Abstract

Chronic Kidney Disease (CKD) affects almost 10-12% of the general population, and in the next years an increase is expected due to the aging of the population and the epidemic of diabetes mellitus type 2. Despite advances in our understanding of the mechanisms of kidney disease and improvements in renal replacement therapy, mortality still remains increased. Furthermore, kidney transplantation—the preferred treatment of CKD—is neither without complications nor widely available due to the lack of kidney donors. Therefore, novel therapies are needed.

Renal regenerative medicine aims at establishing an unlimited supply of autologous renal cells or renal tissue to restore renal function. In contrast to other organs whose cells function as individual units, the kidney is composed of at least 26 terminally differentiated cell types that have to be organized into specific segments with a specific 3D-structure to function properly. Which is the optimal cell for kidney regeneration? The identification, isolation and understanding of the function of the cells that are involved in kidney regeneration would enable the application of novel cell-based technologies to restore kidney function.

Here, a review of the literature regarding the origin of the cells during kidney repair is presented; and emerging cell-based approaches that are currently used to regenerate renal tissue are summarized.

Key words: cell therapy, kidney regeneration, renal progenitor cells.

Introduction

Chronic Kidney Disease (CKD) affects almost 10-12% of the general population, and in the next years an increase is expected due to the aging of the population and the epidemic of diabetes mellitus type 2. The cost of treating CKD patients is enormous, and importantly, in developing countries renal replacement therapy (RRT) is not widely available so renal disease is synonymous to death. Despite advances in our understanding of the mechanisms of kidney disease and improvements in RRT, mortality still remains high. Therefore, novel therapies are needed.

The kidney is a highly terminally differentiated organ that has lower proliferative capacity than other organs such as intestine,
bone marrow and skin. Recovery after acute renal injury depends on the ability of the kidney to replace injured cells with functional cells. Ischemic-reperfusion injury of the kidney leads to detachment of dead tubular cells. Subsequent tubular regeneration restores normal tubular architecture and function. In experimental models of diabetic and non-diabetic nephropathy, the administration of angiotensin converting enzyme inhibitors (ACE inhibitors) and angiotensin II type 1 receptor blockers (ARBs) prevents the progression of renal damage, promotes regression of glomerulosclerosis and is associated with an increase in the number of podocytes. Also, the potential of the human kidney to repair has been shown in patients with diabetes mellitus type 1 whose glomerular lesions ameliorated 10 years after pancreas transplantation. All these findings point towards the capacity of the kidney to regenerate.

Regeneration is a highly conserved process throughout evolution; however, the potential of a tissue to regenerate is different among species. Planarians can regenerate entirely; lower vertebrates have limited regenerative capacity, and lower animals can regenerate internal organs at various degrees. In mammals, most organs have the ability to regenerate functional tissue through a process that is enabled by the presence of stem or progenitor cells. It is well established that renal progenitor cells exist in lower vertebrates. However, the identification, isolation and understanding of the function of the cells that are involved in kidney regeneration in mammals would enable the application of novel cell-based technologies to generate an unlimited supply of autologous renal cells or renal tissue to restore kidney function.

Here, a review of the literature regarding the origin of the cells during kidney repair is presented; and emerging cell-based approaches that are currently used to regenerate renal tissue are summarized.

What is a stem cell?

A stem cell is a cell that is not terminally differentiated. It can divide without limit and it can divide asymmetrically i.e. when it divides each daughter cell can either remain a stem cell or it can commit to terminal differentiation. Stem cells are classified as “totipotent”, if they can give rise to both embryonic and extra-embryonic cells, “pluripotent” if they can form cells of embryonic origin, and “multipotent” if they can produce two or more mature cell types. Progenitor cells have limited capacity of self-renewal and their progeny give rise to specific mature cell types.

During embryogenesis, embryonic stem cells divide into organ-specific stem cells that are resident, multipotent cells that generate lineage-restricted cells; also, they divide asymmetrically so they can regenerate themselves upon mitosis. They are located in a niche that is rich in blood supply, where they are protected from environmental factors and are supplied with factors that prevent their rapid cycling. During adult life and normal organ maintenance or after tissue injury, organ-specific stem cells are the cells that are linked to repair.

Renal progenitors during embryogenesis

There is no doubt that stem cells exist during embryogenesis. The embryo is derived from embryonic stem cells of the inner cell mass of the blastocyst that differentiate into ectoderm, mesoderm and endoderm. Development of the kidney begins when metanephric mesenchyme, a cluster of cells derived from the intermediate mesoderm, interacts reciprocally with the ureteric bud (UB), a derivative of the Wolffian duct. Metanephric mesenchymal cells can self-renew and represent multipotent renal stem cells. Metanephric mesenchyme condenses around the tip of the growing bud, and then is converted into a spherical cyst, the renal vesicle. The renal vesicle continuously elongates and invaginates to form the S-shaped bodies. The proximal end of the S-shaped bodies then become invaded by blood vessels that generate the glomerular tuft. The middle and distal end of the S-shaped bodies elongate and fuse to form the single-layer tubule that is composed of cells that express epithelial proteins. The complete nephron empties into the collecting ducts that are formed through repeated branching and elongation of the ureteric bud. At birth, maturation of the nephron is complete. During the perinatal period, the thin ascending loop of Henle is generated as an outgrowth of the S3 segment of the proximal tubule and of the distal tubule; the pyramidal shape of the
Kidney structure is formed, and cortex and medulla become clearly distinct\(^{19}\).

**Renal progenitors during adult life**

Do renal progenitors exist during adult life? The findings that point towards the ability of the kidney to regenerate\(^{2-10}\) prompted researchers to investigate the origin of the cells that contribute to kidney repair. The identification of the origin of these cells would enable the understanding of their function and their isolation in order to be used as novel therapeutic tools.

Kidney repair could occur through: (a) surviving terminally differentiated cells by a process that is called repopulation; (b) stem cells of extra-renal origin; (c) resident renal stem cells.

**Kidney repair through surviving differentiated cells**

The renal tubule has an extraordinary capacity to undergo regeneration within a few days after acute kidney injury. Kidney regeneration could occur through the proliferation of surviving terminally differentiated cells. In tubular regeneration, surviving fully differentiated tubular cells could proliferate to give rise to cells identical to them that migrate to reline the denuded tubules. To search for evidence of a tubular stem cell system, Vogetseder et al examined the level of differentiation of slow-cycling cells and attempted to detect rapidly cycling tubular cells. They found that all tubular cells had equal potential to enter the cell cycle. When there was an injury, a very rapid increase in cell division occurred, and cells rapidly proliferated\(^{21}\). Also, using genetic fate-mapping techniques in ischemia-reperfusion injury in transgenic mice, it was found that regeneration by surviving tubular epithelial cells was the predominant mechanism of repair\(^{22}\). It was also found that the cell whose fate was to proliferate could be any surviving cell\(^{23}\). Whether these repeated cycles of proliferation could exhaust the regenerative capacity of the tubular epithelial cell compartment, remained unanswered.

To repair the damage, tubular epithelial cells could also dedifferentiate towards a mesenchymal phenotype through epithelial-to-mesenchymal transition (EMT) to re-enter the cell cycle. To examine if tubular regeneration is characterized by re-expression of developmentally important regulatory genes, Imgrund et al induced tubular injury and found that generating proximal tubular epithelial cells transiently re-expressed Pax-2, a transcription factor present during kidney development. This finding indicates that developmental paradigms could be recapitulated to restore mature kidney function\(^{24}\).

**Renal progenitors of extra-renal origin**

The bone marrow is a tissue rich in stem or progenitor cells, since it contains hematopoietic stem cells (HSC) and mesenchymal stem cells (MSC). HSC give rise to erythroid, myeloid and lymphoid cell lines as well as endothelial progenitors and fibrocytes. MSC have the plasticity to form multiple cell-types of mesenchymal and non-mesenchymal lineages such as osteoblasts, adipocytes, chondroblasts and myoblasts. Recent studies have shown that the bone marrow stem cells from both mice and humans have the ability to cross-lineage differentiate into functional components of other tissues such as heart, liver, brain, skeletal muscle, and vascular endothelium\(^{25,26}\). Could bone marrow stem cells represent renal progenitors through cross-lineage differentiation to repopulate injured nephron segments?

During acute renal injury, an increase in white blood cells is observed. To examine if extra-renal cells contribute to the turnover and repair of renal tissues, Poulsom et al analyzed kidneys of female mice that had received a male bone marrow transplant, and demonstrated that circulating stem cells frequently engrafted into the kidney and differentiated into renal parenchymal cells. Female mouse recipients of male bone marrow grafts showed co-localization of Y-chromosomes and tubular epithelial markers; also, Y-chromosome-containing cells were observed within glomeruli, and their morphology and location were similar to those of podocytes\(^{27}\). Also, Kale et al used a mouse model of ischemic renal injury to determine whether bone marrow stem cells can contribute to the repopulation of injured renal tubules. They demonstrated that bone marrow stem cells not only have the capacity to differentiate into renal epithelial cells, but also represent the majority of the cells that reconstitute the necrotic S3
segment of the renal tubule. In addition to these, in a glomerulonephritis model it was shown that 7% of mesangial cells were bone marrow-derived cells and that these cells could contribute to glomerular repair.

On the contrary, others were unable to demonstrate sufficient differentiation of bone marrow-derived cells to renal cells. Also, concern is arising about the capacity of bone marrow stem cells to differentiate into interstitial cells, mainly myofibroblasts that induce renal fibrosis. To draw definite conclusions, further studies are needed to precisely understand the involvement of bone marrow stem cells in kidney regeneration, and to identify which bone marrow stem cells contribute in favor or against kidney repair. If they contribute in favor of kidney repair, approaches based on the mobilization and delivery of the appropriate bone marrow cell to the kidney could be used as a novel therapy to improve the outcome of patients with kidney injury.

**Resident renal progenitors**

Organ specific stem cells were initially recognized in the hematopoietic system, the skin and the intestinal epithelium, where self-renewal is obvious. The kidney is considered to be an organ with limited capacity to regenerate, since in humans new nephrons do not appear after 36 weeks of gestation due to the exhaustion of the progenitor mesenchyme. However, this does not rule out the existence of a renal stem cell system able to induce repair.

To identify stem cells that are present in the kidney, several techniques have been used. Some of them are the expression of stem cell markers on the surface of the kidney stem cell, the label retention by slowly dividing cells, the expression of transcription factors that are expressed during kidney development, the extrusion of fluorescent dyes and several others.

In searching for stem cells in the adult kidney, Oliver et al reasoned that the slow-cycling time of organ specific stem cells could be used as a method to identify them. Slow-cycling cells can be distinguished by retention of a nucleotide label that is incorporated into the DNA during cell division. A pulse of a nucleotide label is administered, and after a long chase period, only slowly dividing cells retain a concentration high enough to allow their staining and therefore their identification. They found that in the kidney, label-retaining cells exist in the interstitium mainly in the renal papilla closest to the urinary pole. Also, they found that during recovery from ischemia, these cells proliferated and were able to leave the region nearest to the urinary space to migrate towards other parts of the kidney where they were incorporated. These results suggested that the kidney papilla is a niche for adult stem cells. In this niche, cells form a compartment of rapidly proliferating cell migrate to play a role in kidney repair after injury.

By assessing the co-expression of CD24 (a surface molecule expressed in metanephric mesenchyme during embryogenesis) and CD133 (a marker of several types of adult tissue stem cells), renal progenitors in adult human kidneys were identified. During kidney development, cells co-expressing CD24 and CD133 represent progenitors of tubular cells and podocytes. They are enriched in the kidneys at 8-9 weeks of gestation and substantially decrease by 10-14 weeks. These renal progenitors can act as precursors to all renal epithelial cells of the cortical nephron, being able to diversify themselves to generate the glomerular or the tubular epithelial cell lineages through a graded series of committed progenitors. Then, podocyte-committed progenitors, localizing along the Bowman’s capsule, display transitional features of progenitors and podocytes, and can only differentiate into podocytes. Finally, tubular-committed progenitors, which display transitional features of progenitors and tubular cells, are scattered along the tubule and can only differentiate into tubular cells. Specifically, progenitors localize within the proximal tubule, particularly in the S3 segment, and co-express CD133 and CD24 as well as markers that are specific of proximal tubular epithelia. Some tubular progenitors localize within the thick ascending limb, the distal convoluted tubule and the connecting segment expressing at the same time markers of the respective tubular compartment.

In adult human kidneys, CD24+CD133+ cells were found at the urinary pole of the Bowman’s capsule the only place in the kidney that appears to be contiguous with both tubular cells and glomerular podocytes. Once isolated, these cells exhibited...
self-renewal properties and could differentiate into both glomerular and tubular epithelial cells. Upon their injection to mouse models of AKI, tubular structures expressing epithelial proteins were formed, and kidney function was improved. A proposed mechanism of kidney regeneration is as follows: when there is tubular injury, the adjacent healthy fully differentiated cells proliferate to reline the denuded basement membrane. Renal progenitors at the urinary pole of the glomerulus help against the exhaustion of the regenerative capacity of the tubular epithelium, since they may provide novel cells to maintain the bulk of the proliferating cells. On the other hand, renal progenitor cells are contiguous with podocytes at the vascular stalk. After podocyte injury, renal progenitors progressively migrate towards the vascular stalk, and differentiate into podocyte-committed progenitors to generate novel podocytes. Under normal conditions, the response of renal progenitors is strictly regulated. However, in severe glomerulopathies, uncontrolled growth of podocyte-committed progenitors can generate hyperplastic glomerular lesions. Also, growing evidence suggests that human tubular progenitors represent the cell of origin of papillary renal cell carcinoma. Thus the precise understanding of the function of these cells is essential to be able to use them as therapeutic targets for the treatment of renal disorders.

Cell therapy for kidney regeneration

The idea of being able to produce functional renal tissue or even a whole kidney de novo is raising hope worldwide. The goal of renal regenerative medicine is to establish an unlimited supply of autologous renal cells or renal tissue to restore renal function. In contrast to other organs whose cells function as individual units (i.e. the hematopoietic system and the pancreas), a kidney is a solid organ composed of at least 26 terminally differentiated cell types that have to be organized into specific segments with a specific three-dimensional structure in order to function properly. Which is the optimal cell for kidney regeneration? Potential cellular sources could include embryonic stem cells, renal progenitors of extra-renal origin, resident renal progenitors or induced pluripotent stem cells (iPS).

Embryonic stem cells in renal regenerative medicine

Embryonic stem cells (ESC) obtained from the inner cell mass of the blastocyst are attractive for cell based therapies since they are “pluripotent”, can give rise to all cell types in the body, and have unlimited capacity for proliferation.

When injected in a mouse model of Alport’s syndrome, murine undifferentiated ESCs were homed in the kidneys, and significant improvement in renal function and histology were observed. When rat fetal kidney cells were injected into the kidney subcapsular space of an AKI model, renal structures were created and renal function was improved.

Recently, Xinaris et al developed a novel approach to renal tissue engineering, and showed that a single cell-derived tissue can become integrated into a host recipient and continue its developmental program. Mouse embryonic kidneys were dissociated into single cells and cultured in vitro. Aggregates, called organoids, were treated with vascular endothelial growth factor (VEGF). Initially, renal organoids consisted of a mixture of embryonic renal cells. Progressively, organoids developed epithelial renal structures, including renal vesicles and S-shaped bodies. Then organoids were implanted beneath the renal capsule of athymic rats subjected to nephrectomy. This was followed by local VEGF injection into the area of organoid implantation. Histology of organoid showed tubuli and a rudimentary glomerular-like structure. Also, implanted organoids exhibited functional renal activities such as production of erythropoietin and endocytosis of low molecular weight proteins by the tubular cells. This system introduced a novel direction for generating donor tissue and offered a significant step towards the replacement of renal function by a tissue-engineered kidney.

The use of ESC is attractive, however important limitations exist. Their unlimited capacity for proliferation has a risk of mal-differentiation into pathologic tissues (teratomas) and uncontrolled growth. Until now, most studies that analyzed growth factor combinations and evaluated the precise point at which ESCs are differentiated to the renal lineage have failed. Precise differentiation protocols and culture conditions are lacking and further studies are needed to establish the exact role
of ESCs in regenerative medicine.\textsuperscript{36-39} Importantly, ethical, political and religious concerns of deriving cells from early human embryos limit their use.

**Resident renal progenitors in renal regenerative medicine**

Due to the limitations of the use of ESC, resident renal progenitors appear to be more attractive. These cells have the advantage of being restricted to kidney-lineage; they have enough differentiation and lower mal-differentiation potential than ESC, and could be derived from either fetal or adult kidneys. However, precise methods for their isolation and culture conditions for expansion are still missing.

**Renal progenitors of extra-renal origin for kidney regeneration**

During normal physiology of tissue maintenance and repair after injury, circulating stem cells can enter the kidney and differentiate into renal cells. Despite contrasting results, most studies showed that bone marrow stem cells were able to replace tubular epithelial, mesangial, endothelial cells and podocytes. Hematopoietic stem cells are relatively difficult to obtain and cannot be cultured and expanded to sufficient levels \textit{in vitro}. Therefore, renal regenerative medicine is shifted towards mesenchymal stem cells, the cells that provide stromal support to hematopoietic stem cells. Mesenchymal stem cells (MSC) are easily derived from bone marrow and also from adipose and other tissues. They are able to self-renew, they easily expand in culture and can easily differentiate into several cell lines.

To investigate the role of MSC in renal fibrosis, Semedo P et al in 2009\textsuperscript{40} administered intravenously MSCs in a model of CKD, and found that MSCs were homed in the interstitium near the vessels. They also found that the fibrotic area was significantly reduced; serum creatinine, serum urea and proteinuria decreased whereas the hematocrit increased.

Bone marrow is the most common tissue source of MSCs. However, their collection is invasive and their number, differentiation potential and life span decreases with age. In searching for new sources of MSCs for renal repair, Morigi M et al in 2010\textsuperscript{41} investigated the potential of human cord blood-MSCs (CB-MSCs) to treat mice with experimental AKI. CB-MSCs are readily available, extremely rich in stem cells, are similar to bone marrow-MSCs regarding their morphology and multipotency, and are autologous to the host. Human CB-MSCs that were infused in AKI mouse models were found in peritubular areas. Also, the infusion was associated to a decrease in capillary changes and neutrophil infiltration, suggesting that intravenous administration of human CB-MSCs protects animals from renal function impairment prolonging their lifespan.

Despite clear benefits, concern about possible side effects exists. Intravenous MSCs administration could lead to pulmonary embolism or infarction and their administration holds risk for organ fibrosis and tumorigenesis. Up to this day, no side effects have been reported in humans and completed clinical trials demonstrated the efficacy and safety of MSCs administration. Currently, safety and efficacy of MSCs are being tested in a clinical trial studying open-heart surgery patients who are at high risk of postoperative AKI.\textsuperscript{36,42-44}

**Induced pluripotent stem cells (iPS) in renal regenerative medicine**

Apart from all cellular sources mentioned already, artificially created renal progenitor cells could be used for kidney regeneration. By manipulating their transcriptional profile, cells can be “forced” to switch from one type into another, so a cellular phenotype can be induced to transform into a desired one. This approach, that is called reprogramming, transforms somatic cells into induced pluripotent stem cells (iPS). Initially, the available cell is de-differentiated into a pluripotent state and then differentiated into the desired cell type. iPS are by definition “pluripotent”; they can give rise to all cell types in the body and are autologous. Human iPS can be generated from several sources including the skin (fibroblasts and keratinocytes), periosteal membrane, adipose tissue and cord blood.\textsuperscript{36}

Recently, iPS were generated from kidney transplant recipients being on immunosuppression. Skin biopsy was performed and dermal keratinocytes were obtained and cultured. Reprogramming generated iPS clones that had morphology similar to that of ESC and could differentiate into cells of all three germ layers. When injected into mice, iPS clones formed teratomas.\textsuperscript{45}
The strategy to develop renal iPS that have reduced mal-differentiation potential is to de-differentiate the mature adult cell only to the stage where renal multipotentiality is achieved. Candidate adult cells could be either extra-renal progenitor cells or adult kidney-derived cells that have the advantage of a small developmental distance. Normal mesangial cells were reprogrammed into renal iPS that had morphology similar to embryonic bodies; and when injected to immunodeficient mice, they developed teratomas. To avoid a renal biopsy to collect renal cells, Zhou et al reasoned that the cells that are normally excreted in urine everyday could be a valuable source for reprogramming. They isolated two types of kidney cells and they were reprogrammed into renal iPSc that could be directly differentiated into neural lineages, hepatocyte-like cells and cardiomyocyte-like cells that exhibited action potentials.

Together these studies show that manipulating adult kidney-derived cells offers the advantage of having a small developmental distance between the cell of origin and the target cell. Reprogramming will be more efficient and successful. Complete reprogramming to the pluripotent state may not be necessary resulting to a decrease in the risk of mal-differentiation. Gene therapy coupled with iPS production could be applied to genetic-based defects; in vitro assays to study kidney diseases and to discover potential drugs can be established.

**Can we regenerate a whole kidney de novo?**

The idea of being able to produce a whole functional kidney is exciting. Lack of donor organs will not be a concern, and CKD could be managed by transplanting de novo functional kidneys. The first attempt to create a functional whole kidney goes back to 1999 where Chan et al tried to develop transplantable pronephros in Xenopus. Using cells from caps of Xenopus, they induced pronephric tubule-like structures that were transplanted into nephrectomized tadpoles. Edema was partially corrected and survival was increased. This was the begging of the attempts to engineer kidneys applicable for mammals. Then, Woolf et al transplanted metanephros into the renal cortex of host mice that continued to grow into a new structure containing glomeruli and tubules.

To investigate if xenotransplantation represents an alternative to organ transplant, Yokuu T et al attempted to construct a functional kidney structure using a developing heterozoic embryo as an “organ factory”. They established a whole-embryo culture system followed by a metanephric organ culture. Rat embryos were isolated from their mothers before the production of the UB and were grown in a culture bottle until the formation of a rudimentary kidney which continued to grow in vitro. Labelled human MSC, genetically engineered to express factors that induce the derivation of the ureteric bud from the Wolffian duct, were injected at the side of budding in the rat embryo. Soon after injection, the embryos were transferred to the whole-embryo culture system. Labeled cells were observed throughout the rudimentary metanephros, were morphologically identical to renal cells and also expressed podocyte and tubule-specific genes. To further investigate whether these constructs were producing urine, kidney primordia were transplanted into the omentum of rats and human MSCs-derived «neokidney» was generated. Several vessels of the omentum appeared to be integrated into the neokidney and red blood cells were observed inside the glomeruli. The vasculature of the neokidney originated from the host and communicated with its circulation, suggesting that this neokidney is viable. They also showed that this neokidney was functional since it developed hydronephrosis.

A putative scenario for the application of stem cell biology to kidney regeneration is as follows: renal stem cells derived either from bone marrow stem cells or skin fibroblasts of an ESRD patient will be cultured in a growing xenoembryo until they become kidney primordia. Then, kidney promordia will be autologously implanted into the omentum of the patient. Kidney primordia will become a self-organ that will perform renal function; and the patient will not require RRT.

**Conclusions**

Potential cellular sources for kidney regeneration could include embryonic stem cells, renal progenitors of extra-renal origin, resident renal progenitors or induced pluripotent stem cells (iPS). Their use is attractive however their capacity for uncontrolled proliferation and risk of mal-differentiation has to be further studied. Precise differentiation protocols and culture conditions are still lacking.
More studies are needed to precisely understand the exact mechanism of function of renal progenitor cells in order to establish their effectiveness and safety. Importantly, ethical, political and religious rules should be clearly established to ensure respect to human rights.

Δήλωση σύγχρονης συμφερόντων
Δεν αναφέρεται σύγχρονη συμφερόντων

Conflict of interest statement
None declared

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Δέξεις κλειδιά: κυτταρική θεραπεία, νεφρική αναγέννηση, προσεγγίσεις νεφρικών κυττάρων.


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*Παρελήφθη στις 26/03/2015
Έγινε αποδεκτή μετά από τροποποιήσεις στις 22/05/2015

* Received for publication 26/03/2015
Accepted in revised form 22/05/2015

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