intrinsic stem cell population.

Maeshima et al. [48] have characterized a different label-retaining population in rat kidney. Rats were pulsed with BrdU continuously for 1 week followed by a 2 weeks chase period. This resulted in predominant labeling of proximal tubular epithelia. BrdU pulse was given to 7-week-old rats, and the chase was only 2 weeks, so these differences may partially explain the different pattern of BrdU labeling compared to Oliver et al. After renal IRI, 75% of the label-retaining tubular cells (LRTC) were also positive for PCNA, and only 14% of PCNA positive cells were BrdU negative, suggesting that the majority of proliferating cells in the postischemic kidney derive from the LRTC pool. The authors were able to isolate and culture the LRTC and showed that they formed branching tubes in three-dimensional culture after exposure to growth factors. Additionally, a subset of the cultured cells was incorporated into epithelial structures after injection into metanephric kidney cultures. These experiments, along with others from groups who have isolated renal progenitor cell lines, provide additional evidence for a population of adult renal stem cells [49,50,60].

Identification of renal stem cell markers

The need to identify renal stem cell markers is being addressed by several groups using complementary approaches. Challen and Little used a microarray approach to identify molecules expressed in uninduced metanephric mesenchyme, the embryonic tissue containing a renal progenitor population responsible for formation of most of the nephron [51,52]. After isolating uninduced metanephric mesenchyme from embryonic day 10.5 ($E_{10.5}$) mice, this tissue was subjected to microarray analysis. The authors found preferential expression of 21 genes in metanephric mesenchyme. Two of these proteins, CD24 and cadherin-11, are cell surface proteins and therefore may be exploited for purification of renal progenitors by FACS.

Barasch and colleagues have used a similar strategy to identify secreted proteins derived from the ureteric bud that may regulate nephrogenesis. In this case, they isolated ureteric bud from $E_{12.5}$ mice or $E_{13.5}$ rats and identified the transcriptional profile of this tissue using microarrays [53]. These investigations yielded 20 different transcripts encoding putative secreted molecules including known regulators of nephrogenesis such as BMP-7 as well as novel factors such as cytokine-like factor-1 (CLF-1). Collectively, these studies validate microarray technologies for the generation of lists of candidate markers for renal stem cells/progenitors.

A comprehensive approach toward identification of renal progenitor markers has been undertaken by Andrew McMahon's group at Harvard University. He has launched the Kidney Molecular Anatomy Project (KMAP), which aims to map the expression patterns of all major transcriptional regulators, signaling factors and their receptors in the developing mouse kidney [54]. This exciting project will define the molecular anatomy of kidney development and should provide many candidate stem cell markers.

Another strategy to isolate renal progenitor cells is the property of many progenitor cells to secrete dyes such as Hoechst 33342 and Rhodamine 123. Such "Hoechst low" cells were first identified as HSCs called the SP due to their position at the bottom left side of FACS sorting profiles after staining with Hoechst 33342 [55]. The SP fraction of embryonic and adult kidney was recently reported to represent 0.1–0.2% of the total kidney cell population. SP cells gave rise to mesodermal derivatives in vitro, and exhibited a significantly enhanced ability to engraft into developing kidney than main population cells. Expression profiling revealed many candidate marker genes for SP including members of the Notch signaling pathway [44]. Although the SP was heterogeneous on a cellular level, this study validates use of the SP for enrichment of renal progenitors and identification of candidate renal stem cell markers.

Conclusions

Stem cells hold enormous promise for the treatment of human kidney diseases. Current challenges to the understanding of the roles and potential therapeutic implications of stem cells in repair of the kidney include developing robust lineage tracing methodologies and identifying markers for the localization and purification of renal stem cells. Surmounting these challenges will lead to a better understanding of the cells involved with the repair process and to the identification of novel therapeutic strategies to facilitate renal repair after acute kidney injury in humans.

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References

[1] Osawa M, Hanada K, Hamada H, Nakauchi H. Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. Science 1996;273(5272):242–5.

[2] Morrison SJ, Weissman IL. The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. Immunity 1994;1(8):661–73.

[3] Reynolds BA, Weiss S. Clonal and population analyses demonstrate that an EGF-responsive mammalian embryonic CNS precursor is a stem cell. Dev Biol 1996;175(1):1–13.

[4] Wagers AJ, Conboy IM. Cellular and molecular signatures of muscle regeneration: current concepts and controversies in adult myogenesis. Cell 2005;122(5):659–67.

[5] Crosnier C, Stamataki D, Lewis J. Organizing cell renewal in the intestine: stem cells, signals and combinatorial control. Nat Rev Genet 2006;7(5):349–59.
[6] Ming GL, Song H. Adult neurogenesis in the mammalian central nervous system. Annu Rev Neurosci 2005;28:223–50.
[7] Cotsarelis G. Epithelial stem cells: a folliculocentric view. J Invest Dermatol 2006;126(7):1459–68.
[8] Tumbar T, Guasch G, Greco V, Blanpain C, Lowry WE, Rendl M, et al. Defining the epithelial stem cell niche in skin. Science 2004;303(5656):359–63.
[9] Ito M, Liu Y, Yang Z, Nguyen J, Liang F, Morris RJ, et al. Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. Nat Med 2005;11(12): 1351–4.
[10] Cantley LG. Adult stem cells in the repair of the injured renal tubule. Nature Clinical Practice Nephrology 2005;1(1):22–32.
[11] Lin F. Stem cells in kidney regeneration following acute renal injury. Pediatr Res 2006.
[12] Brodie JC, Humes HD. Stem cell approaches for the treatment of renal failure. Pharmacol Rev 2005;57(3):299–313.
[13] Ricardo SD, Deane JA. Adult stem cells in renal injury and repair. Nephrology (Carlton) 2005;10(3):276–82.
[14] Little MH. Regrow or repair: potential regenerative therapies for the kidney. J Am Soc Nephrol 2006;17(9):2390–401.
[15] Xue JL, Ma JZ, Louis TA, Collins AJ. Forecast of the number of patients with end-stage renal disease in the United States to the year 2010. J Am Soc Nephrol 2001;12(12):2753–8.
[16] Danovitch GM, Hariharan S, Pirsch JD, Rush D, Roth D, Ramos E, et al. Management of the waiting list for cadaveric kidney transplants: report of a survey and recommendations by the Clinical Practice Guidelines Committee of the American Society of Transplantation. J Am Soc Nephrol 2002;13(2):528–35.
[17] Humes HD, Weitzel WF, Bartlett RH, Swaniker FC, Paganini EP, Luderer JR, et al. Initial clinical results of the bioartificial kidney containing human cells in ICU patients with acute renal failure. Kidney Int 2004;66(4):1578–88.
[18] Fissell WH, Humes HD. Cell therapy of renal failure. Transplant Proc 2003;35(8):2837–42.
[19] Humes HD. Stem cells: the next therapeutic frontier. Trans Am Clin Climatol Assoc 2005;116:167–83 (discussion 183-164).
[20] Cortesini R. Progress in tissue engineering and organogenesis in transplantation medicine. Exp Clin Transplant 2003;1(2): 102–11.
[21] Thadhani R, Pascual M, Bonventre JV. Acute renal failure. N Engl J Med 1996;334(22):1448–60.
[22] Sheridan AM, Bonventre JV. Pathophysiology of ischemic acute renal failure. Contrib Nephro 2001;132:7–21.
[23] Witzgall R, Brown D, Schwarz C, Bonventre JV. Localization of proliferating cell nuclear antigen, vimentin, c-Fos, and clusterin in the postischemic kidney. Evidence for a heterogenous genetic response among nephron segments, and a large pool of mitotically active and dedifferentiated cells. J Clin Invest 1994;93(5):2175–88.
[24] Villanueva S, Cespedes C, Vio CP. Ischemic acute renal failure induces the expression of a wide range of nephrogenic proteins. Am J Physiol Regul Integr Comp Physiol 2006;290(4): R861–R870.
[25] Lin F, Moran A, Igarashi P. Intrarenal cells, not bone marrow-derived cells, are the major source for regeneration in postischemic kidney. J Clin Invest 2005;115(7):1756–64.
[26] Bonventre JV. Dedifferentiation and proliferation of surviving epithelial cells in acute renal failure. J Am Soc Nephrol 2003; 14(Suppl 1):S55–61.
[27] Abbate M, Brown D, Bonventre JV. Expression of NCAM recapitulates tubulogenic development in kidneys recovering from acute ischemia. Am J Physiol 1999;277(3 Pt 2):F454–F463.
[28] Duffield JS, Park KM, Hsiao LL, Kelley VR, Scadden DT, Ichimura T, et al. Restoration of tubular epithelial cells during repair of the postischemic kidney occurs independently of bone marrow-derived stem cells. J Clin Invest 2005;115(7): 1743–55.
[29] Bonventre JV. Molecular response to cytotoxic injury: role of inflammation, MAP kinases, and endoplasmic reticulum stress response. Semin Nephrol 2003;23(5):439–48.
[30] Schena FP. Role of growth factors in acute renal failure. Kidney Int Suppl 1998;66:S11–5.
[31] Vogetseder A, Karadeniz A, Kaissling B, Le Hir M. Tubular cell proliferation in the healthy rat kidney. Histochem Cell Biol 2005;124(2):97–104.
[32] Kim CF, Jackson EL, Woolfenden AE, Lawrence S, Babar I, Vogel S, et al. Identification of bronchioalveolar stem cells in normal lung and lung cancer. Cell 2005;121(6):823–35.
[33] Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, et al. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell 2001;105(3): 369–77.
[34] Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, et al. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nat Med 2000;6(11):1229–34.
[35] Herzog EL, Chai L, Krause DS. Plasticity of marrow-derived stem cells. Blood 2003;102(10):3483–93.
[36] Poulsom R, Forbes SJ, Hodivala-Dilke K, Ryan E, Wyles S, Navaratnarasah S, et al. Bone marrow contributes to renal parenchymal turnover and regeneration. J Pathol 2001;195 (2):229–35.
[37] Kale S, Karihaloo A, Clark PR, Kashgarian M, Krause DS, Cantley LG. Bone marrow stem cells contribute to repair of the ischemically injured renal tubule. J Clin Invest 2003;112(1): 42–9.
[38] Lin F, Cordes K, Li L, Hood L, Couser WG, Shankland SJ, et al. Hematopoietic stem cells contribute to the regeneration of renal tubules after renal ischemia–reperfusion injury in mice. J Am Soc Nephrol 2003;14(5):1188–99.
[39] Duffield JS, Bonventre JV. Kidney tubular epithelium is restored without replacement with bone marrow-derived cells during repair after ischemic injury. Kidney Int 2005;68 (5):1956–61.
[40] Rizvi AZ, Swain JR, Davies PS, Bailey AS, Decker AD, Willenbring H, et al. Bone marrow-derived cells fuse with normal and transformed intestinal stem cells. Proc Natl Acad Sci USA 2006;103(16):6321–5.
[41] Grompe M. Bone marrow-derived hepatocytes. Novartis Found Symp 2005;265:20–7 (discussion 28–34, 92-27).
[42] Held PK, Al-Dhalimy M, Willenbring H, Akkari Y, Jiang S, Torimaru Y, et al. In vivo genetic selection of renal proximal tubules. Mol Ther 2006;13(1):49–58.
[43] Togel F, Hu Z, Weiss K, Isaac J, Lange C, Westenfelder C. Administered mesenchymal stem cells protect against ischemic acute renal failure through differentiation-independent mechanisms. Am J Physiol Renal Physiol 2005; 289(1):F31–F42.
[44] Challen GA, Bertoncello I, Deane JA, Ricardo SD, Little MH. Kidney side population reveals multilineage potential and renal functional capacity but also cellular heterogeneity. J Am Soc Nephrol 2006;17(7):1896–912.
[45] 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 2004;114(6):795–804.
[46] Burns TC, Ortiz-Gonzalez XR, Gutierrez-Perez M, Keene CD, Sharda R, Demorest ZL, et al. Thymidine analogs are transferred from pre-labeled donor to host cells in the central nervous system after transplantation: a word of caution. stem cells. 2006.

[47] Kuan CY, Schloemer AJ, Lu A, Burns KA, Weng WL, Williams MT, et al. Hypoxia-ischemia induces DNA synthesis without cell proliferation in dying neurons in adult rodent brain. J Neurosci 2004;24(47):10763–72.

[48] 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 2003;14(12): 3138–46.

[49] Kitamura S, Yamasaki Y, Kinomura M, Sugaya T, Sugiyama H, Maeshima Y, et al. Establishment and characterization of renal progenitor like cells from S3 segment of nephron in rat adult kidney. FASEB J 2005;19(13):1789–97.

[50] Maeshima A, Sakurai H, Nigam SK. Adult kidney tubular cell population showing phenotypic plasticity, tubulogenic capacity, and integration capability into developing kidney. J Am Soc Nephrol 2006;17(1):188–98.

[51] Challen GA, Martinez G, Davis MJ, Taylor DF, Crowe M, Teasdale RD, et al. Identifying the molecular phenotype of renal progenitor cells. J Am Soc Nephrol 2004;15(9):2344–57.

[52] Herzlinger D, Koseki C, Mikawa T, al-Awqati Q. Metanephric mesenchyme contains multipotent stem cells whose fate is restricted after induction. Development 1992;114(3):565–72.

[53] Schmidt-Ott KM, Yang J, Chen X, Wang H, Paragas N, Mori K, et al. Novel regulators of kidney development from the tips of the ureteric bud. J Am Soc Nephrol 2005;16(7):1993–2002.

[54] McMahon A. Patterning of the mammalian kidney. Kidney Int 2005;68(5):1962–72.

[55] Challen GA, Little MH. A side order of stem cells: the SP phenotype. Stem Cells 2006;24(1):3–12.

[56] Morrison SJ, Wandycz AM, Hemmati HD, Wright DE, Weissman IL. Identification of a lineage of multipotent hematopoietic progenitors. Development 1997;124(10):1929–39.

[57] Kayahara T, Sawada M, Takaishi S, Fukui H, Seno H, Fukuzawa H, et al. Candidate markers for stem and early progenitor cells, Musashi-1 and Hes1, are expressed in crypt base columnar cells of mouse small intestine. FEBS Lett 2003;535(1–3): 131–5.

[58] Montarras D, Morgan J, Collins C, Relaix F, Zaffran S, Cumano A, et al. Direct isolation of satellite cells for skeletal muscle regeneration. Science 2005;309(5743):2064–7.

[59] Shafritz DA, Oertel M, Menthena A, Nierhoff D, Dabeva MD. Liver stem cells and prospects for liver reconstitution by transplanted cells. Hepatology 2006;43:S89–98 (2 Suppl 1).

[60] Gupta S, Verfaillie C, Chmielewski D, Kren S, Eidman K, Connaire J, et al. Isolation and characterization of kidney-derived stem cells. J Am Soc Nephrol 2006;17(11):3028–40.