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Progenitor cells in the kidney: Biology and therapeutic perspectives

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Progenitor cells in the kidney: Biology and therapeutic perspectives. The stem cell may be viewed as an engineer who can read the blue print and become the building. The role of this fascinating cell in physiology and pathophysiology has recently attracted a great deal of interest. The archetype of stem cells is the zygote: one cell capable of endless proliferation and differentiation into all tissue types in the human body. Historically, the differentiation of embryonic stem cells is seen as an irreversible process with restricting possibilities for differentiation leading finally to a terminally differentiated cell type. Stem cells have also been described in the adult. They were first defined in tissues with a high cell turnover like skin and gut. Today, stem cells have also been shown in tissues with no or low regenerative potential and turnover, like the kidney. Traditionally, adult stem cells were thought to be restricted in their differentiative and regenerative potential to the tissues in which they reside. However, the stem cell concept is changing rapidly as evidence is mounting that adult stem cells not only reside locally in specific niches, but may also be recruited from the circulation to actively participate in the regeneration of various tissues. Furthermore, reverse differentiation has been demonstrated. This means that highly specialized cell types are able to dedifferentiate and engage in stem cell like activities. Moreover, transdifferentiation of mature cells into different cell types has been reported. This paper will review our current knowledge on renal stem cells and progenitor cells. Specifically, it will discuss the role of progenitor cells and transdifferentiation in renal repair and maintenance. Finally, the potential clinical implications of these findings will be discussed.

Most renal diseases can be envisioned as the consequence of a dysbalance between tissue damage and repair. Hypoxia, infection, immune reactions, and toxic substances can damage renal tissue. On the other hand, regenerative mechanisms counteracting the damage inflicted on renal tissues have been reported as well, both in tubuli (reviewed in [1]) and the glomeruli [2]. Insights into the nature of these regenerative mechanisms have evolved over the years. In tissues with a high cell turnover like the gut or the hematopoietic system, organ- or tissue-restricted stem cells have been shown to replace cells that have completed their life cycle. It is becoming increasingly apparent that in organs with a relatively low rate of cellular turnover like the liver and kidney similar regenerative mechanisms are operational [3–5]. The cellular players responsible for regenerative mechanisms in the kidney include not only proliferating mature renal cells but recent reports also suggest a renal role for stem cells, both from local pools as well as from the circulation.

Key words: endothelial progenitor cells, multipotent adult progenitor cells, vascular endothelial growth factor.

STEM CELLS OR PROGENITOR CELLS

Stem cells and progenitor cells are terms often used and confused. By definition stem cells are clonogenic cells capable of both self-renewal and multilineage differentiation [6]. According to the classical view, these cells become increasingly restricted in their differentiation potential during proliferation and development. Progenitor cells are defined as immature and proliferative cells which are limited in their differentiation potential to only one cell type. According to the classical view, the stem cell is the multipotent ancestor of the unipotent progenitor cell.

Few studies have addressed the potential of multipotent adult stem cells to differentiate into renal tissues. In one of these experiments, Jiang et al [7] isolated a multipotent adult progenitor cell (MAPC) from adult murine bone marrow and showed that these MAPC contributed to virtually all somatic tissues of the developing embryos, including several renal cell types, like endothelial and tubular cells. Although this proof of principle is fascinating, the physiologic and clinical relevance of these multipotent stem cells is still uncertain. In these studies single cell assays have been used to prove clonogenic expansion and pluripotency of stem cells. In these assays single cells are cultured and their progeny is differentiated into various cell types. This extensive in vitro processing might have influenced the cells in a nonphysiologic way, thereby extending their developmental repertoire.

To study renal regeneration and maintenance, pluripotency of the proliferating cells is not a primary issue that is therefore not often addressed. Consequently, these cells should be considered as progenitor cells. Most renal studies have focused on the role of progenitor cells in basal cell turnover and repair in (reversible) renal injury models. Resident progenitor cells are typically identified using techniques that trace foci of proliferating cells in conjunction with immunohistochemical characterization of

these cells. Participation of extrarenal progenitor cells is mostly studied in renal or bone marrow transplantation models, using methods to specifically stain for cells derived from the kidney recipient or bone marrow donor. Although transplantation experiments provide an excellent tool to investigate whether extrarenal progenitors contribute to renal regeneration, accurate quantification of the extent to which these extrarenal progenitors participate in this process may not be possible from this type of experiment. The total body radiation required for bone marrow transplantation experiments, combined with rejection involved in both transplantation models, may cause increased damage and subsequently a higher renal cell turnover. Furthermore, radiation might influence the proliferating capacity of resident renal progenitor cells, favoring the role of bone marrow–derived renal progenitor cells.

Notwithstanding the limitations of experimental approaches, evidence is accumulating suggesting that progenitor cells play a role in maintenance and repair of the adult kidney. In the following sections we will outline recent observations supporting the existence of progenitors for the major renal cell types.

INVOLVEMENT OF PROGENITOR CELLS IN THE KIDNEY

Endothelium

The kidney harbors several different types of endothelial cells, in particular the endothelium of the macrovasculature, the peritubular capillary endothelium, and the glomerular endothelium. Besides participation in the filtration, reabsorption, and nutrition of the renal tissue, endothelial cells play a key role in recovery from several renal diseases. The evident question therefore is whether endothelial progenitor cells (EPC) can also participate in regeneration and maintenance of these renal endothelial cell types. Evidence for the existence of EPC has mostly been derived from cardiovascular research on ischemia and angiogenesis [8]. Circulating progenitor cells for endothelial cells were first shown by Asahara et al [9]. In their experiments CD34+ leukocytes were isolated from human adult peripheral blood. These cells were subsequently cultured and differentiated into an endothelial cell–like phenotype. That these EPC are different from circulating mature endothelial cells was elegantly shown by Lin et al [10]. In bone marrow transplant patients, circulating endothelial cells from both donor (bone marrow–derived) and acceptor were cultured from peripheral blood. Interestingly, the bone marrow–derived cells were markedly more mitogenic than the acceptor cells and quickly outgrew circulating mature endothelial cells. EPC can stimulate vascular repair processes in different ways. Several authors have shown that EPC are involved in new vessel formation by incorporating in the vessel wall [9]. Furthermore, EPC stimulate vessel formation by secreting proangiogenetic factors like vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), which subsequently enhance proliferation and migration of resident cells [11].

A number of studies have addressed maintenance and regeneration of the specialized renal glomerular capillaries. Pabst and Sterzel [3] have reported that, in normal rats, the rate of total glomerular cell renewal is about 1% per day with the endothelial fraction being the most predominant cell type. However, in response to injury, the rate of vascular regeneration could well be increased. An established model to study glomerular injury and repair in rats is experimental anti-Thy1.1 glomerulonephritis. Injection of a complement-fixing antibody to the mesangial cell antigen Thy1.1 causes acute mesangiolysis and matrix dissolution, leading to ballooning of glomerular capillaries, formation of aneurysms, and loss of endothelial cells. In the subsequent repair phase increased proliferation and migration of endothelial and mesangial cells is observed resulting in (partial) restoration of glomerular structure and function. Using this model it was shown that glomerular capillary repair is associated with a marked increase in endothelial cell proliferation [12]. Several studies have provided evidence that circulating EPC may contribute to glomerular capillary repair. Recent experiments in our laboratory with rat hematopoietic chimeras demonstrated low levels of bone marrow–derived cells staining for the rat endothelial cell antigen RECA-1 [13]. The number of these cells gradually increased over time suggesting that EPC contribute to normal physiologic glomerular endothelial cell turnover. Following anti-Thy-1.1–induced glomerular injury we observed a fourfold increase in bone marrow–derived endothelial cells in the glomeruli. These data indicate that glomerular repair cannot only be attributed to migration and proliferation of resident endothelial cells but also involves bone marrow–derived cells.

Participation of circulating EPC to renal regeneration has also been demonstrated in human adults. Williams and Alvarez [14] were the first to report the presence of acceptor endothelial cells in kidney allografts. Lagaaij et al [15] reported that in human renal transplants the extent of replacement of donor endothelial cells lining the peritubular capillaries by those of the acceptor was related to the severity of vascular injury. They suggested that this endothelial replacement could be explained by the involvement of acceptor-derived EPC. Recently, our group demonstrated male, donor-derived endothelial cells in the renal macrovasculature of a female patient who developed thrombotic microangiopathy after gender-mismatched bone marrow transplantation [16]. Taken together these observations confirm a novel role for bone marrow–derived endothelial cells in maintenance and repair of renal endothelium.

Mesangium

Glomerular mesangial cells provide structural capillary support to the glomerulus and display a pericyte- or smooth muscle cell–like phenotype. They play a central role in the pathogenesis of a number of human and experimental glomerular inflammatory diseases. In particular, mesangial hyperplasia is a prominent histopathologic feature associated with impaired glomerular function. Although transient hyperplasia is thought to reflect a physiologic response required for successful glomerular reconstitution and renal tissue repair, tight regulation of mesangial proliferation, function, and apoptosis is needed for recovery without fibrosis.

Initially, mesangial maintenance and repair after injury was thought to depend solely on proliferation of viable resident intraglomerular mesangial cells. These mature mesangial cells dedifferentiate before they proliferate, as is described by El Nahas et al [17]. Like the glomerular endothelial cells, in normal rats, mesangial cell turnover amounts to less then 1% per day [3]. Hugo et al [18] demonstrated that during recovery of anti-Thy1.1 glomerulonephritis, proliferating immature mesangial cells migrated from the juxtaglomerular apparatus and hilar region into the glomerulus.

Reminiscent to mesangial cell recruitment during embryonic glomerulogenesis, the involvement of extraglomerular mesangial progenitor cells in glomerular repair was reported by several investigators [19]. Imasawa et al [5] demonstrated the involvement of bone marrow–derived cells in normal mesangial cell turnover. Lethally irradiated mice given transplants of T-cell–depleted bone marrow cells from syngeneic donor transgenic for green fluorescent protein (GFP) manifested a time-dependent increase in GFP-positive cells in their glomeruli. When isolated and cultured, these cells stained positive for the mesangial cell marker desmin and the cells contracted in response to angiotensin II, confirming that bone marrow–derived cells have the potential to differentiate into glomerular mesangial cells. Similar experiments with mice transplanted with purified clonally expanded hematopoietic progenitor cells were carried out by Masuya et al [20] to confirm the hematopoietic origin of bone marrow–derived mesangial cells.

In similar experiments, using a rat allogenic bone marrow transplant model and antibodies to the mesangial cell–specific antigen Thy-1 (ox7), we confirmed this time-dependent increase of bone marrow–derived mesangial cells in the glomerulus [13]. Both Ito et al [21] and our laboratory observed a major increase of bone marrow–derived mesangial cells during recovery from anti-Thy-1.1–induced mesangiolysis in bone marrow transplantation models in rats.

Cornacchia et al [22] demonstrated that glomerulosclerosis can be transmitted by bone marrow transplantation in mice. Transplantation of bone marrow cells from sclerosis-prone mice in normal background mice invoked glomerulosclerosis in the recipients. These data not only point to the contribution of bone marrow–derived cells to glomerular maintenance and repair but also show that dysfunctional or diseased mesangial progenitor cells can have a negative influence on the kidney.

Tubules

The renal tubule is known for its high capacity for regeneration. Acute tubular necrosis, as a result of ischemia or toxic substances, can be followed by active migration and proliferation to restore normal tissue architecture and function [1]. Different sources of these proliferating progenitor cells have been reported. Humes et al [23] isolated resident proliferative epithelial cells from the tubuli of mature rabbit kindeys and demonstrated that these cells displayed a high capacity for self renewal and differentiated into complete three-dimensional tubular structures in vitro. Similar experiments were later performed with human epithelial cells [24].

Bone marrow–derived extrarenal tubular progenitor cells were reported by Poulsom et al [4]. In female mouse recipients of male bone marrow grafts colocalization was observed of Y chromosomes and tubular epithelial cell markers, suggesting participation of bone marrow–derived cells in normal tubular cell turnover. The potential importance of the role of bone marrow–derived cells in tubular repair was demonstrated by Kale et al [25]. When LacZ gene-positive bone marrow cells from Rosa26 mice were transplanted into wild-type mice, renal ischemia was associated with the occurence of LacZ-positive (bone marrow–derived) tubular cells. It was estimated that the majority of the tubular cells after tubular repair were bone marrow–derived. Moreover, bone marrow ablation diminished functional recovery after tubular ischemia, while infusion of a progenitor cell reversed this effect, suggesting an important functional role for the hematopoietic stem cell in tubular repair.

Also in humans there are some indications for bone marrow–derived tubular repair [26]. When male kidney transplant patients who received a female kidney and who recovered from acute tubular necrosis were studied, a Y chromosome could be demonstrated in few (less than 1%) of the tubular cells. Although the functional importance of this phenomenon in the human situation is still dubious, these experiments do provide us with a proof-of-principle observation on bone marrow–derived tubular repair.

Fibroblasts

Renal fibrosis is a common denominator of most chronic renal diseases that progress to end-stage renal

failure and is associated with major alterations in the tubular interstitial compartment. Increased numbers of fibroblasts producing excessive amounts of extracellular matrix molecules characterize interstitial pathology. Whether interstitial fibrosis is a cause or a consequence of renal pathology remains unclear. Recently, it was hypothesized that expansion of the cellular content of the interstitium could be an attempt to restore an embryonic environment supporting the repair of injured tubules [27]. Understanding the origin of interstitial fibroblasts may well contribute to our understanding of the role of these cells in renal pathology.

One hypothesis for the origin of interstitial fibroblasts proposes an epithelial-mesenchymal transition in the local formation of fibroblast from organ epithelium [28]. These transitions are particularly apparent during fibrogenesis: fibrotic tissue repair following injury. Strutz et al [29] first demonstrated de novo expression of a murine fibroblast specific marker (FSP-1) in selected tubular epithelial cells during late stages of renal fibrogenesis. Ng et al [30] showed that progressive renal failure is associated with the transdifferentiation of tubular cells into myofibroblasts. A second hypothesis for the origin of adult fibroblasts argues that marrow stromal cells are fibroblast progenitors that shuttle from the bone marrow through the circulation to populate peripheral organs. The presence of circulating fibroblast progenitor cells in animals was shown in 1867 by Cohnheim [31]. When circulating blood cells were labeled, they could be traced to sites of active wound healing and displaying a fibroblastic morphology. Bucala et al [32] described a distinct population of human leukocytes that was able to differentiate into fibroblasts. In an animal wound healing model, the ability of fibroblast progenitor cells to home at sites of tissue injury and participate in scar formation was demonstrated. In a human study, Grimm et al [33] assessed the relative participation of extrarenal derived α-actin–positive cells in the kidney. In renal transplanted patients kidney biopsies were taken approximately 2 months after transplantation. The amount of acceptor-derived α-actin–positive cells in the kidney varied between 77% and 30%, indicating the existence of a circulating mesenchymal progenitor cell.

Finally, Iwano et al [34] assessed the relative contribution of fibroblasts from both origins to the interstitial fibroblast content in normal and fibrotic murine kidneys. In the normal kidney, bone marrow–derived cells were responsible for 12% of total renal fibroblasts, whereas the remaining 88% were resident fibroblasts. In renal fibrosis, as a consequence of unilateral ureteral obstruction, these numbers were 15% and 49%, respectively. The remaining 36% was derived from epithelial-mesenchymal transdifferentiation. Furthermore, they showed that these transdifferentiated cells were actively involved in the fibrosis, producing abnormal amounts and types of collagen.

CLINICAL IMPLICATIONS

Most therapies in renal medicine focus on reducing renal damage. However, insight in renal repair and maintenance may offer new therapeutic strategies. As demonstrated above, progenitor cells participate in renal repair and turnover of the major renal cell types. Therefore, renal progenitor cells may comprise a new target for therapeutic strategies aimed at the reduction or even prevention of renal disease.

Such strategies could be directed toward different populations of progenitor cells. The advantage of circulating progenitor cells may be that they are more accessible for isolation in comparison to the resident progenitor cells. Autologous progenitor cells from the patient are preferable to allogenic progenitor cells because of possible rejection. Obviously, in case of inherited progenitor cell disease, allogenic cells should be considered.

One approach to harness progenitor cells for therapeutic purposes is to increase the available pool of progenitor cells. Such expansion can be achieved by growth factor therapy both in vivo and ex vivo. VEGF and erythropoietin are probably good candidates to stimulate progenitor cell–mediated endothelial repair. Both have EPC mobilizing and proangiogenetic activities [35, 36]. It was shown that VEGF enhanced renal vascular repair in rats with thrombotic microangiopathy and experimentally induced glomerulonephritis [37]. Although a proliferative effect on resident cells was shown, mobilization and stimulation of circulating cells may also have been involved. Next to in vivo growth factor therapy, the progenitor cell pool can also be amplified by isolation of the progenitor cells from the circulation or the kidney and expansion in tissue cultures. Ex vivo expansion of progenitor cells has already been performed by Weitzel, Fissell, and Humes [24]. They isolated tubular progenitor cells from cadaveric kidneys and were able to expand these cells in vitro.

Another approach to enhance cellular repair and maintenance is reinforcement of progenitor cell function. EPC dysfunction has been shown in diabetic mice [38]. The decreased angiogenetic capacity of diabetic mice was restored after infusion of EPC from nondiabetic mice. Mesangial and mesangial progenitor cell dysfunction has been described too (see above) [22, 39]. Replacement of these prosclerotic cells by healthy allogenic mesangial progenitor cells may potentially reduce or even prevent progressive renal disease. The relatively low turnover rate of mesangial cells of 1% per day might however hamper this strategy [3]. Controlled mesangial injury by pharmacologic agents combined with healthy allogenic or transfected autologous mesangial precursor cell infusion might increase mesangial turnover and improve cell replacement.

Finally, progenitor cells can be used as so-called "magic bullets." Progenitor cells are able to home and participate

in their target tissue. This ability can be used to deliver certain gene products very locally. Gene therapy has already successfully been used. Transfection of skeletal muscles with the gene of a transforming growth factor-β1 (TGF-β1) inhibitor was able to reduce glomerulosclerosis in a rat nephritis model [40]. Transfection of renal progenitor cells might provide a more local therapy, preventing possible systemic side effects.

CONCLUSION

For most renal cell types, progenitor cells have now been described. Besides a role in the normal cellular turnover, progenitor cells are also involved in repair processes. In contrast, dysfunctional progenitor cells can hamper repair or even actively inflict damage. These characteristics make these cells interesting as therapeutic targets either by increasing their number or by improving their function.

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