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Acute Kidney Injury as a Condition of Renal Senescence

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Abstract

Acute kidney injury (AKI), characterized by a sharp drop in glomerular filtration, continues to be a significant health burden because it is associated with high initial mortality, morbidity, and substantial health-care costs. There is a strong connection between AKI and mechanisms of senescence activation. After ischemic or nephrotoxic insults, a wide range of pathophysiological events occur. Renal tubular cell injury is characterized by cell membrane damage, cytoskeleton disruption, and DNA degradation, leading to tubular cell death by necrosis and apoptosis. The senescence mechanism involves interstitial fibrosis, tubular atrophy, and capillary rarefaction, all of which impede the morphological and functional recovery of the kidneys, suggesting a strong link between AKI and the progression of chronic kidney disease. During abnormal kidney repair, tubular epithelial cells can assume a senescence-like phenotype. Cellular senescence can occur as a result of cell cycle arrest due to increased expression of cyclin kinase inhibitors (mainly p21), downregulation of Klotho expression, and telomere shortening. In AKI, cellular senescence is aggravated by other factors including oxidative stress and autophagy. Given this scenario, the main question is whether AKI can be repaired and how to avoid the senescence process. Stem cells might constitute a new therapeutic approach. Mesenchymal stem cells (MSCs) can ameliorate kidney injury through angiogenesis, immunomodulation, and fibrosis pathway blockade, as well as through antiapoptotic and promitotic processes. Young umbilical cord–derived MSCs are better at increasing Klotho levels, and thus protecting tissues from senescence, than are adipose-derived MSCs. Umbilical cord–derived MSCs improve glomerular filtration and tubular function to a greater degree than do those obtained from adult tissue. Although senescence-related proteins and microRNA are upregulated in AKI, they can be downregulated by treatment with umbilical cord–derived MSCs. In summary, stem cells derived from young tissues, such as umbilical cord–derived MSCs, could slow the post-AKI senescence process.

Keywords

acute kidney injury, cell cycle arrest, Klotho, telomeres, oxidative stress, mesenchymal stromal cells

Introduction

Acute kidney injury (AKI), previously known as acute renal failure, is a syndrome characterized by a sharp drop in the glomerular filtration rate with consequent deterioration of renal function, ultimately leading to the need for dialysis in a great portion of cases. The concept of AKI has undergone significant reexamination in recent years. Traditionally, emphasis has been placed on a pronounced acute reduction in renal function, manifested by azotemia accompanied by oliguria or anuria, with consequent fluid overload and electrolyte abnormalities. However, recent evidence suggests that even relatively mild injury and impairment of renal function, manifested by small changes in serum creatinine or decreased urine output, are predictors of serious clinical consequences. Many patients with AKI have a mixed etiology, often consisting of the coexistence of sepsis, ischemia–reperfusion injury (IRI), and the use of nephrotoxic medications. AKI, a condition that is becoming increasingly prevalent, represents a significant health burden: It affects approximately 25% of hospitalized patients,

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especially critically ill patients in intensive care units, and is associated with high mortality and morbidity, as well as having a substantial impact on health-care costs[1,2]. To date, no single therapy has been shown to improve the outcome of AKI.

Unless a fatal outcome occurs, renal function impairment after AKI has been considered to be a reversible process. In the past, the resolution of AKI and the recovery of renal function have generally been considered to be efficient processes, the traditional view being that they would have no impact on long-term renal function in surviving patients. However, recent studies suggest that there is a strong correlation between AKI and the progression to chronic kidney disease (CKD)[3,4]. Although the mechanisms involved in CKD progression after AKI are still largely unknown, there is growing evidence that the reduced regenerative capacity is linked to a process of senescence activation. In this review, we will focus on the understanding of AKI as a condition of renal senescence and the emerging stem cell therapies that might play a role as treatment strategies.

AKI as a Condition for Accelerated Kidney Aging

After an ischemic or nephrotoxic AKI insult, a wide range of pathophysiological events occur, particularly in the proximal tubule cells, the kidney cells that are most vulnerable to hypoxia and nephrotoxins[5]. Cell injury, in this setting, is characterized by cell membrane damage, cytoskeleton disruption, and DNA degradation, leading to tubular cell death by necrosis and apoptosis[6]. Complete kidney recovery has been observed in the majority of surviving patients, mainly in cases of mild kidney injury. However, in severe injuries or in previously damaged or aged kidneys, abnormal tubular regeneration can occur, defining a maladaptive response of the kidney to AKI[4,7]. The maladaptive response is characterized by interstitial fibrosis, tubular atrophy, and capillary rarefaction, which impede the complete morphological and functional recovery of the kidneys, thus indicating a strong link between AKI and the progression to CKD. In fact, seminal studies have shown an intriguing association between the development of fibrosis and cell cycle arrest of proximal tubular epithelial cells after acute injury[8]. This fibrogenic process is likely mediated by upregulated production of profibrotic factors, such as transforming growth factor-β (TGF-β) and connective tissue growth factor, characterized by activation and proliferation of fibroblasts and perivascular pericytes, which in turn induce extracellular matrix production and tubulointerstitial inflammation, with chronic activation of macrophages[7,9,10]. During the process of abnormal kidney repair, tubular epithelial cells can assume a senescence-like phenotype[8].

Cellular senescence may occur as result of cell cycle arrest due to increased expression of cyclin kinase inhibitors, downregulation of Klotho expression, and telomere shortening. Cells can also be induced to senescence in response to elevated oxidative stress[11]. In this review, we will discuss the mechanisms potentially involved in post-AKI renal senescence.

AKI-induced Cell Cycle Arrest as a Condition of Renal Senescence

Under normal conditions, kidney cells have a low turnover, remaining in the G0 phase, a quiescent state. Ischemic or toxic insults trigger a cascade of cellular events leading to subsequent tubular epithelial cell death by necrosis and apoptosis. Remnant quiescent surviving renal tubular cells enter the cell cycle, proliferating and dedifferentiating into new tubular cells, a process that is important for repopulating the tubules[12]. Shortly after AKI, when the acute stress drives the damaged renal tubular cells to enter the cell cycle, there is rapid, massive induction of cyclin-dependent kinase inhibitor ($p21^{Waf1/Cip1}$), a protein recognized as a cell cycle inhibitor, blocking the cell cycle at the G1/S phase[7,13]. The induction of this early antiproliferative response due to cell cycle arrest after an acute kidney insult, an apparently paradoxical phenomenon, provides more time for DNA damage repair, avoiding uncontrolled progression toward cell death or malignant transformation. In addition, $p21^{Waf1/Cip1}$ activation modulates apoptosis and necrosis in the kidney[14], helping mitigate injury. Hypoxia and other stresses can also activate the ataxia telangiectasia mutated/ataxia telangiectasia (ATM ATR) signaling pathway[15], which can block cell cycle progression through $p21^{Waf1/Cip1}$ or by inducing the protein kinases, Checkpoint kinase 1 (Chk1) and Checkpoint kinase 2 (Chk2), which can promote cell cycle arrest at the G2/M checkpoint[16–18].

The cell cycle is highly regulated by different classes of proteins (Fig. 1). Cyclins and cyclin-dependent kinases (CDKs) are two important classes of proteins that, uncoupled, have a low level of kinase activity. However, when they bind to each other to form heterocomplexes, the kinase subunit is activated, inducing progression through the cell cycle. The third class of proteins consists of CDK inhibitors, which act by inhibiting the CDK complex, consequently inhibiting the cell cycle. In addition, the retinoblastoma protein (pRb) represents an important substrate for CDK function during the cell cycle, at the G1 checkpoint, providing a negative control of the cell cycle. The pRb, a tumor suppressor protein, prevents excessive cell growth by inhibiting cell cycle progression. When the pRb is inactivated by phosphorylation, the E2F transcription factor is released, allowing cell cycle progression.

There are two important families of CDK inhibitors:—the Cip/Kip and inhibitor of cyclin-dependent kinase 4 (INK4) families. Members of both families inhibit the cell cycle, maintaining cells in the G1 phase.

The most well-known member of the Cip/Kip family is the previously mentioned $p21^{Waf1/Cip1}$, a 21-kDa protein that binds to CDK2, inhibiting the CDK2–cyclin E complex, thus promoting cell cycle arrest[13,19]. In addition to participating in cell cycle progression, CDK2 is required in order to trigger pathways of necrosis and apoptosis[14]. In the kidney, nuclear p21 is located in the proximal and distal tubules.

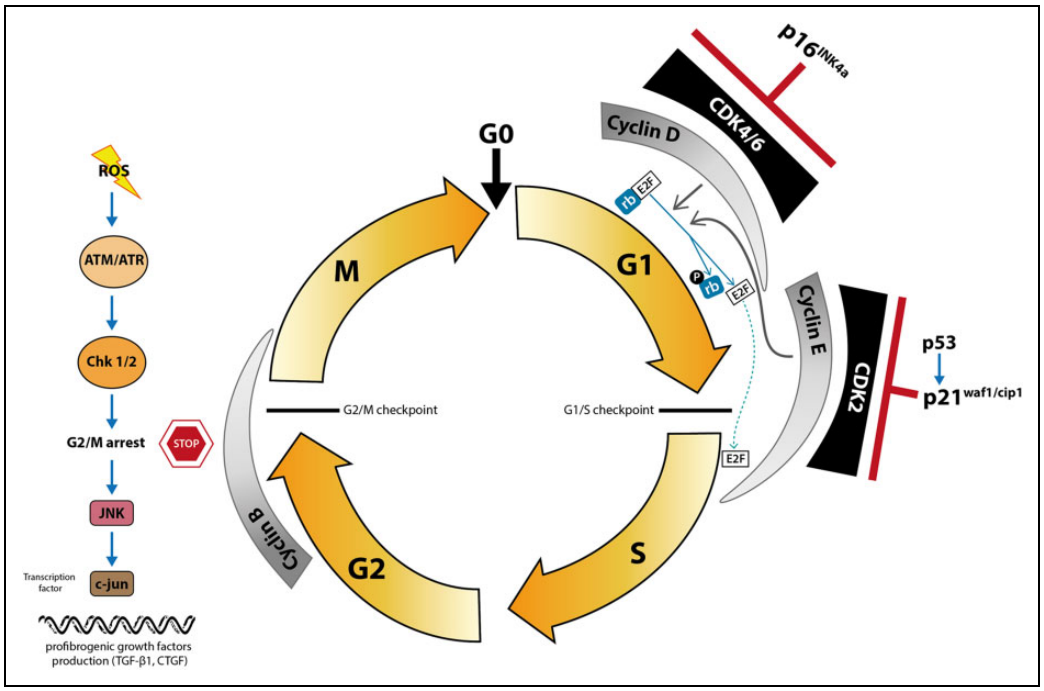

Fig. 1. Mechanism of cell cycle arrest induced fibrosis. In addition to the quiescent state (G0), the cell cycle includes 4 tightly controlled phases: G1, S (DNA synthesis), G2, and M (mitosis). Cyclin D and cyclin E are cell cycle regulatory proteins synthesized in the G1 phase (in its early and late portions, respectively) but degraded when the cells enter the S phase. Cyclin B is required for progression from G2 to M, and an increased cyclin B/cyclin D ratio might represent accumulation of cells in G2/M, which can occur in maladaptative repair. The $p16^{INK4a}$ and $p21^{Waf1/Cip1}$ proteins bind to cyclin-dependent kinase–cyclin heterodimers, causing cell cycle arrest in the G0 to G1 phase, inhibiting cell proliferation. Hypoxia and reactive oxygen species may activate protein kinases Chk1 and Chk2 through ataxia telangiectasia mutated/ataxia telangiectasia and Rad3-related protein kinase signaling pathway, promoting cell cycle arrest in G2/M checkpoint. An excess of G2/M-arrested cells activates the Jun N-terminal kinase pathway, increasing levels of the transcription factor c-jun, which upregulates profibrotic cytokine production.

The p21 gene is induced after DNA damage, leading to activation of p53 and induction of $p21^{Waf1/Cip114}$. After the initial insult, there is a rapid upregulation of $p21^{Waf1/Cip1}$ in the kidney, although not of other Cip/Kip family members such as p27 and p57[20]. The upregulated expression of $p21^{Waf1/Cip1}$ observed in different models of AKI[20,21] prevents DNA-damaged cells from entering the cell cycle by directly inhibiting CDK2 activity[22], thus avoiding cell death by necrosis or apoptosis[21]. In fact, p21 knockout mice induced to AKI show increased susceptibility to ischemia and nephrotoxins, characterized by more severe renal function impairment, morphologic changes, and overall mortality[21,23]. Once activated, $p21^{Waf1/Cip1}$ can promote protection against a subsequent renal insult[24]. $p21^{Waf1/Cip1}$ proteins have a wide spectrum of activities, depending on the cell type and the circumstances of their induction[14]. In addition to the beneficial effects on tubular cells, $p21^{Waf1/Cip1}$ might play a role in enhancing the progression to CKD by inducing TGF-β production, ultimately leading to fibrosis[25], as reported for other cell cycle inhibitory factors. In the renal ablation model, a lack of $p21^{Waf1/Cip1}$ diminishes cell cycle arrest, avoiding long-term renal dysfunction and interstitial fibrosis[26].

The INK4 family consists of 2 proteins: $p16^{INK4a}$, a cyclin kinase inhibitor, and $p19^{ARF}$, a p53 stabilizer. $p16^{INK4a}$ binds to the CDK4/6 kinase subunit of cyclin D, causing cell cycle arrest in the G0 to G1 phase, thus reducing the proliferation of tubular epithelial cells. In AKI models, the absence of $p16^{INK4a}$ promotes regenerative cell proliferation and better outcomes after kidney injury[27,28].

Cellular senescence is characterized by permanent growth arrest, accompanied by characteristic morphological remodeling and metabolic changes, with a pro-inflammatory secretome phenotype, referred to as the senescence-associated secretory phenotype or senescence-messaging secretome, as described by van Deursen[18]. Senescent cells stain positive for senescence-associated activity of β-galactosidase (β-gal).

The role of the cell cycle inhibitory proteins $p21^{Waf1/Cip1}$ and $p16^{INK4a}$-Rb as potent early cell cycle arrest mediators is considered a crucial mechanism of cellular senescence. In cultured cells undergoing senescence, $p21^{Waf1/Cip1}$ protein has been shown to be overexpressed[29]. On the other hand, decreased expression of $p16^{INK4a}$ and p19 results in decreased senescence with extended cell life span. Studies involving human kidney biopsy samples have shown that $p16^{INK4a}$ expression is low to undetectable in young individuals, whereas it is markedly increased in adult and elderly individuals[30]. Because the levels of INK4a proteins increase

with age, they are recognized as biomarkers of aging[31]. Therefore, $p16^{INK4a}$, like β-gal, is considered a biomarker of cellular senescence.

Both aging and AKI can upregulate the expression of cell cycle inhibitory proteins, such as $p21^{Waf1/Cip1}$, p53, and $p16^{INK4a}$, blocking the cell cycle and thus promoting cell cycle arrest[32]. However, the exact mechanisms involved in AKI, which is apparently a transient and reversible process, with possible late changes, are still largely unknown. Senescence, rather than representing a static end point, seems to be a dynamic process. It has long been known that, although $p16^{INK4a}$ expression leads to cell cycle arrest within 24 h after induction, sustained $p16^{INK4a}$ expression (for $\geq$6 d) is required in order to induce senescence in human cells[33]. In our previous studies using the IRI model, we have shown overexpression of $p16^{INK4a}$, $p21^{Waf1/Cip1}$, and TGF-β within 2 d after the ischemic insult, as well as that the expression of β-gal and $p16^{INK4a}$ remains high in ischemic animals, even at 7 d after of the insult[34]. Therefore, it is likely that AKI, through cell stress and DNA damage, triggers cell cycle arrest, leading to a sustained process of senescence. Post-AKI senescence could, therefore, be a consequence of maladaptative repair[35,36].

Yang et al. demonstrated that distinct types and severity of kidney injury can behave differently regarding cell cycle arrest[8]. When moderate, reversible IRI is induced, mice present abrupt renal dysfunction, although kidney function returns to normal levels in 7 d. That model of AKI results in transient cell cycle changes, with increased numbers of G2/M phase cells only from day 1 to day 5. The authors showed that mice thus induced to AKI do not present kidney interstitial fibrosis in the long term[8]. Kidney injuries that are more severe such as severe IRI and nephrotoxic insult (acute aristolochic acid toxic nephropathy) feature abrupt renal dysfunction and delayed recovery. In those models of AKI, G2/M-arrested cells are prominent in the long term, persisting in large numbers up to day 42, and leading to significant interstitial fibrosis in multiple organs[8].

An excess of G2/M-arrested tubular cells represents the maladaptative response to AKI because such cells can activate mitogen-activated protein kinase pathways, such as the Jun N-terminal kinase (JNK) pathway, leading to increased levels of the transcription factor c-jun, which ultimately upregulates profibrotic cytokine production[8], as illustrated in Fig. 1. The evidence that interstitial fibrosis is not merely a consequence of kidney dysfunction that is more severe comes from models of unilateral kidney injury. When unilateral IRI or unilateral ureteral obstruction is employed, there is no kidney dysfunction as defined by the serum creatinine level. However, the affected kidneys develop persistent proximal tubule G2/M populations, which remain large until at least 1 mo after the insult. Even in the absence of initial renal dysfunction, such kidneys develop pronounced interstitial fibrosis over time[8]. In addition, inhibition of the ATM gene reduces G2/M arrest and inhibits JNK, which reduces the influence of downstream pathways activated by G2/M-arrested cells, thus decreasing profibrogenic factor production. Inhibition of p53, which can permit cell cycle progression, might also diminish renal fibrosis after AKI. Consistently, induction of G2/M arrest with drugs (such as a CDK1 inhibitor or paclitaxel, a microtubule stabilizing agent) increases profibrogenic gene expression, and that condition can reverse as the drugs wash out[8]. In mice null for proteins that promote the G2/M transition, recovery after AKI is delayed and there is significant interstitial fibrosis[37].

Deficiency of the Klotho Gene as a Mechanism of AKI-induced Premature Senescence

In 1997, Kuro-o et al. identified an aging-suppressor gene designated Klotho. The authors demonstrated that disruption of this gene in mice, which leads to reduced Klotho protein expression, induces diverse aging-associated features such as a shortened life span, growth retardation, skin atrophy, hearing loss, reduced cognitive function, decreased bone mineral density, and premature arteriosclerosis[38]. In contrast, overexpression of the Klotho gene in transgenic mice has been associated with a longer life span[39]. The Klotho gene is also involved in human aging, having been associated with longevity[40]. Serum protein levels of Klotho decrease with age, and low levels are considered early predictors of atherosclerosis[41]. Low levels of circulating Klotho protein have also been identified in humans and animals with CKD[42,43].

The Klotho gene is predominantly expressed in the kidney, specifically in the kidney tubules (distal and proximal convoluted tubules)[38,44,45]. Cell lines derived from the inner medullary collecting duct also express the *Klotho* gene. In addition, the kidney represents an important target of the Klotho protein, which has a broad range of renal effects, including regulation of renal 1,25-$(OH)_2$-vitamin D_3 production, as well as phosphate, calcium, and potassium homeostasis (Fig. 2).

Klotho is a transmembrane protein, expressed on the cell surface, that interacts with the fibroblast growth factor (FGF) receptor as a co-receptor for FGF-23 (Fig. 2). Alternative cleavages of transmembrane Klotho generate cleaved (130 kDa) Klotho, which is released in blood and urine, and secreted (65-kDa) Klotho, which can have multiple functions[46] (Fig. 2). Phosphate homeostasis is regulated by the interaction of Klotho protein with FGF-23, which induces renal phosphate excretion (phosphaturia). Phosphate homeostasis likely plays an important role in the aging process, an assumption supported by reports that control of hyperphosphatemia in Klotho-deficient (*kl*/+) and FGF-23-deficient mice can prevent premature aging syndrome[47].

Several other mechanisms have been shown to play a role in Klotho depletion-induced cell senescence. Knockdown of endogenous Klotho promotes augmentation of senescence in the cultured cells. Administration of exogenous Klotho significantly decreases senescence in the endothelial cells[48]. Klotho deficiency also enhances cell senescence by

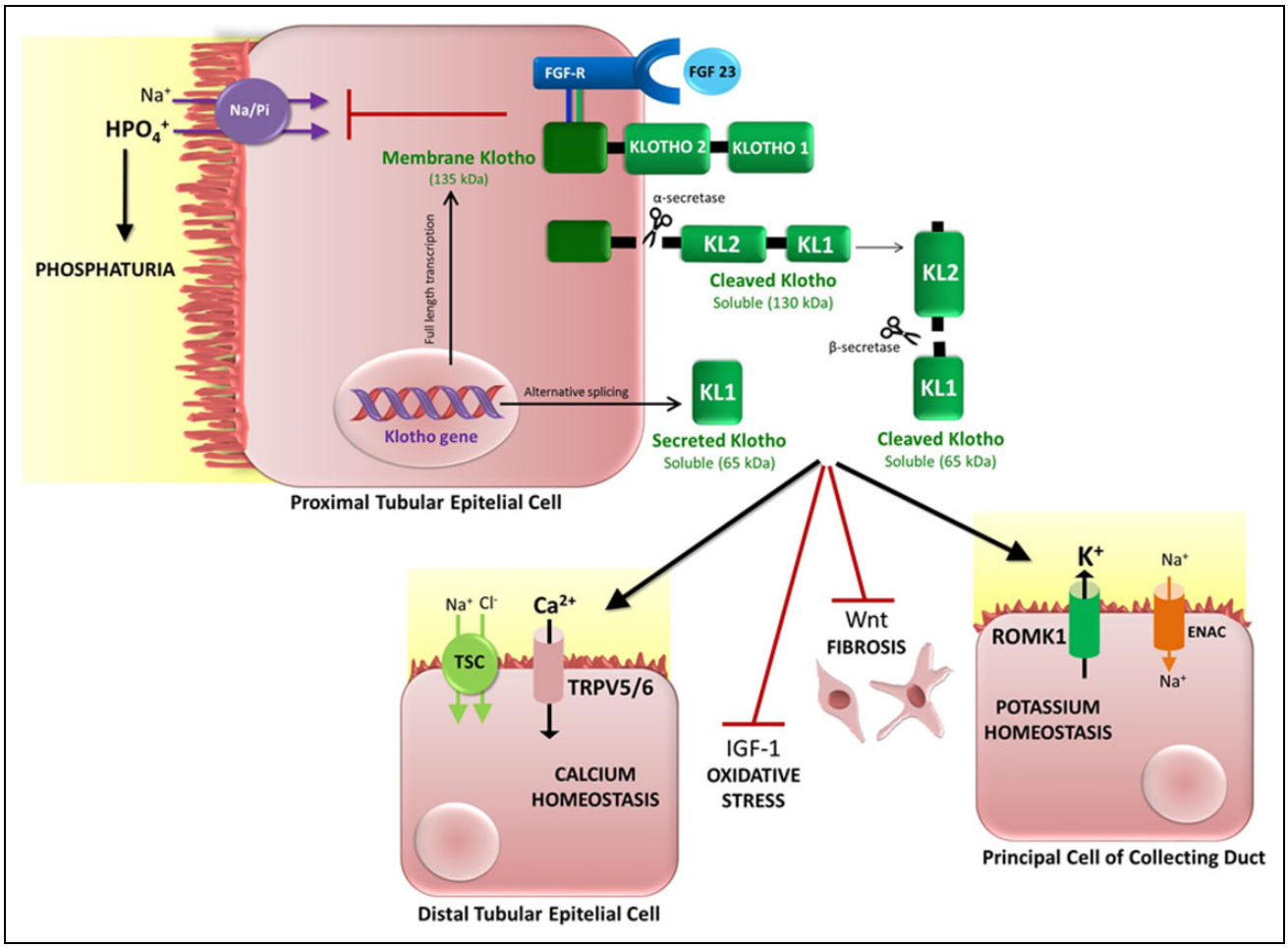

Fig. 2. Main functions of the Klotho protein. Membrane Klotho is a 135-kDa transmembrane protein that interacts with the fibroblast growth factor receptor (FGF-R) as a co-receptor for FGF-23, regulating renal phosphate excretion and metabolism. Once cleaved by α-secretases, membrane Klotho becomes a soluble, 130-kDa peptide that is released from the cell surface, subsequently entering the bloodstream and the urine. At these sites, 130-kDa Klotho can undergo further cleavage by β-secretases, producing 65-kDa soluble peptides. Alternative splicing of the Klotho gene also creates a 65-kDa soluble Klotho, known as "secreted Klotho," which is released into the bloodstream and urine with no need for enzymatic cleavages. All forms of soluble Klotho develop paracrine and systemic functions: In the distal nephron, soluble Klotho activates the transient receptor potential cation channel subfamily V (TRPV) calcium channels TRPV5 and TRPV6, increasing calcium reabsorption, and the renal outer medullary I potassium channel, increasing potassium secretion. In addition, Klotho inhibits Wnt signaling activity, thus contributing to the blocking of fibrogenic mechanisms. Klotho protein also regulates the oxidative stress through insulin growth factor I (IGF-I) signaling. Inhibition of IGF-I permits forkhead box O3 action, elevating levels of the antioxidant enzymes manganese superoxide dismutase and catalase.

increasing oxidative stress[49]. The biological activity of Wnt in Klotho knockout mice may contribute to increasing senescence in progenitor cells as described by Liu et al[50].

Klotho is an antiapoptotic protein, as demonstrated in human umbilical vein endothelial cells[51]. In the kidney, Klotho deficiency significantly increases apoptosis[49]. In contrast, enhancement of Klotho expression by genetic manipulation decreases the number of apoptotic cells, as well as improving renal function and morphology, after acute and chronic kidney damage[49,52].

In a mouse model of IRI-induced AKI, Hu et al. found reduced Klotho expression in the kidneys as well as in urine and blood, although Klotho expression was restored upon recovery[53]. Reductions in kidney and plasma levels of Klotho occur earlier than does the production of neutrophil gelatinase-associated lipocalin (NGAL), a known biomarker of kidney injury[53]. Patients with AKI show drastic reductions in urinary Klotho levels. To determine whether Klotho plays a pathogenic role, the authors induced ischemic AKI in mice with different levels of endogenous Klotho expression, ranging from heterozygous Klotho-haploinsufficient mice to wild-type mice and transgenic mice overexpressing Klotho[53]. In comparison with what was observed in wild-type mice, the levels of Klotho protein during AKI were found to be lower in haploinsufficient mice and higher in transgenic mice. The authors also found that the haploinsufficient mice showed functional and histological alterations that were more extensive than those seen in the wild-type

mice, whereas those changes were milder in the transgenic mice, implying that Klotho is renoprotective. Among the rats with AKI, those receiving recombinant Klotho showed higher Klotho protein expression, less kidney damage, and lower NGAL levels than did the vehicle-treated control group[53]. Therefore, Klotho deficiency likely renders the kidney more susceptible to injury, accelerates renal fibrogenesis, retards renal tissue regeneration, and, in some cases, promotes chronic progression. Klotho supplementation can provide benefits, preventing or slowing the progression to CKD and renal senescence.

Telomere Shortening

Telomeres are noncoding nucleotide TTAGGG sequences repeated at the end of the chromosomes, acting as defensive caps that protect genetic material from damage and consequently from cell death triggered by DNA repair pathways. Telomerase is the enzyme responsible for maintaining telomeres. Most human cells do not express telomerase, the exceptions being highly proliferative cells such as germ cells, skin cells, and bone marrow[54]. At each cell division, some base pairs are lost and telomeres shorten progressively[55,56]. Therefore, telomere length declines with age, characterizing replicative senescence. Activation of DNA repair pathways triggered by telomere shortening can enhance $p21^{Waf1/Cip1}$ and $p16^{INK4a}$ to halt proliferation and minimize replicative stress[53,57,58]. Telomere attrition causes chromosome instability, cellular senescence, and apoptosis[59].

In addition to replicative senescence, cells can become senescent after a noxious stimulus, and this stress-induced senescence can lead to telomere shortening[59]. In short-term evaluations, telomere shortening is not a feature of ischemic AKI in the first 2 d after the insult despite the presence of greater oxidative stress[34]. However, renal IRI can lead to significant telomere shortening at 30 d after AKI induction, that shortening being more pronounced in telomerase-deficient mice[60]. Telomere attrition and telomerase deficiency have been shown to aggravate the course of kidney injury[60]. In aging kidneys, the increased susceptibility to injury and lack of sufficient repair might involve telomere shortening and other mechanisms of increased senescence. Although telomerase-deficient mice can present the same degree of AKI severity as do their wild-type littermates after IRI, the recovery of renal function is significantly delayed in the former[60,61].

Other Mechanisms

Oxidative injury can be a mechanism involved in stress-induced cell senescence. In the kidney, mitochondrial electron transport chain and reduced nicotinamide–adenine dinucleotide–ubiquinone oxidases are the main sources of reactive oxygen species (ROS)[62]. The mitochondria constitute a target of ROS effects, because mitochondrial dysfunction and loss of mitochondrial membrane could be consequences of oxidative stress, resulting in altered mitochondrial permeability and release of cytochrome c, thus inducing cell death[63]. Highly reactive oxygen molecules can cause significant modification of lipids, DNA, and proteins[64]. In addition, oxidative stress can activate injury mitogen-activated protein kinase pathways as well as p53 and $p21^{Waf1/Cip165}$, leading to higher cell apoptosis rates, activation of inflammation, and the development of stress-induced senescence. Autophagy pathways become activated[66], resulting in increased cell expression of β-gal[67]. In mitochondrial dysfunction, respiratory chain disruption results in superoxide overproduction[65,68]. In that context, the mitochondrial antioxidant manganese superoxide dismutase (MnSOD) is particularly important for promoting cytoprotection[69]. In ischemic AKI, the antioxidant enzyme heme oxygenase-1 is stimulated, which increases MnSOD levels. In models of AKI, the disease is more severe and mortality is higher in mice lacking heme oxygenase-1 than in wild-type mice[70]. However, AKI can also make heme oxygenase-1 unable to stimulate MnSOD[34], thus impairing ROS scavenging. Aging mesangial cells exhibit downregulation of the mitochondrial antioxidants MnSOD and thioredoxin reductase 2, accompanied by upregulation of the microRNA (miR)-335 and miR-34a, together with high levels of ROS. Overexpression of miR-335 and miR-34a can induce oxidative-stress related premature senescence of young mesangial cells, whereas antisense miR-335 and miR-34a inhibit senescence of old mesangial cells[71]. Antioxidant compounds, such as N-acetylcysteine, can delay stress-induced senescence[72,73].

Another pathway that could be involved in renal senescence after AKI is inflammation. Inflammation is a multifactorial response that is needed in order to eradicate harmful pathogens and mediate tissue repair after injury. However, excess unresolved inflammation can promote fibrosis, tissue damage, and early senescence[74]. The release of cytokines and neutrophil/macrophage recruitment to the site of injury are considered hallmark features of the early inflammatory response, which is followed by adaptive immunity cell recruitment in later stages. Recent studies have suggested that T cells also participate in early inflammatory responses in AKI[75]. The recruitment of immune effector cells is facilitated by the upregulation of adhesion molecules in various cell types within the kidney[76]. It has been suggested that the immune signature of inflammation during IRI has many similarities with that of inflammation occurring in response to a microbial pathogen[77]. Cellular damage and its associated molecular products are thought to be key triggers of inflammation after acute tissue injury. Necrotic cells present damage-associated molecular patterns in the extracellular spaces, which subsequently activate pattern recognition receptors, such as Toll-like receptors and pyrin domain-containing 3 inflammasome, which are expressed in epithelial cells, endothelial cells, dendritic cells, macrophages, and lymphocytes[78,79]. Activated renal parenchyma cells and

dendritic cells also secrete chemokines, and changes in the expression of pro-inflammatory and anti-inflammatory mediators by resident and recruited cell populations are important determinants of the injury and repair phases[80]. A balance between pro-inflammatory and anti-inflammatory factors is extremely important for tissue repair. However, AKI often results in an abnormal repair process resulting from sustained secretion of profibrotic cytokines and leading to post-AKI fibrosis and kidney senescence[78]. Virtually, all immune cells have been implicated in AKI, some—such as neutrophils, monocytes/macrophages, dendritic cells, natural killer T cells, natural killer cells, and B cells—being thought to be deleterious, whereas others—such as Tregs—are likely protective[81]. M1 macrophages contribute to inflammation and tissue injury in the injury phase, whereas M2 macrophages exert anti-inflammatory effects in postischemic kidneys and facilitate renal tubular regeneration during the recovery phase. In addition, increased numbers of activated and effector memory T cells have been found in the postischemic kidneys as late as 6 wk after IRI, suggesting that T cells are also involved in long-term structural changes in postischemic kidneys[82]. Numerous studies have suggested that a robust inflammatory process engaging innate and adaptive immune responses causes initial renal injury and mediates long-term structural changes including interstitial fibrosis or repair. It is known that acute injury results in microvascular damage and vessel loss in the kidney and that such changes are typically persistent. Various studies of biopsies of renal transplants have suggested that ischemia imposes early sustained loss of peritubular capillaries in the transplanted graft. The loss of peritubular capillaries might represent a single, common pathway toward progressive damage and senescence[83].

Autophagy is a cell process triggered by lysosome-mediated degradation of damaged organelles and plays a protective role after cell stress situations. This process can be affected by aging, autophagy having been shown to be impaired in aged murine macrophages[84]. Removal of damaged organelles and protein aggregates occurs when those substrates are circumscribed into double-membrane vesicles called autophagosomes, which subsequently fuse with lysosomes[62]. Formation of an autophagosome requires the coordinated action of several protein complexes such as the so-called autophagy-related proteins and phosphatidylinositide-3 kinase. Successful autophagosome formation can be marked by increased expression of the protein light chain 3-II[85]. ROS can trigger mitochondrial autophagy[86], although extremely severe insults can lead to lysosomal permeabilization, impairing autophagic mechanisms[62], and clearance of damaged organelles indirectly inhibits excessive ROS production, protecting injured tissues[62]. The p62 protein recognizes cellular waste and initiates an autophagy response in cells[87]. There is evidence of p62-dependent Kelch-like ECH-associated protein 1 (Keap1) degradation that enhances nuclear factor (erythroid-derived 2)-Like 2 (Nrf2) to exert antioxidant effects[88]. Although other molecules from apoptotic pathways, such as those in the B-cell lymphoma-2 family, hypoxia-inducible factor (HIF), and p53, can induce autophagy[89], the mechanisms involved in triggering this process in AKI are still unknown[85]. The mammalian target of rapamycin (mTOR) pathways can also modulate autophagy, the mTOR complex 1 (mTORC1) pathway inducing autophagy and the mTORC2 pathway acting as a negative regulator of autophagy in response to starvation. However, in the setting of AKI, the mTORC2 pathway can induce autophagy by activating serine/threonine kinase signaling, thus promoting cell survival as well as protecting against tubular cell apoptosis and AKI.

Autophagy is induced in response to kidney injury. In septic AKI, autophagy increases in the early stages, although the decline that follows is associated with proximal tubular dysfunction[90]. Inhibition of the autophagy pathway aggravates necrosis, apoptosis, and kidney injury in short-term evaluations after ischemia reperfusion[91]. In addition, autophagy can reduce the level of mature TGF-β1, which would theoretically suppress kidney fibrosis[92]. However, in long-term evaluations, a lack of autophagy can promote better kidney healing after ischemic injury[91]. Dramatically injured cells typically do not survive after noxious stimulus; if they do, maladaptative repair, a pro-inflammatory state, and the senescent phenotype usually follow. Healthy healing occurs when potentially viable cells proliferate and contribute to tubular repair. Thus, autophagy can protect cells from death in an acute scenario, although it might subsequently allow more fibrosis and senescence. In fact, the enzyme β-gal, which is expressed in autophagy activation, is a hallmark of cell senescence.

AKI as a Condition for Progression to CKD

Clinical data show that patients with AKI, even after complete recovery, progress to a decline in renal function that is more pronounced than that occurring in individuals without a history of AKI[93]. Patients who survive AKI are 9 times more susceptible to developing CKD and 3 times more likely to develop end-stage renal disease (ESRD) than are those with no history AKI or of requiring dialysis during hospitalization[94,95]. A single AKI episode increases the risk of ESRD by 28 times and doubles the mortality risk[96]. It is estimated that nearly one fourth of the increase in ESRD prevalence between 1988 and 2002 was attributable to AKI[97].

CKD is an independent predictor of AKI development in intensive care patients[98], and acute illness will frequently accelerate progression of preexisting CKD. It is possible that, if the acute injury develops in CKD patients with minimal renal reserve, in whom repair mechanisms are already disabled, the progression to ESRD will occur more rapidly, even if the adaptive repair occurs after AKI. Otherwise, maladaptive repair can also trigger fibrosis and capillary rarefaction, thus rapidly worsening the preexisting CKD[7].

Although fibrosis is not necessarily progressive, fibrotic tissue can reduce renal mass and functional reserve and can even can induce systemic hypertension[99]. Therefore, the additional loss of renal mass caused by unsuccessful repair of AKI can trigger hemodynamically mediated processes that will damage the nephrons, thus hastening the progression to CKD[100]. Shear stress in the remaining nephrons can result in further loss of glomeruli, and marked proteinuria demonstrates significant podocyte injury. Activation of the mTORC2 pathway and consequent activation of serine/threonine kinase 2 can protect podocytes from apoptosis and foot process effacement, promoting podocyte survival and perhaps further slowing the progression to CKD[101]. Other well-established pathways of progression in CKD, such as the renin–angiotensin–aldosterone axis, probably play an important role in determining post-AKI progression to CKD. In fact, some studies have demonstrated that inhibition of renin–angiotensin–aldosterone can confer protection against CKD progression in the setting of AKI[102–104].

In AKI, the behavior of HIF constitutes an important mechanism. It can be protective, given that HIF activation before the induction of AKI has been shown to reduce the degree of kidney injury in experimental models[105]. However, when chronic kidney hypoxia is already present, as it is in CKD, activation of HIF can actually exacerbate tissue injury[106]. Therefore, preexisting CKD can contribute to the exacerbation of fibrosis in some AKI scenarios.

Klotho deficiency activates the expression of Wnt, activating the tubulointerstitial renal fibrosis process[107]. Activation of Wnt/β-catenin signaling due to Klotho deficiency can arrest cells at the G2/M phase of the cell cycle, inducing the production of TGF-β and connective tissue growth factor[108].

It is known that aging individuals and CKD patients are more susceptible to CKD progression after AKI. Individuals in either situation are in a state of Klotho deficiency. The decrease in Klotho expression is dependent on the severity of the insult (duration of ischemia) and on the time since the insult[109], demonstrated that, in their ≥30-min bilateral IRI model (Bi-IRI group) and unilateral nephrectomy plus contralateral IRI model (Npx-IRI group), Klotho expression normalized by day 10 after ischemia–reperfusion injury, decreased again around day 14, and declined even further by day 28. Although renal Klotho (protein and messenger RNA [mRNA]) expression was reduced in both models, renal Klotho protein expression at 20 wk after IRI was slightly higher in the Bi-IRI group mice. Kidney histology showed glomerular collapse, tubular dilation, tubulointerstitial infiltration, and fibrosis. The levels of fibrotic markers (α-smooth muscle actin, connective tissue growth factor, and collagen I) showed a robust increase in the Npx-IRI group and a less severe but still significant increase in the Bi-IRI group. Therefore, at 20 wk, there was a difference in severity between the Npx-IRI and Bi-IRI models, although all of the animals had CKD with predominant fibrotic components. The authors also applied the Bi-IRI model in *kl*/+ mice and mice of the transgenic mouse line Tg-Kl (in which there is ubiquitous overexpression of mouse Klotho, with Klotho levels approximately 150% of normal). Notably, they found that, at 20 wk, renal Klotho protein levels in the Tg-Kl mice were comparable to those seen in the wild-type mice not subjected to AKI, although they were much lower in the *kl*/+ mice than in the wild-type mice. The authors also reported that, in comparison with what they observed in the wild-type mice, renal α-smooth muscle actin, connective tissue growth factor, and collagen I were lower in the Tg-Kl mice and higher (consistent with renal fibrosis) in the *kl*/+ mice. Therefore, *kl*/+ mice have a higher risk of developing fibrosis (similar to that of aging individuals and patients with CKD). In a previous study, our group also demonstrated that, by day 49 after IRI, senescence was more apparent in the rats submitted to IRI alone than in those submitted to IRI and treated with human umbilical cord–derived mesenchymal stem cells (MSCs), Klotho expression being significantly lower in the former group[34].

Cell Therapy Perspectives for the Treatment of Premature Renal Senescence

There is a great body of evidence showing that the pathophysiological mechanisms triggered after AKI-induced cell damage are associated with activation of the senescence machinery. There is yet no effective treatment for AKI, with supportive therapy being the only option.

In this context, adult stem cells—derived from the bone marrow, from other sources, or even from the kidneys—can play a role in this healing process, creating prospects for new therapeutic approaches such as cell therapy. The potential regenerative properties of stem cells have opened opportunities for investigation of the potential beneficial effects of the administration of stem cells in improving the renal outcomes of AKI in animal models[110,111].

The bone marrow was the first tissue to be explored as a source of stem cells. Although studies have shown that bone marrow–derived stem cells can engraft into the kidney and participate in normal tubular epithelial cell turnover and repair after AKI, the main mechanism involves paracrine effects rather than cell transdifferentiation[111]. It has become quite clear that the expected potential ability of stem cells to transdifferentiate into renal cells and replace damaged cells is a rare phenomenon.

Besides hematopoietic stem cells, the bone marrow contains MSCs that have been shown to provide a wide range of beneficial effects when administered in different experimental models of kidney diseases and in some clinical trials[112,113]. Adipose-derived MSCs (ADMSCs) have also become a very attractive source of MSCs, with regenerative capacity similar to bone marrow–derived MSCs. ADMSCs have additional advantages considering that their harvesting is minimally invasive, there are no ethical issues regarding their use, and there are fewer safety concerns. Furthermore, some authors have suggested that ADMSCs have more robust anti-inflammatory and immunomodulatory effects than do bone marrow–derived MSCs[114,115].

Table 1. Cell Therapy and Senescence Markers in Kidney Tissue.

Model	Recipients	Cell Treatment	Senescence Markers	Klotho	Ref.
AKI-IRI	Adult rats	WJ-hMSCs	↓ p16 ↓ p21 ↓ TGF-β ↓ β-galactosidase ↓ miR-29a ↓ miR-34a	↑	Rodrigues et al.[34]
Aging	Old mice	Old BMCs	↑ Collagen IV ↑ PAI-1 ↓ PDGF-B	↓	Yang et al.[127]
Aging	Old mice	Young BMCs	↓ Collagen IV ↓ PAI-1 ↑ PDGF-B	↑	Yang et al.[127]

Abbreviations: AKI, acute kidney injury; IRI, ischemia–reperfusion injury; WJ-hMSCS, Wharton's jelly-derived human mesenchymal stem cells; BMCs, bone marrow–derived cells; PAI-1, plasminogen activator inhibitor-1; PDGF-B, platelet-derived growth factor subunit B; microRNA, *miR*.

It is well known that MSCs possess the ability to secrete a variety of soluble factors in a paracrine fashion[116]. Experimental studies have shown that MSCs can ameliorate kidney injury through different mechanisms, such as anti-inflammatory, antiapoptotic, and other immunomodulatory effects, besides enhancing angiogenesis. Alternatively, horizontal cell-to-cell communication may occur through the release of extracellular microvesicles derived from stem cells, which can mediate the transfer of proteins, mRNA, miR, and other molecules[117], with potential application for therapeutic intervention.

A number of studies have shown that MSCs are efficient in recovering renal function in experimental models of AKI[34,118–125]. The significant findings of small animal studies served as the basis for preclinical and clinical trials. A phase I clinical trial was designed to determine whether the administration of allogeneic MSCs is safe in patients who are at a high risk of developing AKI after undergoing on-pump cardiac surgery[112]. Preliminary data from that trial show that kidney function was preserved for up to 16 mo and that none of the patients required dialysis. Another phase I study involving three patients who developed AKI after cisplatin treatment for a solid tumor demonstrated that intravenous injection of autologous MSCs was safe and improved renal function (NCT01275612). Nevertheless, additional clinical studies are needed in order to show the real benefit of stem cells for post-AKI recovery of renal function.

Another key aspect of regeneration in which stem cell therapy could play a role is avoiding senescence. As discussed above, AKI likely induces cell aging, possibly through mechanisms closely related to cell cycle arrest and Klotho downregulation. In this setting, it is of note that umbilical cord–derived MSCs are more effective in reducing the expression of cell cycle inhibitors than do bone marrow–derived MSCs or ADMSCs[126]. In experimental IRI, our group has recently reported that treatment with umbilical cord–derived MSCs, besides ameliorating kidney function, downregulates the upregulated expression of senescence markers (β-gal, $p21^{Waf1/Cip1}$, and $p16^{INK4a}$) and miRs (miR-29a and miR-34a)[34]. In comparison with adult ADMSCs, young (postnatal) cells appear to be more effective in the treatment of AKI[34]. Our study also showed that Wharton's jelly-derived MSCs have a superior capacity to increase Klotho protein levels and therefore to protect the tissue against senescence when compared with ADMSCs. In mechanistic terms, this study showed that Wharton's jelly-derived MSCs protect renal tissues against senescence resulting from oxidative stress, as well as increasing the renal expression of Klotho protein and MnSOD (Table 1).

There is also some evidence that young cells can restore youthful features in aged tissues, possibly due to some regenerative factors that are not present in older cells. In parabiosis studies involving heterochronic couples (aged and young animals that share a common circulation), the proliferative and regenerative capacity of aged tissues has been shown to improve when they are put in contact with young tissues[128]. These youth-associated factors can be RNAs, and some miRs that are responsible for the inhibition of antioxidants and consequently heightening of oxidative stress can promote the cell senescence phenotype, because the anti-miR effects can direct older cells back toward the young phenotype[71]. In addition, when aged mice are exposed to a lethal dose of radiation and then treated with bone marrow–derived cells, age-matched cells, or cells from young donors, differences can be observed among the groups. Recipients of cells from young donors are more successful in maintaining a younger phenotype, whereas recipients of age-matched cells show higher renal expression of senescence-related proteins (such as β-gal, plasminogen activator inhibitor-1, platelet-derived growth factor subunit B, fibroblast-specific protein-1, $p21^{Waf1/Cip1}$, and $p16^{INK4a}$), elevated deposition of collagen IV in the mesangium, and low Klotho expression[127], as shown in Table 2.

In fact, the younger the cells are, the more youth-related factors are present. A comparison among cells harvested from the umbilical cord, from neonates at fourth day after birth, from their mothers, and from adult volunteers showed that levels of soluble Klotho are markedly more elevated in the cells harvested from the umbilical cord. Furthermore, we

Table 2. Expression of Markers of Aging by Stem Cell Source.

Source of Stem Cells						
Markers of Aging	Umbilical Cord	Neonates	Mothers	Adult Volunteers	Adipose Tissue	Ref.
Soluble α-Klotho	↑↑↑	↑	↑	↑	—	Ohata et al.[129]
Klotho	↑↑↑				↑	Rodrigues et al.[34]
Cell cycle inhibitors (p16, p21, and p53)	↑				↑↑	Jin et al.[126]
β-galactosidase	↑				↑↑	Rodrigues et al.[34]

found that umbilical cord–derived MSCs presented more Klotho expression than did ADMSCs (see Table 2).

Hematopoietic cells derived from umbilical cord blood present longer telomeres than do bone marrow–derived cells, and studies of hematopoietic stem cell transplantation have shown that telomeres are longer in recipients of umbilical cord cells than in recipients of cells from other sources[54]. That would lead us to think that longer telomeres could be a benefit of using younger cells in cytotherapy. However, comparable telomere lengths have been demonstrated in MSCs from different sources such as dental papilla tissue, umbilical cord matrix, and adipose tissue[130].

Telomerase activity presents a noncanonical enhancing activity of antioxidant enzymes such as SODs and catalases, conferring protection against oxidative stress in a telomere-length independent function[131]. Nevertheless, telomerase activity does not seem to differ between cells from young tissues and those from older tissues[130]. Therefore, it seems that, despite the fact that shorter telomeres and lower telomerase activity can confer a poorer prognosis in AKI, the mechanism by which young stem cells are better in repairing AKI does not seem to include telomere biology.

Some important studies have demonstrated that *kl*/+ mice show impaired progenitor cell function in various tissues including renal tissue. In the literature, there is convincing evidence that raising Klotho protein levels can ameliorate AKI and perhaps CKD[53]. Nevertheless, all of the studies involving Klotho have been conducted at the experimental level, and it is quite possible that upregulation of Klotho protein expression by exogenous Klotho can become a new therapeutic tool in kidney disease.

In this regard, stem cells/progenitor cells for kidney regeneration are more likely to target a cascade of mechanisms, whereas the use of one pharmacological agent targets only a limited number of pathogenic pathways. Therefore, perhaps a combination of the two could lead a better therapeutic approach. Further experimental and clinical studies, as well as phase I, II, and III trials, are needed. The use of younger MSCs, such as umbilical cord–derived MSCs and kidney progenitor cells (isolated from amniotic fluid or preterm neonate urine), could be an interesting alternative to the use of other tissue-specific renal progenitor cells[132,133] and could also play a role in kidney regeneration.

Conclusion

In conclusion, the post-AKI progression of renal function includes mechanisms of senescence activation. There is as yet no single therapy that has been shown to alter the outcome of AKI. Stem cell therapy might play a role as a treatment strategy and provide improved outcomes in the coming years.

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