The Role of Renal Progenitors in Renal Regeneration

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Key Words

Podocyte · Renal · Renal regeneration · Tubular regeneration

Abstract

The adult kidney has a remarkable ability to survive injury and restore function despite a limited turnover of cells under physiologic conditions. This accounts both for the tubular and to a lesser extent for the glomerular compartment. It is an ongoing debate whether renal repair is carried out by self-duplication/de-differentiation of mature resident renal cells, or by specialized renal progenitors residing in specific niches or by circulating bone marrow-derived stem cells. In this review, the existence of renal progenitor cells and their contribution for regeneration of the tubular and the glomerular compartment are discussed, highlighting landmark publications of recent years.

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Bone Marrow Derived Stem Cells

The contribution of circulating bone marrow derived stem cells (BMSCs) in renal repair is now recognized to be a rare event. For the tubular compartment, several groups could demonstrate with non-transgenic [1, 2] and transgenic approaches [3–5] that BMSC-derived cells

were found in the tubular epithelium following injury. However, careful examination showed that less than 1% of tubular cells originated from BMSCs [3] and that the majority of cells involved in tubular repair most likely originated from endogenous tubular cells [5, 6]. With the help of lineage tracing experiments, it was demonstrated that tubular regeneration occurred from intrinsic surviving tubular epithelial cells and not from interstitial cells [7, 8].

Despite the rare occurrence of BMSCs in regenerating tubular epithelia, BMSCs have also been implicated in glomerular repair in mouse models with a damaged glomerular basement membrane [9, 10] but with limited findings in humans [1, 11] and negative findings in rats [12] and mice [13]. Complicating the interpretation of these results, cell fusion between BMSCs and differentiated cells has been observed in adult tissues [14, 15], giving rise to the possibility that integrated BMSCs in the tubular and glomerular compartment are a result of cell fusion rather than cell replacement. Despite the rare occurrence of BMSC incorporation in glomerular or tubular structures, a therapeutic effect has been noted following an injection of BMSC after tubular kidney injury such as ischemia [4], cisplatin-induced renal injury [16], ischemia and reperfusion injury [3, 17–19], glycerol-induced nephropathy [20] or after glomerular injury such as anti-Thy1.1 antibody-induced glomerulonephritis [21]. An injection of BMSCs prevented interstitial fibro-

sis in mice with Alport syndrome (COL4a3-null mice) [22] and in rats following 5/6 nephrectomy [23]. It is believed that BMSCs are protective in renal injury through paracrine effects such as the secretion of growth factors [24, 25] or by immune-modulatory functions [23].

Renal Progenitor Cells

In kidney development, single metanephric mesenchymal stem cells can regenerate all of the epithelial cell types of the nephron (excluding those of the collecting ducts, which arise from the ureteric bud) [26], but it is a matter of debate whether such progenitor cells persist in adult life. Different methods with their own intrinsic limitations exist to identify resident progenitor cells from differentiated adult cells in the kidney such as (1) detection of label-retaining properties (so-called label-retaining cells) due to low proliferative activity of stem cells, (2) detection of Hoechst dye extrusion via ATP binding cassette transporters by fluorescent-activated cell-sorting analysis [27], (3) selective culture conditions [28], (4) stem cell marker expression analyses (CD133, CD24, CD106, stem cell antigen-1, and the stem cell-specific transcription factors Bmi-1 and Oct-4), and (5) genetic reporter systems [29–31].

Based on these methods, different kinds of putative progenitor cell niches were identified in human and/or rodent kidneys. For example, label-retaining cells were identified in the renal papilla of rats and mice [32, 33] and in proximal tubules of rats [34, 35]. The drawback of this identification technique is that labels such as BrdU and Histone-2B-GFP might be released from dying cells and taken up from adjacent dividing cells [36]. Additionally, label-retaining cells can be fully differentiated cells and not stem/progenitor cells [37, 38].

Through the use of stem cell marker expression analyses, renal progenitor cells were identified in proximal tubules [39, 40] and in Bowman's capsule [41] of human kidneys (both characterized by CD133 expression, normally found in embryonic kidney [42, 43]). The use of stem cell markers is problematic, as they are scarce and rarely linked to their function and are not equally expressed in human and rodents. Moreover, gene expression levels fluctuate in stem cells in response to environmental cues and intrinsic factors, making it very difficult to discern stem cells from dedifferentiated cells.

Lineage tracing reporter systems using a putative parietal epithelial cell (PEC) promoter have led to the suggestion that podocyte progenitor cells were located in Bow-

man's capsule of mice [30]. This promoter also led to the labeling of proximal tubular cells (PTCs), which had concomitantly been identified due to their positivity for the stem cell marker CD133 [39, 40]. Cells of renin lineage were recently described as an additional niche for podocyte and PEC progenitor cells using this technique [31]. Lineage tracing is a powerful technique to mark cells with a heritable molecular mark and thereafter trace their behavior in physiological and pathophysiological settings. However, care has to be taken regarding the design of lineage tracing experiments and the subsequent interpretation of results. Of importance, the choice of promoter, of the molecular switch controlling Cre-recombinase activity, of the genetic marker that ultimately labels the target cell, and of the timing and duration of labeling can result in unequivocal results between mice lines [31, 44]. Furthermore, some lineage tracing systems can show background expression of Cre-recombinase in the absence of a Cre-inducing drug [44].

Renal Progenitor Cells in Tubules

In an attempt to investigate, whether renal progenitor cells identified by the expression of stem cell markers fulfilled the characteristics of progenitor cells such as the capacity of self-expansion, limited self-renewal and differentiation, CD133-positive cells were isolated from human kidneys. Once cultured, human CD133-positive renal cells exhibited progenitor characteristics and differentiated to endothelial and epithelial cells. Additionally, injection of these cells into mice with glycerol-induced acute renal injury resulted in their incorporation into tubules [45]. More precise investigations localized CD133⁺ CD24⁺ double positive cells in Bowman's capsule [41] and in tubules [40] of the human kidney; the latter were named scattered tubular cells (STCs). The addition of CD106 (vascular cell adhesion molecule 1) as an additional marker differentiated CD133⁺, CD24⁺ STCs (CD106⁻) with a low proliferation rate and the propensity to propagate tubular cells from CD133⁺, CD24⁺ PECs (CD106⁺) with a high proliferation rate and the propensity to differentiate towards tubular cells and podocytes in culture [46]. Injection of both of these human cell populations into mice following glycerol-induced renal injury attenuated tubulointerstitial fibrosis and clinical disease [46]. Despite these promising results, further studies are required to demonstrate, whether injured resident murine renal cells are truly replaced by the injected human progenitor cells, or whether the observed disease at-

Fig. 1. Scheme summarizing possible modes of tubular and glomerular (podocyte) regeneration. (1) Regeneration by dedifferentiation of resident cells and subsequent proliferation. (2) Regeneration by resident progenitor cells such as PECs (2a) especially in postnatal glomerular development, cells of renin lineage (2b), and STCs (2c). (3) Regeneration by BMSCs, which is considered to be a rare event. MD = Macula densa; MC = mesangial cell; DTC = distal tubular cell.

tenuating effects are due to cell fusion, paracrine and/or immune-modulatory effects as described above for BMSC.

Interestingly, the existence of tubular progenitor cells was questioned by the use of lineage tracing experiments in *SLC3Aa1-CreER^{T2}* mice, in which differentiated PTCs were terminally labeled upon tamoxifen-dependent recombination. When mice with completely labeled PTCs were subjected to acute renal ischemia and repair, there was no dilution of the fate marker despite substantial proliferation, indicating that unlabeled progenitors do not contribute to kidney repair [47]. These findings were supported by experiments using the doxycycline-inducible variant of the PEC-specific-transgenic mouse, which results in terminal labeling of PECs and SCTs. In these mice, no increase of labeled tubular cells was noted, when terminal labeling was induced prior to tubular injury, whereas terminally labeling SCTs during tubular injury resulted in an increase of labeled cells [48]. Further argu-

mentation against a tubular progenitor cell niche were findings that human and rodent SCTs shared a common transcriptional program with activated PECs (as in crescentic glomerulonephritis) characterized by the common expression of CD133, CD24, CD44, vimentin and kidney injury marker-1 [39]. Additionally, injury to labeled proximal tubule epithelia as well as culture of PTCs induced the expression of the above-mentioned putative stem cell markers [47]. Taken together, these findings in mice question the existence of an intra-tubular progenitor cell niche, but rather indicate that terminally differentiated tubular epithelia re-express apparent stem-cell markers during injury-induced dedifferentiation and repair, potentially rendering them more resistant to injury and providing the needed intrinsic proliferative activity to replace injured and lost tubular cells [39, 47, 48].

Renal Progenitor Cells in the Glomerulus

There appear to be several basic responses of the glomerulus to injury such as cellular proliferation, changes in glomerular cell phenotypes, and increased deposition of extracellular matrix. The cellular compartment of the glomerulus, which predominantly exhibits these basic responses, is in part determined by the nature of glomerular injury [49]. The potential of glomerular regeneration from injury is widely thought to be limited. Nonetheless, in human and in rat kidney, an expression of stem cell markers was found in PECs of Bowman's capsule [50, 51]. In human kidney, the stem cell markers CD133, CD24 and the podocyte protein podocalyxin (PDX) were utilized to subdivide human PECs into 3 subgroups: first, CD133⁺ CD24⁺ PDX⁻ cells at the urinary pole with the potential to differentiate towards podocytes and tubular cells; second, CD133⁺ CD24⁺ PDX⁺ intermediate cells with the potential to differentiate to podocytes and third CD133⁻, CD24⁻ PDX⁺ cells at the vascular pole, which resembled differentiated podocytes [51]. Injection of human CD133⁺, CD24⁺, PDX⁻ cells into mice with adriamycin-induced podocyte injury was shown to reduce glomerular damage and proteinuria [51]. Supporting the notion, that PECs could function as resident progenitor cells for podocytes, lineage-tracing experiments in juvenile mice demonstrated that PECs generated to a certain extent novel and fully developed podocytes during kidney maturation [13, 30, 52]. It was suggested that PECs could migrate onto the vascular tuft and differentiate into podocytes.

Despite these compelling data, it remains unclear, whether podocyte regeneration exists in adults during re-

nal aging or in pathophysiologic situations and, if podocyte regeneration exists, from what source of resident progenitor cells the novel podocytes originate. Podocytes are post-mitotic cells. Several studies indicate that loss of podocytes plays an important role in age-related glomerulosclerosis in humans and rodents [53–56]. A loss of 20% of podocytes is tolerated by rats [57] and mice [13] before glomerular fibrosis occurs. A putative regenerative increase of podocyte numbers following acute glomerular injury was described in a transgenic model using diphtheria toxin-induced podocyte ablation [13], in a model of focalsegmental glomerulosclerosis (FSGS) following glucocorticoid treatment [58, 59], in a leptin-deficient model of diabetic nephropathy [59] and following angiotensin converting enzyme inhibition in a rat model of spontaneous glomerular injury [60]. These studies were limited by the fact that they could not identify the source of the observed novel podocyte population and that they relied on indirect observations of podocyte repopulation such as the expression of proliferation and of cell markers like WT-1, p27^{Kip1}, p57, and synaptopodin in PECs, which may not be stable in pathophysiologic situations such as disease.

From recent studies it has become evident, that PECs and podocytes not only share a common embryologic background but also remain closely related after kidney maturation. PECs display a lineage switch towards podocytes during nephrogenesis following conditional β -catenin knockdown under the control of the Pax8 promoter [61]. Further underlining the common ancestry with podocytes, PECs have the capacity to express podocyte proteins in human disease [62–64] in culture [65] and in rodent disease models [66, 67]. Additionally, naïve rat PECs express mRNA encoding for podocyte proteins at low levels [68]. Investigations in cultured PECs demonstrate that post-translational mechanisms are involved in the regulation of podocyte proteins in PECs. Levels of podocyte-specific transcripts are modulated in cultured human PECs by microRNA-193a [69], which downregulates WT-1 [70], a major transcription factor responsible for the regulation of podocyte-specific transcripts [71]. Transgenic upregulation of microRNA-193a in mice was shown to result in podocyte dedifferentiation with loss of podocyte-specific marker expression, podocyte loss and subsequently in the development of focal segmental glomerulosclerosis [70]. Additionally, levels of podocyte-specific proteins could be altered in cultured rat PECs by interfering with the proteasomal and lysosomal degradation pathways [68]. Therefore, adult PECs and podocytes still share a common tran-

scriptional program and post-translational mechanisms are involved in maintaining PECs in a less differentiated state than podocytes. It is therefore conceivable that marker proteins attributed as being PEC-specific are expressed by podocytes in the course of injury-induced dedifferentiation as is the case for mesenchymal markers under the influence of TGF β [72] or in a model of FSGS in mice with genetically tagged podocytes [73] and that this changed protein expression pattern is not a sign of podocyte regeneration from PECs.

To overcome the limitations of marker expression studies in the evaluation of podocyte regeneration from renal progenitor cells, several groups have made use of lineage-tracing approaches in mouse models, summarized in table 1. In most studies published to date, PECs were genetically traced in adult mice using the putative PEC promoter first published in 2009 [30], which consists of the truncated human PDX and 0.3 kb of rabbit PDX 5' UTR. To permanently tag PECs, the rtTA-transactivator system was used in which gene-transcription was activated by administration of doxycycline. Interestingly, several groups failed to detect podocyte regeneration from permanently labeled PECs using this lineage-tracing approach in aging mice and in the pathophysiologic situation of nephron loss secondary to unilateral nephrectomy and the aggravated form of 5/6 nephrectomy and DOCA/salt [13, 74]. These and other studies demonstrated an involvement of PECs in the generation of crescents and sclerotic scars [75, 76] rather than in regeneration. On the contrary, in more accelerated models of podocyte loss, podocyte replacement from permanently labeled PECs was observed following the administration of a sheep polyclonal antibody directed against rabbit glomeruli [77], whereas mild podocyte injury using immunotoxin-induced glomerular injury failed to observe podocyte regeneration [78]. In a recent study, podocyte regeneration was demonstrated following adriamycin nephropathy using *NPHS2.iCreERT2;mT/mG* mice to permanently label podocytes. Further, the authors permanently tagged PECs using *Pax2.rtTA;TetO.Cre;R26.Confetti* mice, and demonstrated that podocytes were regenerated from PECs in mice with disease remission, whereas the sclerotic lesions from mice with persistent proteinuria were populated by PECs [52].

Therefore, the setting and rate of podocyte loss in mice seem to be an important determinant, whether podocyte replacement is observed in mice with genetically tagged podocytes or PECs. Recent work suggested that in the setting of proteinuria, the sequestration of retinoic acid by albumin impaired podocyte regeneration from human

Table 1. Summary of lineage tracing approaches used to investigate for glomerular regeneration in different physiological and pathophysiological settings

Lineage tracing approach	Model	Labeling	Reference	Findings
Postnatal development	<i>hPODXL.rTTA;LC1;R26R</i>	PECs, STC	Appel et al. [30], 2009	PEC lineage derived cells give rise to podocytes in postnatal growth
	<i>hPODXL.rTTA;TetO.Cre;mT/mG</i>	PECs, STC	Wanner et al. [13], 2014	PEC lineage derived cells give rise to podocytes in postnatal growth (1%)
	<i>hPODXL.rTTA;LC1;R26R;H2B-eGFP</i> <i>NPHS2.rTTA;LC1;R26R;H2B-eGFP</i>	PECs, STC Podocytes	Berger et al. [48, 74], 2014	PEC lineage derived cells at vascular stalk give rise to podocytes in postnatal growth
	<i>Pax2.rTTA;TetO.Cre;R26.Confetti</i>	PECs, STC	Lasagni et al. [52], 2015	Recruitment of 8% podocytes from PAX2+ cells in postnatal growth
	<i>hPODXL.rTTA;LC1;R26R</i>	PECs, STC	Berger et al. [48, 74], 2014	No podocyte regeneration from PECs
Ageing	<i>hNPHS2.rTTA;TetO.Cre;mT/mG</i>	Podocytes	Wanner et al. [13], 2014	No podocyte regeneration
	<i>hPODXL.rTTA;TetO.Cre;mT/mG</i>	PECs, STC	Wanner et al. [13], 2014	No podocyte regeneration from PECs
	<i>hPODXL.rTTA;LC1;R26R</i> <i>hPODXL.rTTA;LC1;R26R;H2B-eGFP</i>	PECs, STC	Berger et al. [48, 74], 2014	No podocyte regeneration from PECs, PECs populate sclerotic lesions
Glomerular hypertrophy/mild podocyte injury	<i>hNPHS2.rTTA;TetO.Cre;mT/mG</i>	Podocytes	Wanner et al. [13], 2014	No podocyte regeneration (2.6%)
	<i>hPODXL.rTTA;TetO.Cre;mT/mG</i>	PECs, STC	Wanner et al. [13], 2014	No podocyte recruitment from PECs
	Transfer of mG labeled bone marrow to irradiated mT/mG wildtype mice	Bone marrow	Wanner et al. [13], 2014	No podocyte recruitment from bone marrow
	<i>NPHS2Cre;R26R^{tm4(CreB-tdTomato-EGFP)Lox}</i> <i>NPHS2.iCreERT2;R26R^{tm4(CreB-tdTomato-EGFP)Lox}</i> <i>NPHS2Cre;R26.Confetti</i>	Podocytes	Hackl et al. [80], 2013	Podocytes replace PECs
	<i>PEPCKCre;R26R^{tm4(CreB-tdTomato-EGFP)Lox}</i>	PECs, PTC	Hackl et al. [80], 2013	Propagation of visceral and parietal epithelial cells at the vascular pole
Acute podocyte depletion	<i>Ren1CreER;Rs-IdTomato-R</i> <i>Ren1Cre;Rs-ZsGreen-R</i> <i>Ren1dCre;Z/EG</i> <i>RenGFP</i>	Cells of JGA	Pippin et al. [31], 2013	Cells of juxtaglomerular compartment migrate to Bowman's capsule and glomerular tuft to replace PECs and podocytes
	<i>hNPHS2.rTTA;TetO.Cre;mT/mG</i>	Podocytes	Wanner et al. [13], 2014	7.33% podocyte regeneration
	<i>hPODXL.rTTA;LC1;R26R</i>	PECs, STC	Eng et al. [77], 2015	Podocyte recruitment from PECs
	<i>Pax2.rTTA;TetO.Cre;mT/mG</i>	PECs, STC	Lasagni et al. [52], 2015	Pax2+ cells populate glomerular hyperplastic lesions in mice with no remission of proteinuria
	<i>Pax2.rTTA;TetO.Cre;mT/mG</i>	PECs, STC	Lasagni et al. [52], 2015	24.7% podocyte regeneration from Pax2+ cells in mice with remission of proteinuria
NPHS2.iCreERT2;mT/mG	<i>Pulse-chase W5-d28 after adriamycin nephropathy</i>	Podocytes	Lasagni et al. [52], 2015	30.4% (5.9%) podocyte regeneration in mice with remission of proteinuria
	<i>Pulse-chase W5-d28 after adriamycin nephropathy</i>	Podocytes	Lasagni et al. [52], 2015	30.4% (5.9%) podocyte regeneration in mice with remission of proteinuria

P = Postnatal day; W = week; M = month; d = day.

renal progenitor cells by impairing retinoic acid response element-mediated transcription of podocyte-specific genes [79]. Further studies questioning podocyte replacement by PECs suggested that podocytes moved from the tuft to Bowman's capsule in a murine model of unilateral ureteral ligation [80] and that PECs and podocytes might be replaced by cells from a new potential podocyte progenitor niche: cells of renin lineage of the juxtaglomerular apparatus (JGA) [31]. In this study, lineage-tracing of cells of the JGA with 4 different murine reporter systems suggested a replacement of PECs and podocytes by renin lineage cells in a model of experimental FSGS. In this model of acute podocyte loss, intraglomerular cells of renin lineage were shown to express podocyte proteins, and Bowman's capsule cells were shown to express PEC proteins in addition to the reporter fluorochrome, but not renin [31]. Interestingly, the quantity of intraglomerular cells of renin lineage varied between the mouse systems used and was lowest in the inducible *Ren1cCreER;Rs-td-Tomato-R* mice in comparison to the 3 other constitutive reporter systems used [31]. It remains unresolved how cells of renin lineage breach the parietal and/or glomerular basement membrane, and whether they functionally replace podocytes, or whether they play a role in podocyte and PEC replacement in homeostasis. Also, the question remains through what cellular program and in which sequence the mesenchymal-derived cells of renin lineage transdifferentiate to PECs and podocytes.

Taken together, different groups and experimental approaches have demonstrated that the Bowman's capsule contains PECs with intermediate and/or 'less differentiated' phenotypes at the vascular and tubular pole. The transcriptional program of PECs is closely related to podocytes resulting in the capacity to express podocyte proteins by PECs and vice versa PEC proteins by podocytes under pathologic conditions. This phenotypic flexibility of PECs and podocytes could explain the observed confounding results from different groups. Data are strong regarding the involvement of PECs in postnatal podocyte generation in the time frame of glomerular development and in the involvement of PECs in glomerular scarring during disease. Data are, however, conflicting regarding the existence and quantity of podocyte regeneration from PECs or cells of renin lineage in the setting of glomerular injury with acute podocyte loss. Therefore, future efforts should lie on analyzing the proteome of PECs and of cells of renin lineage to identify specific markers, which will allow new lineage tracing approaches. Together with techniques that enable an objective quantification of podocyte replacement, these new ap-

proaches would reinforce existing findings and shed new light on the questions regarding if and to what extent podocyte replacement occurs. Furthermore, it remains to be solved, why podocyte replacement is mainly observed in models characterized by acute podocyte loss. These models are characterized by considerable cellular stress within glomeruli, giving rise to the possibility of confounding variables such as aberrant promoter activities leading to false-positive results. Furthermore, it needs to be established, whether regenerated podocytes in these models are fully differentiated and functional, and how movement of podocyte precursors from their respective niches to the glomerular tuft occurs.

Conclusion

Much knowledge has been gained about the embryonic origin of renal cells, and about the cellular and molecular mechanisms regulating tubular and glomerular regeneration. These data tell us to bear in mind that in the kidney, as in other mesenchymal tissues, regeneration by a stem cell compartment might be less important than regeneration by resident renal cells (summarized in figure 1). Accumulating data support the notion that resident renal cells maintain a considerable phenotypic flexibility, which is modulated by intrinsic post-translational mechanisms and by environmental factors under physiologic and pathophysiologic conditions. This phenotypic flexibility could be sufficient to provide the majority of observed regeneration during ageing and tissue repair. Understanding the factors that modulate the phenotypic flexibility of tubular and glomerular cells could in the future enable us to enhance the potential of renal regeneration in the setting of ageing and injury.

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