Peripheral blood as a source of stem cells for regenerative medicine

"Stem cell therapy for kidney disease"

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Introduction: Kidney diseases are a global public health problem whose incidence is rapidly growing due to a global rise in the aged population and the increasing prevalence of cardiovascular disease, hypertension and diabetes. With the emergence of stem cells as potential therapeutic agents, attempts in using them to significantly reduce the burden of these diseases have increased.
Areas covered: Several types of stem cells have been proven to be likely candidates for treating kidney diseases. We discuss in detail the potential use of mesenchymal stem cells in preclinical and clinical works, with additional populations that have been studied briefly described. Moreover, we discuss current knowledge on endogenous kidney regeneration ability and on the possibility to modulate it using chemical and biological agents.
Expert opinion: Stem cell therapy is a promising new treatment for kidney disease documented in many animal studies. Mesenchymal stem cells have emerged as a promising cell type, but their efficacy in clinical trials is still controversial. Identification of progenitor cells in the adult kidney is another step forward in regenerative medicine, suggesting the repair potential of the adult kidney and the possible modulation of renal progenitors *in situ* using pharmacological approaches.

Keywords: mesenchymal stem cells, regenerative medicine, renal progenitor cells, stem cells

1. Introduction

Kidney diseases are a heterogeneous group of disorders affecting kidney structure and function with variable clinical presentation and course. Kidney diseases can be defined as acute or chronic, depending on duration (£ 3 vs > 3 months). In 2002, a uniform definition and staging system for chronic kidney disease (CKD) was launched in the medical community [1]. The introduction of these guidelines for definition and classification of this disease showed that CKD is far more fre- quent than previously appreciated, affecting > 10% of the world's population [2,3]. The prevalence of CKD increases with age, exceeding 20% in individuals older than 60 years and further increasing to 35% in those older than 70 years [4]. Although multidrug treatment titrated to urinary protein excretion could be successfully applied for preventing progression of chronic nephropathies [5], kidney failure, which symptoms are usually caused by complications of reduced kidney function, it remains the most serious outcome of CKD. For patients in end-stage kidney failure, dialysis or kidney transplant remains the last resort, these approaches being however hampered by severe shortage of organ donors, potential organ rejection and high medical costs.

Consequently, new methods to alleviate, cure or prevent renal disease are urgently required to reduce the exponentially growing burden due to acute kidney disease and CKD and to improve patients' survival and quality of life. Development of cell-based therapies for human therapeutic application is an important emerging challenge. Several potential regenerative cell-based therapies for the treatment of renal failure are currently under develop- ment. The first one is the direct application of stem cells (SCs) to the diseased kidney. The therapeutic success of this treat- ment depends on the natural capabilities of SCs to differenti- ate, organize and integrate into the existing tissues to restore function and/or on the capacity of SCs to release renoprotec- tive factors. A wide range of adult SCs and progenitor cell types, including bone marrow mesenchymal SCs (MSCs), endothelial progenitor cells (EPCs) and renal progenitor cells (RPCs) have been tested in experimental kidney damage models. In addition, embryonic SCs (ESCs) and induced pluripotent SCs (iPSCs) have also been recently studied. Although findings from a few clinical trials suggested that administration of MSCs to patients suffering from CKD is feasible and well tolerated, the effective efficacy of these cells in clinical settings is still uncertain and several critical points, namely the choice of the cell type, method of administration and timing of delivery, still need to be addressed. Another SC-based strategy is based on the prospective design of a therapeutic approach focused on modulation of endogenous kidney regenerative properties by conventional chemical and biological agents capable of modulating the activity of resi- dent progenitor cells. Indeed, the classical definition of the human kidney as a non-proliferative and non-regenerative organ has been recently called into question by emerging evidence that the kidney has the potential to regenerate itself starting from populations of resident progenitors. In this review, we discuss the field of SC-based therapy for kidney disease and focus particularly on the administration of MSCs and on the modulation of the endogenous regenerative potential.

2. Which cell type is the best candidate for cell-based therapy of kidney disease?

Multiple different SC types have been investigated for their potential to repair damaged kidney, with the not surprising result that the optimal cell type contributing to renal repair still remains disputed. In principle, ESCs appear as the best candidate because their pluripotent nature confers them the potential to regenerate any cell type in the kidney. However, the pluripotent potential of ESCs also constitutes the major obstacle to their safe clinical use due to the possible increased risk of neoplasms arising from transplanted cells. To control and reduce this risk, several groups have developed protocols to induce differentiation of ESCs into renal lineage using retinoic acid (RA), activin A and bone morphogenic proteins [6-9]. The differentiation process is usually relatively inefficient with a low yield of differentiated cells; however, recently Mae *et al.* established a more efficient differentiation protocol of human ESCs into intermediate mesoderm [10]. Experimental works demonstrated that it is possible to obtain renal cells from ESCs, and that these cells can be differenti- ated into glomerular-like structures and integrated into renal proximal tubules in animal models [8-11]. However, the utility of this cell type is hampered by the risk of teratoma formation and immunological rejection, in addition to ethical considerations.

A further significant advancement in cell-based therapy of renal disease is the generation of iPSCs [12]. Undifferentiated murine iPSCs attenuate kidney injury in ischemia reperfusion (I/R)-induced acute kidney injury (AKI) only when adminis- tered via intra-arterial route, and this beneficial effect is mediated by paracrine action [13]. However, as discussed for ESCs, the pluripotent nature of iPSCs raises concerns on post-transplantation safety when these cells are administered without pre-differentiation. Several studies described protocols to differentiate iPSCs into nephrogenic cells, renal proximal tubular cells and podocytes [14-20]. Surprisingly, no beneficial effect on kidney function nor tissue engraftment has ever been reported using human pre-differentiated iPSCs in animal models of AKI. On the contrary, Imberti *et al.* recently reported that iPSCs differentiated toward RPCs using an efficient two-step differentiation protocol [21], robustly engrafted into damaged tubules and restored renal function and structure in a mouse model of cisplatin-induced AKI. In the same experiment, injection of human undifferentiated iPSCs, used as control, failed to exert any protective effect on renal function and histology. Interestingly, no signs of inappropriate differentiation and tumor formation by iPSC- derived RPCs were observed in the renal tissues either at 4 days or 8 weeks after injection [21]. ESCs and iPSCs differentiated toward renal phenotypes have been recently demonstrated to generate three-dimensional kidney structures *in vitro* [14,22-24]. However, the possibility to obtain functional kidney structures has not been directly tested.

Amniotic fluid-derived SCs (AFSCs) have intermediate characteristics between ESCs and adult SCs [25] and have the potential to differentiate *in vitro* toward renal lineages acquir- ing the expression of epithelial and podocyte markers when cultured in appropriate differentiating medium [26,27]. Human AFSCs (hAFSCs) injected directly into mouse kidneys with glycerol-induced AKI are still present in the kidney after 21 days and are specifically located among the tubules where some cells are able to commit toward renal differentiation [28]. However, a beneficial effect on serum creatinine and blood urea nitrogen (BUN) levels could be observed only when hAFSCs were injected into the kidney on the same day of glycerol injection. This effect correlated with a significant increase in proliferative activity and with a decreased apopto- sis of tubular epithelial cells [28]. Moreover, hAFSCs exerted immunomodulatory effects that appeared to control the local immune response in favor of a tissue cytokine and cellular milieu that promotes prevention or resolution of tissue dam- age [28]. Similar results have been reported by Hauser *et al.*: injection of hAFSCs in an acute tubular necrosis mouse model induced reduction of creatinine and BUN levels, associated with amelioration of tissue damage, reduction of apoptosis and promotion of tubular cell proliferation [29]. Comparison of the regenerative potential of hAFSCs with that of MSCs in this model of AKI demonstrated that MSCs were more efficient in inducing proliferation than hAFSCs, which, in contrast, were more antiapoptotic [29]. These beneficial effects are probably mediated through para- crine mechanisms. Indeed, Rota *et al.* demonstrated that in the cisplatin-injured kidney, administration of hAFSCs improved renal function and limited tubular damage, but they did not differentiate into renal tissue, engrafting predom- inantly in the peritubular region without acquiring tubular epithelial markers [30]. Similarly, AFSCs were capable of prolonging the animals' survival, ameliorating the decline in kidney function and delaying the progression of glomerular sclerosis and interstitial fibrosis also in mouse models of CKD (Col4a5$^{-/-}$ mice, unilateral ureteral obstruction [UUO]) [31,32].

The use of a variety of adult stem or progenitor cells for renal disease, in particular EPCs, RPCs and MSCs, has been widely reported. Improvement of renal function after exoge- nous adoptive transfer of EPCs has been observed in Adriamycin-induced nephropathy, and in other models of AKI such as the renal I/R injury model and the sepsis-induced AKI [33-36]. Administration of EPCs by intravenous injection is limited by the high number of cells that become trapped in the pulmonary vasculature or suffer from anoikis. One of the most studied ways to protect administered EPCs and to maximize their therapeutic potential is to deliver them embedded in bioengineered scaffolds, such as hyaluronic acid (HA) hydrogels [37], implanted either superficially into ears or subcapsularly into kidneys. In comparison with intravenous administration, delivery of cells in HA hydrogels provides a microenvironment conducive for viability and expansion of embedded cells and protection from circulating cyto- and endo-toxins and allows mobilization of embedded cells on demand through administration of digestive enzymes directly into the implanted HA hydrogels or taking advantage of the intrinsic release of hyaluronidase when kidneys are injured [37]. A beneficial effect of EPC injection has also been reported in a 5/6 nephrectomy model of CKD [38] and in the UUO model [39]. Indeed, EPCs were successfully

incor- porated into the capillary network of the damaged kidney, increasing the number of capillary density and alleviating the development of renal fibrosis [39].

Potential candidate SCs have been detected in the adult mammalian kidney using different identification methods. Using *in vivo* bromodeoxyuridine labeling, tubular cell populations (label-retaining tubular cell), which showed tubu- logenic capacity, integrated into the developing kidney and contributed actively to the regeneration of the kidney after ischemic injury were identified in normal adult kidneys [40-42]. Alternatively, using the culture of rat kidney or the dissection of single nephron from adult rat kidney, researchers demon- strated the existence of highly proliferative cells, especially in S3 segment of nephron, which generated cell lines that expressed both immature and mature tubular cell markers. These cells engrafted to kidney in rat I/R model, replaced cells in injured tubules and improved renal function [43,44]. In addi- tion, mouse kidney progenitor cells, isolated from medulla and papilla interstitium, showed characteristics consistent with renal SCs, as demonstrated by their ability to rescue renal damage, incorporating in renal tubules and forming new ves- sels after their intrarenal injection in mice with ischemic injury [45]. Accordingly, Bussolati *et al.* described a multipo- tent resident SC population in the renal interstitium of human adult kidney, which was capable of clonal expansion *in vitro* and of homing into the injured kidney, and integra- tion in tubules after intravenous injection in severely com- bined immunodeficiency mice with glycerol-induced tubular damage [46].

In the most recent years a population of RPCs was identi- fied at the urinary pole of Bowman's capsule of adult human kidney. RPCs were identified by co-expression of progenitor markers, $CD133^{+}CD24^{+}CD106^{+}$, in absence of lineage markers. *In vitro*, these cells displayed typical characteristics of adult SCs, such as expression of SC markers, clonogenicity and the potential to differentiate toward renal lineages when cultured in appropriate differentiating medium [47]. Injection of human RPCs in an acute tubular necrosis mouse model induced reduction of BUN levels, associated with ameliora- tion of tissue damage and reduction of fibrosis. These benefi- cial effects were due to the engraftment of RPCs into injured tubular structures followed by their differentiation in tubular cells [47,48]. Moreover, injection of RPCs into mice with Adriamycin-induced nephropathy reduced proteinuria and improved chronic glomerular damage, through their engraftment into the glomerular tuft and differentiation into podocytes [49].

Mazzinghi *et al.* described the mechanism responsible for RPCs' homing to injured kidney [50], demonstrating that the homing and the engraftment of RPCs in an acute tubular necrosis mouse model are ruled by the CXCR4 -- CXCR7/ stromal cell-derived factor 1 or CXCL12 pathway. Indeed, treatment of RPCs before injection with a selective CXCR4 or CXCR7 antagonist induced a dramatic reduction in the number of engrafted RPCs. However, although CXCL12-in- duced migration of RPCs was only abolished by pretreating cells with an anti-CXCR4 antibody, transendothelial migra- tion was mediated by the activity of both CXCR4 and CXCR7 and, last, CXCR7 was essential for RPCs adhesion to endothelial cells and for the CXCL12-mediated RPCs survival [50].

3. Are MSCs the right cell type for renal regeneration?

The description of the use of MSCs for SC-based therapy of kidney injury merits a separate section. MSCs are undifferen- tiated adult cells defined by adherence to plastic in culture, multipotentiality, expression of typical surface markers (CD105, CD73 and CD90) and the absence of expression of hematopoietic lineage markers (CD34, CD45, CD14 or CD11, CD79*a* or CD19, and human leukocyte antigen class II), as suggested by the International Society for Cellular Therapy [51]. MSCs can be isolated primarily from bone marrow stroma but it can also be extracted from a variety of tissues, including kidney [52-54]. MSCs are easily cultured and expanded to obtain clinically useful number of cells. Functional properties of MSCs are their capacity to differen- tiate into cells of the mesenchymal lineage such as osteocytes, adipocytes and chondrocytes and potentially other cell types and their immunomodulatory properties [55,56]. All these properties make MSCs as one of the most promising cell type for cell-based therapy in several injured organs/tissues. A large number of studies reported evidence that MSCs can promote regenerative responses in the injured kidney, leading to tissue repair and improvement of renal function [57-59]. These beneficial effects have been initially attributed to the migration of MSCs to the injured kidney, a process mediated by stromal cell-derived factor 1/CXCR4

axis [60], and the sub- sequent transdifferentiation of MSCs into renal-specific cells [61,62]. The possibility of direct engraftment of MSCs into the kidney is also supported by the demonstration that MSCs directly injected into developing kidney followed by subsequent embryo and organ culture incorporated into glo- merulus, tubule and interstitium [63]. However, several other studies showed protection from injury by MSCs, but very lit- tle or no tubular incorporation was observed [64-68]. Even if these differences may be explained by different injury models and protocols, or by the method used for tracking injected MSCs, several data argue against the hypothesis that the beneficial effect of MSCs on renal repair is mediated by their engraftment and direct repopulation of the tubule. First, in most studies, the protective effect of injected MSCs is observed within 24 -- 48 h, a timing that seems too rapid to be explained by transdifferentiation of MSCs into epithelial cells [67]. Second, the numbers of MSC-derived epithelial cells appear to be so low (£ 0.1%) that they could not have func- tionally contributed to repairing the nephron, at least by direct engraftment [67]. Thus, there is a growing consensus that kidney-protective effects of MSCs can be attributed mainly to paracrine and endocrine mechanisms (Table 1). In support of this hypothesis are recent data obtained using intravenous injection of nestin+ renal-resident MSCs in acute kidney I/R injury; this treatment induced functional improve- ment by significantly decreasing the serum creatinine and BUN, and reducing the cell apoptosis, but conditioned medium from nestin+ cells could equally protect against ischemic renal failure at least partially through the paracrine factor VEGF [68].

The immunomodulatory properties of MSCs may play a role in renoprotection, because inflammation is of great importance in the pathophysiology of AKI. Allogeneic MSCs, indeed, express low levels of MHC class I and class II molecules, do not express major costimulatory molecules such as CD40, CD80 and CD86 and inhibit dendritic cell alloantigen-induced differentiation and activation [69]. More- over, MSCs produce prostaglandin E2 that modulates T-cell function, decreasing secretion of proinflammatory cytokines TNF-*a*, and IFN-*g* and increasing secretion of suppressive and tolerance-promoting cytokines IL-10 [69]. In accordance, high levels of anti-inflammatory cytokines have been found in kidney extracts from MSC-treated animals after I/R injury [70]. Prostaglandin E2 released by MSCs also acts on monocytes and/or macrophages through the prostaglandin EP2 and EP4 receptors increasing their IL-10 production [71]. Through this mechanism, intravenous injection of MSCs beneficially modulated the response of the host immune sys- tem to sepsis and improved survival in a mouse model of cecal ligation and puncture [71]. Exposure of MSCs to proinflam- matory stimuli promotes the immunosuppressive activity of MSCs, resulting in protection from natural killer-mediated cytolysis, inhibition of IL-2-induced natural killer prolifera- tion, induction of hepatocyte growth factor and TGF-*b* secre- tion and induction of indoleamine 2,3-dioxygenase (Table 1) [56,72-74].

MSCs can secrete a broad repertoire of growth factors and cytokines with considerable positive potential for the treatment of kidney disease: G-CSF, SC factor, leukemia- inhibitory factor, macrophage-colony stimulating factor, IL-6 and IL-11, VEGF, basic fibroblast growth factor, monocyte-chemoattractant protein-1, hepatocyte growth fac- tor and IGF-1 [75,76]. Some of these molecules act on epithelial cells promoting proliferation, others promote angiogenesis and exert vasculoprotective actions [77], yet others are anti- inflammatory and antiapoptotic, but all can protect the kidney from further injury and accelerate repair. It is of inter- est that MSCs also confer protection from I/R injury when injected intraperitoneally, that is, in absence of MSCs homing to the kidney [78]. In this case, the beneficial effect must be attributed to the low systemic concentrations of factors released from extrarenal MSCs.

MSCs have been tested successfully in experimental models of CKD. A single intravenous injection of MSCs prevented renal damage in streptozotocin-induced type 1 diabetes in C57BL/6 mice, and treated animals showed histologically normal glomeruli and a significant decrease in albumin- uria [79]. Similar results have been obtained in not obese diabetic/severe combined immunodeficiency mice: in this model, intracardiac infusion of human MSCs decreased mesangial thickening and macrophage infiltration with a few injected MSCs differentiating into glomerular endothelial cells [80]. MSCs injected in rats with modified 5/6 nephrec- tomy persisted within the kidney 1 day after nephrectomy [81], but no significant differences in BUN and creatinine concen- tration were observed between the MSCs group and the con- trol group. However, proteinuria in the MSCs group was lower than that in the control group. Interestingly, VEGF lev- els were substantially higher in MSC-treated animals 1

month after MSC injection, thus suggesting that the beneficial effects were mediated by secretion of paracrine factors [81]. This con- clusion is also supported by recent data in rats subjected to UUO: indeed, the intravenous administration of both MSCs and MSC-conditioned medium improved fibrosis progression [82]. Intrarenal infusion of MSCs isolated from subcutaneous adipose tissue protected the stenotic kidney despite sustained hypertension [83]. Remarkably, MSCs also attenuated renal inflammation, endoplasmic reticulum stress and apoptosis through cell-contact-mediated mechanisms. Furthermore, MSCs improved renal function and structure after renal revascularization and reduced inflammation, oxida- tive stress, apoptosis, microvascular remodeling and fibrosis in the stenotic kidney [84]. Recently, Papadimou *et al.* found that human MSCs could be reprogrammed into renal proximal tubular cells by exposure to HK-2-cell-free extracts. These reprogrammed cells acquired an antigenic profile and func- tional properties of proximal tubular-like epithelial cells *in vitro*, integrated into developing nephrons *ex vivo* and protected mice from AKI [85].

All these preclinical murine models offer the proof of concept that the use of MSCs in the management of AKI is rational and feasible. However, further studies are required to meet the quality and safety criteria before applying MSC administration in clinical studies. We need to validate different models and standardize different protocols of MSC application to facilitate the comparison among results. Indeed, several issues have to be solved, first the better route of MSCs administration. To this aim, studies should be focused on the direct comparison of the administration through the tail vein, carotid artery or renal artery in different animal models. In rat model of renal I/R injury, authors observed that 1 × 10(5) MSCs injected through renal artery produces the most dramatic improvement in renal function and morphology [86]. Second, MSCs from different sources (bone marrow, adipose tissue, kidney) have to be compared in terms of accessibility, yield and clinical efficacy [87]. Another point to fix is the use of autologous or allogeneic cells. T€ogel *et al.* [88] investigated the long-term outcome of treatment after injecting autologous or allogeneic bone marrow MSCs in a rat model of AKI. Identical doses of autologous MSCs were more effective than allogeneic, but both autologous and allogeneic cells were able to reduce late renal fibrosis and loss of renal function in surviving animals. However, age or systemic disease may influence and decrease the regenerative potential of autologous MSCs, as demon- strated by Klinkhammer *et al.* who investigated the negative effects of CKD on MSCs' function and concluded that CKD/uremia lead to a sustained loss of *in vitro* and *in vivo* functionality in MSCs, likely due to premature cellular senes- cence [89]. This finding raises doubts on the possibility of obtaining beneficial effects from autologous transplantation in CKD patients.

Recently, a systematic review and meta-analysis to evaluate the efficacy of cell-based therapy in preclinical studies of CKD, and determined factors affecting cell-based therapy efficacy in order to guide future clinical trials, has been published [90]. Analysis of 71 articles with difference in the selection and preparation of cells, administration route and choice of disease model and model species showed that cell- based therapy reduced the development and progression of experimental CKD. This was most evident when urinary pro- teins and urea levels were analyzed. Sub-analysis showed that cell type (bone marrow-derived progenitors and mesenchymal stromal cells being most effective) and administration route (intravenous or renal artery injection) were significant predic- tors of therapeutic efficacy. On the contrary, the timing of therapy in relation to clinical manifestation of disease, and cell origin and dose, did not influence the efficacy of the treat- ment [90]. The differences observed in the functional efficacy of cell-based therapy in CKD appeared to be model-depen- dent, thus reflecting the different pathogenesis of CKD when initiated by subtotal nephrectomy *versus*, for example, toxic injury, whereas for structural efficacy, most models showed improvement of all outcome variables, perhaps reflecting the common pathway to end-stage kidney failure. Such differences might also be relevant when designing cell therapy studies in specific patient populations.

4. MSCs in human clinical trials of cell-based therapy for kidney repair

The interesting results obtained in preclinical studies of MSC administration in kidney diseases prompted to the translation of MSC-based treatments in humans. The clinical studies are still limited. A Phase I clinical trial (NCT00733876) has been performed to determine if the administration of allogeneic MSCs at defined doses is safe in patients who are at high risk of developing AKI after undergoing on-pump cardiac sur- gery. Preliminary data on the first five treated patients showed that

kidney function is preserved up to 16 months and that none of the patients required dialysis [91]. On the contrary, 20% of case controls developed acute renal failure. The length of hospital stay and readmission rates in study patients were reduced by 40%. No therapy-related adverse events were noted in these patients [91,92]. The Phase I explorative study (NCT 01275612) on three patients with acute renal failure induced by cisplatin treatment for solid organ cancers has demonstrated that intravenous infusion of autologous *ex vivo*-expanded MSCs improves renal function and that the procedure is safe. The Phase II trial NCT 01602328 enrolled subjects who experienced kidney injury (defined by a creatinine rise ≠ 0.5 mg/dl) within 48 h of cardiac surgery to assess safety and efficacy of single administration of human MSCs. More than 150 subjects were enrolled and the trial was terminated because no beneficial effects were observed [93]. However, the use of a rise in creatinine provides late information on the possibility of evolving AKI; a more specific and early biomarker, such as kidney injury molecule-1, neutrophil gelatinase-associated lipocalin or IL-18 would be more appropriate to diagnose AKI and thus allow to start the treatment earlier with a higher probability of success.

A Phase I, single-group assignment, open-label study (NCT01840540) is recruiting participants to determine the safety and toxicity of intra-arterial-infused autologous adipose-derived mesenchymal stromal (stem) cells in the treat- ment of 15 patients with atherosclerotic renal artery stenosis affecting one or both kidneys and serum creatinine < 2.5 mg/100 ml.

The preliminary results presently available confirm that MSC treatment is safe, but other data are required to evaluate clinical benefit and the effects observed in clinical trials must be critically analyzed to understand when negative results could be due to an inadequately designed trial. Moreover, the long-term safety of MSC treatment must be verified in view of the preclinical data published by Kunter *et al.* [94]. The authors injected high number of MSCs intrarenally into rats in a model of glomerulonephritis and reported a beneficial therapeutic effect on day 60, but ~ 20% of the glomeruli of MSC-treated rats contained large adipocytes derived from maldifferentiation of MSCs with pronounced surrounding fibrosis.

5. Does the endogenous regenerative capacity of the kidney exist and how can it be modulated?

Exciting concepts have emerged over the past decade on the regenerative capacity of the kidney, and this field now appears able to advance rapidly showing some fundamental principles of kidney regenerative biology. One of the most interesting

recent finding was the indisputable demonstration that the kidneys of lower vertebrates such as the elasmobranch skate and zebrafish could generate entire new nephrons after injury [95,96], thereby demonstrating the existence of adult progenitors capable of kidney regeneration. In contrast to lower vertebrates, the mammalian kidney shows a limited capacity for regeneration. Indeed, a partial nephrectomy can induce the formation of new nephrons during development, but this capacity seems to be lost shortly after birth [97]. How- ever, the knowledge of how the adult kidney repairs itself is a remarkable promise and this is the reason why many debates, regarding the identification of key cellular players in renal regeneration, characterize this field of intense research. How- ever, even if several recent lines of evidence confirmed that parietal epithelial cells (PECs) of Bowman capsule may serve as progenitor cells for podocytes during kidney growth [98] and may have a role in glomerular repair by replacing lost podocytes [49,97,99], controversial data in adult mouse kidney rather suggested that PECs' activation is harmful and drives generation of hyperplastic intraglomerular cellular lesions leading to nephron degeneration [100]. In addition, other cell types such as renin cells of the juxtaglomerular apparatus have been reported as a source [101] of both PECs and podo- cytes in a murine model of focal segmental glomerulosclerosis (FSGS). However, Starke *et al.* demonstrated that renin line- age cells represent a major source for reconstitution of the intraglomerular mesangial cells and not endothelial, podocyte or PECs after injury [102]. A similar debate regards the regenerative capacity of the tubular compartment, which shows evidence of a certain proliferation responsible of cellu- lar repair and tissue remodeling, causing a quick recovery of kidney function in response to AKI. Some researchers pro- posed that fully differentiated tubular cells would transiently undergo a cycle of dedifferentiation, proliferation and, ultimately, redifferentiation [103-106], whereas others argued on the existence of an intratubular progenitor population which, poised in a quiescent state, would re-enter

the cell cycle with the aim of replacing tubular cells [41,44,97]. Resolution of these debates is essential for developing new therapeutic strat- egies to prevent AKI, as well as CKD. In the most recent years, the existence of a renal progenitor system consisting of a heterogeneous population of RPCs with different com- mitment in adult human kidney has been demonstrated [47-49] and has been proposed as the main source to replace podocyte and/or tubular cell lost during an insult, at least in humans [97]. For this reason, it is becoming mandatory to clarify which tools we could use to manage the regenerative potential and how to enhance the endogenous regenerative processes to achieve kidney repair. Nowadays, the existence of a renal progenitor system seems to be the most hopeful discovery to set up new potential therapeutic treatments for kidney disease and to identify new targets in order to accelerate their regenerative response.

5.1 How current pharmacological treatments can enhance kidney regeneration

The clinical benefits of the current pharmacological treatments such as corticosteroids and inhibitors of the renin-angiotensin (Ang) system such as ACE inhibitors (ACEis) and Ang II type I receptor blockers (ARBs) to halt the progression of CKD are well described. The study of molecular mechanisms of glucocorticoids and ACE inhibition has increased knowledge on the action of these molecules, clarifying their benefic role in the treatment of kidney disease. Of note, several evidences have demonstrated that these mol- ecules can be, surprisingly, a selective way to potentiate the regeneration of the glomerulus by increasing the differentia- tion of RPCs toward podocyte lineage at least in adult mice and rats [107,108]. Indeed, a recent study demonstrates that prednisone administration in mice with experimental FSGS has a dual biological effect on both podocytes and RPCs. Of note, this effect was either the reduction of the magnitude of podocyte apoptosis or the increase of podocyte markers acquired by RPCs, determining a final enhanced number of podocytes that correlated directly with decreased glomerulo- sclerosis and reduced proteinuria [107]. Moreover, animal models of non-diabetic and diabetic nephropathy have clearly shown that treatment with ACEis and ARBs or their combi- nation not only prevented progressive renal damage but also promoted the regression of glomerulosclerosis and vascular lesions [109]. By performing three-dimensional analysis to estimate the volume of glomerular tuft, a reduction in exten- siveness of sclerotic lesions and regeneration of new normal capillary tuft was demonstrated, suggesting that remodeling of glomerular architecture is possible, and that some forms of regeneration can occur. Based on these findings, one possi- ble mechanism that elucidated the effects observed after ACEi treatment could involve the restoration of podocyte number by increasing their number per capillary tuft [108]. The hypo- thetical population responsible for the regression of glomeru- lar lesions was considered to be a subset of PEC in Bowman's capsule of rat kidney, which was characterized by the expression of the marker Neural cell adhesion molecule (NCAM). These cells showed outstanding ability to acquire phenotypic features of differentiated podocytes *in vitro* [110], as also reported for the human RPCs, suggesting their determinant role in the restoration of podocyte number *in vivo*. Moreover, another effect observed following treatment with ACEi was the reduction of PEC proliferation in the formation of cres- cents, preventing the evolution toward glomerulosclerosis, and demonstrating that this treatment can reestablish a nor- mal glomerular architecture [110]. Mechanisms responsible for RPCs' proliferation seem to involve the expression of Ang II receptor, AT1, which is upregulated in the hyperplastic lesions [111]. These results suggest a crucial contribution of Ang II/AT1 receptor pathway in promoting abnormal RPCs' migration and proliferation in proliferative diseases [109,111] and give another explanation to the beneficial effects observed
after ARBs treatment. Indeed, ACEi therapy given to a patient with crescentic glomerulonephritis limited progenitor cell proliferation and normalized AT1 receptor expression on RPCs, concomitant with the regression of hyperplastic lesions. However, although treatment of glomerular diseases with ACEis or ARBs slows the progression of kidney disease, the mechanisms mediating their renoprotective effects are only partially known [112].

5.2 Future challenges to enhance endogenous kidney regeneration

Further to drugs acting on immune system and on renin-Ang system, several other compounds were proposed to target glo- merulosclerosis by improving podocyte regeneration by RPCs. Such drugs

include chemokines or chemokine receptor antagonists [113], retinoids [114], Notch inhibitors [115], *b*-catenin/Wnt molecules [116] and IFNs [117]. The dual antagonism of the chemokine ligand monocyte-chemoattractant protein- 1 or CCL2 and CXCL12 showed additive and synergistic pro- tective effects on the progression of diabetic nephropathy [113], combining the protective effect on CCL2-mediated glomeru- lar leukocyte recruitment with that on CXCL12-mediated loss of podocytes. *In vitro* studies demonstrated that the effect of CXCL12 blockade increased podocyte number and neph- rin or podocin mRNA expression, enhancing RPC differenti- ation toward the podocyte lineage, and thus, enhancing podocyte regeneration [113]. The rationale of the clinical ben- efit of CXCL12 blockade also resides in direct evidence about the contribution of CXCR4/CXCL12 pathways to the abnor- mal behavior of RPCs in proliferative diseases [111]. Indeed, the interaction of CXCR4, expressed by PECs, with its ligand CXCL12, produced by podocytes, triggers and enhances RPCs proliferation in the Bowman's capsule, leading to hyperplasia. These findings promote CXCL12 blockade as a novel strategy to more efficiently prevent crescentic lesions and glomerulosclerosis and to foster the regenerative capacity of RPCs.

Retinoids can be added to the number of molecules with a renoprotective function that were widely investigated. How- ever, the potential role of these molecules in promoting kidney regeneration has been unveiled only recently. RA represents the key inducer of RPCs differentiation toward the podocyte lineage *in vitro*, allowing the acquisition of spe- cific podocyte markers and proper features of podocytes [114]. However, exposure to albumin, which represents the specific carrier of RA, can impair RPCs differentiation into podocytes by sequestering RA. In agreement with *in vitro* results, block- ing endogenous RA synthesis in experimental FSGS, markedly increased albuminuria and mortality, whereas the exogenous administration of RA neutralizing the sequestering activity of albumin allowed the regenerative response of RPCs, determining an increase in podocyte number and the improvement of renal function [114]. These data suggest that endogenous RA is essential for podocyte number maintenance and the presence of even a small amount of albumin within the Bowman's space can be detrimental for an appropriate regenerative process to occur [114]. However, once podocyte damage is severe and leads to a progressive increase of protein- uria within the Bowman's space, RPCs' response to RA is impaired, causing a failure of differentiation into podocytes, and an aberrant RPC proliferation that generates hyperplastic glomerular lesions [100]. Recently, new pathways such as Notch and *b*-catenin/Wnt signaling involved in growth/ differentiation of RPCs toward the podocyte lineage were investigated, providing evidence that a regulated balance between podocyte loss and regeneration by RPCs influences the severity of glomerular disorders. *In vitro* experiments have shown that Notch activation in human RPCs leads to entry into the S-phase of the cell cycle and successive cell divi- sion, whereas its downregulation is essential for RPC differen- tiation into podocytes [115]. Accordingly, inhibition of Notch signaling throughout the regenerative phases leads to a worsened podocyte loss resulting in glomerulosclerosis and a worsening of the albuminuria in a mouse model of FSGS [115]. Conditional *b*-catenin knockout mice, in which a Wnt down-target has been deleted, form abnormal kidneys and have reduced renal function. Tracing nephrogenesis in embryonic knockout mice revealed that the Wnt pathway drives the differentiation of RPCs into podocytes in the pari- etal layer of the S-shaped body by direct lineage switch [116]. Furthermore, recent evidence demonstrates the distinct effects of IFN-*a* and IFN-*b* on podocytes and RPCs *in vitro* and in an Adriamycin-induced nephropathy mouse model. Of note, IFN-*b* specifically promoted podocyte loss by inducing mitotic catastrophe of podocytes, IFN-*a* affected proliferation and migration of RPCs *in vitro* and *in vivo*, and both IFNs impaired the differentiation of RPCs into mature podocytes *in vitro* [117]. Further studies are needed to determine the role and contribution of specific signaling pathways in the balance between podocytes loss and the physiologic regenera- tive capacity of RPCs. The identification of novel targets will open the previously unthinkable possibility that in patients with glomerular disorders this balance might be manipulated to trigger podocyte regeneration (Table 2).

Expert opinion

SC therapy is potentially a promising new treatment for kid- ney disease. The benefit of cell therapy has been documented in many animal studies [90-92], and MSCs have emerged as a promising cell type, due to the allogeneic potential of trans- plantation, the ability to undergo *in vivo* renal differentiation

(albeit at low levels) and the paracrine mechanisms of action [57-70,77-87]. Potentiating MSCs migration, engraftment, survival and paracrine effects using preconditioning or genetic modification strategies could optimize the beneficial effect of this kind of therapy [118]. In clinical trials no major adverse effect has been attributed to the injection of MSCs [91-93]. However, the efficacy of these cells is still controversial at least
in part due to differences in protocols used such as the num- ber of administered cells and the time of administration. Other important aspects that can influence the therapeutic potential of MSCs are donor age and functional abnormality of MSCs isolated from patients with renal diseases. Moreover, there is also a need to carry out appropriately designed exper- imental studies to verify the long-term safety of these therapies and to examine the risks of fibrosis, maldifferentiation or malignancy and only the results of larger, well powered rigor- ously designed clinical trials will determine the real clinical efficacy of MSC therapy in kidney diseases.
In our opinion, a progenitor cell of a particular tissue is perhaps the right source to treat tissue-specific diseases. This kind of cell can be obtained through differentiation of ESCs or iPSCs. However, research toward the kidney regeneration strategy using ESCs and iPSCs is in its infancy. Continued
efforts are necessary for the establishment of step-by-step differentiation protocols directing the pluripotent SCs first to form intermediate mesoderm, then renal progenitors, followed by the formation of mature, functional renal cells and to try to overcome the current problems with these cell types: heterogeneity in the differentiation potential, interline variability and low frequency and inefficient transduction of iPSCs.
Adult kidney-specific stem/progenitor cells are an alterna- tive approach of SC-based therapy in renal disease. However, at present, the possible application of these cells cannot be envisaged, for several reasons. RPCs are not easily accessible and need to follow an ex vivo expansion process before implantation; moreover, age or CKD may influence and decrease the regenerative potential of autologous RPCs; thus, we need to verify the compatibility among patients to use allogeneic RPCs.
In any case, identification of progenitor cells in the adult kidney was another step forward in regenerative medicine, suggesting that the repair potential of the adult kidney also exists in mammals. Targeting this function in situ using pharmacological approaches or targeting potential pathways in order to accelerate the regenerative response may be a near-term application of regenerative approaches because they are amenable to traditional drug and biologic therapies. Experimental data suggest that reconstitution of functional tissue by renal progenitors could theoretically be confined to the early phase of the disease, becoming limited as the damage increases, as we demonstrated for podocyte replacement, which was completely abolished when albumin within the Bowman's space increases even slightly, sequestering RA, the key molecule for RPC differentiation toward the podo- cyte [114]. This phenomenon is not ineluctable, as underlined by the occurrence of glomerular regeneration when protein- lowering drugs such as ACEis are used, and can be even phar- macologically enhanced, turning failure into success [108-110]. On the contrary, in the advanced phases of the disease, the endogenous regeneration of the podocytes operated by RPCs seems unable to correctly and successfully replace a sig- nificant loss of cells, but RPCs rather chaotically proliferate and migrate, leading to lesion formation and subsequent sclerosis [111,114,118]. The enhancement of the repair process operated by RPCs toward regeneration could be obtained through the delivery of drugs [107-110] or targeting other puta- tive renoprotective pathways, such as chemokine receptor antagonists, Notch, Wnt and IFNs [113-117]. Targeting of these pathways, however, might exert negative effects on other renal and extrarenal cell types. Thus, future studies will likely identify more candidate signaling pathways implicated in RPCs' maintenance, regulation and response after injury. Genetic lineage-tracing approaches will facilitate this work improving progenitor cell characterization and enabling us to examine their differentiation potential in vivo and their response to pharmacological agents. These combined efforts will favor the translation of experimental results into clinical practice.

Declaration of interest

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discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents, received or pending, or royaltie

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Table 1. Indirect mechanisms of MSCs-mediated renoprotection and repair of kidney damage.

Target cells	Effects	Refs.
Trophic actions		
Secretion of bioactive factors: SDF-1, VEGF, HGF, G-CSF, stem cell factor, leukemia-inhibitory factor, macrophage-colony stimulating factor, IL-6, IL-11, basic fibroblast	Tubular cells Antiapoptotic Mitogenic Endothelial cells Vasoprotective Angiogenic	[57,60,66-68,76-78,91,92]
growth factor, monocyte-chemoattractant protein-1, IGF-1	Endogenous progenitor cells Unknown	
Release of microvesicles or exosomes containing:	Tubular cells Antiapoptotic Mitogenic	[57,58]
protein mRNA (i.e., IGF-1R) miRNA	Endogenous progenitor cells Unknown	
Anti-inflammatory and immune-modulating actions		
Release of soluble factors: PGE-2, TGF-*b*, HGF, IL-10, nitric oxide, soluble TNF-receptor 1	Dendritic cells T cells NK cells B cells Treg Neutrophils	Inhibition of differentiation and activation Inhibition of proliferation, activation and release of TNF-*a* and IFN-*g* increased secretion of suppressive cytokine IL-10 Inhibition of proliferation Inhibition of proliferation, antibody production and chemotaxis Increase the number of Tregs Reduce infiltration

HGF: Hepatocyte growth factor; MSC: Mesenchymal stem cell; NK: Natural killer; SDF-1: Stromal cell-derived factor 1; Treg: Regulatory T cell

Table 2. Endogenous renal regeneration can be modulated targeting both RPC differentiation and/or proliferation.

Effect on	Treatment	Observation	Model	Ref.
Differentiation	Corticosteroids	Increase of podocyte number	Animal model of FSGS	[107]
	ACE inhibitors	Increase of podocyte number	Animal models of non-diabetic and diabetic nephropathy MWF rat Human proliferative glomerulonephritis	[108,110]
	CXCL12 antagonist	Increase of podocyte number	Animal model of diabetic nephropathy	[113]
		Upregulation of nephrin and podocin mRNA expression by RPCs	Primary culture of human RPCs	
	Retinoic acid	Acquisition of specific podocyte markers and proper features of podocytes by RPCs	Primary culture of human RPCs	[114]
	DAPT (Notch inhibitor)	Increase of podocyte number Differentiation of RPCs into podocytes	Animal model of Adriamycin nephropathy Primary culture of human RPCs	[115]
	Conditional *b*-catenin knockout	Switch in lineage differentiation of RPCs toward a podocyte-like cell fate	Animal model of FSGS *b*-catenin--deficient mice	[116]
	IFN-*a*; IFN-*b*	Suppression of RPC differentiation into podocytes Reduction of podocyte number	Primary culture of human RPCs Animal model of adriamycin-induced nephropathy	[117]
Proliferation	ACE inhibitors	Reduction of RPCs proliferation	Animal models of non-diabetic and diabetic nephropathy MWF rat Human proliferative glomerulonephritis	[110]
	Angiotensin II type I receptor blockers	Reduction of RPCs proliferation	Animal models of non-diabetic and diabetic nephropathy MWF rat Human proliferative glomerulonephritis	[111]
	IFN-*a*	Reduction of RPCs proliferation	Animal model of adriamycin-induced nephropathy Primary culture of human RPCs	[117]

DAPT: *N*-[*N*-(3,5-difluorophenacetyl)-L-alanyl]-*S*-phenylglycine *t*-butyl ester; FSGS: Focal segmental glomerulosclerosis; MWF: Munich-Wistar Fromter; RPCs: Renal progenitor cells.

Article highlights.

- Stem cell-based therapy for kidney diseases is an exciting research area.
- Embryonic stem cells, induced pluripotent stem cells, amniotic fluid-derived stem cells, endothelial progenitor cells, mesenchymal stem cells (MSCs) and renal progenitor cells (RPCs) are some of the cell types that have been associated with a benefit in several animal models of kidney disease.
- MSCs have emerged as a promising cell type for treatment of kidney disease.
- The benefit of MSCs is mediated mainly through paracrine and immunomodulatory mechanisms rather than engraftment and differentiation.
- MSCs' delivery to human subjects in clinical trial settings is safe but the efficacy of these cells is still controversial.
- RPCs are present within the kidney and are involved in renal repair.