The impact of aging on kidney repair

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Schmitt R, Cantley LG. The impact of aging on kidney repair. Am J Physiol Renal Physiol 294: F1265–F1272, 2008. First published 20 February 2008; doi:10.1152/ajprenal.00543.2007.—The process of normal aging affects organ homeostasis as well as responses to acute and chronic injury. In view of the rapid growth in the elderly population, it is increasingly important for us to develop a mechanistic understanding of how these age-dependent changes can impact the susceptibility and response of the kidney to injurious stimuli. In this overview, we focus on the current understanding of those mechanisms by reviewing how cellular changes in the aging kidney might lead to a diminished proliferative reserve, an increased tendency for apoptosis, alterations in growth factor profiles, and changes in potential progenitor and immune cell functions. A better understanding of these processes may help us to define new targets for studying kidney repair and could ultimately lead to new therapeutic strategies that are specifically tailored for treatment of the elderly population.

Tissue repair and regeneration are processes that are essential for maintaining the integrity and survival of complex organisms. The goal of all tissue repair is the elimination of potentially harmful defects and the reconstitution of organ function. However, some of the mechanisms required become less reliable with aging, resulting in a decrease in repair capacity. This functional decline in the potential to repair and regenerate is often considered a hallmark of the aging phenotype.

In patients with acute kidney injury (AKI), this is reflected in more prolonged and less successful functional recovery in the elderly. According to our recent meta-analysis, it is estimated that the population over the age of 65 yr is at a 20–30% higher risk of failing to completely recover renal function after surviving AKI (Schmitt R, unpublished observations). Similarly, kidneys from old organ donors have a higher risk of delayed graft function (DGF), which is indicative of delayed or unsuccessful repair after transplantation-induced AKI. According to the 2006 United States Renal Data System report, chances of DGF almost double when the transplant comes from a living donor above the age of 65 yr compared with a younger donor (99). At least in part, this can be regarded as a result of an impaired ability for renal repair (22).

The diminished repair ability of the aged kidney coincides with a significantly increased susceptibility of the old kidney to develop AKI. This has been observed in humans with AKI (65, 104) and has been corroborated in rodent studies of renal ischemia-reperfusion (I/R) which demonstrate significantly greater injury in older animals (71, 78, 81, 106). Mechanisms that are thought to contribute to this higher susceptibility include structural and functional changes in the renal vascular system leading to diminished perfusion (78) and a lower epithelial stress resistance that has been ascribed to an accelerated rate of ATP depletion due to mitochondrial alterations (1, 14, 58, 84). It has also been proposed that parenchymal loss in the aging kidney directly confers a higher susceptibility to acute damage, although this is not supported by experimental data in which the reduction of renal mass surprisingly protected against I/R injury in a rat model of 5/6 nephrectomy (100). As we gain more mechanistic insight into the complex events that occur during aging, it is clear that many of these changes not only increase the susceptibility to acute damage but also impact the repair phase after injury (14, 81).

In addition to the inherent differences in susceptibility of the aged kidney to injury, the systemic impact of the aging process often results in exposure of patients to renal stressors such as nephrotoxic drugs, invasive interventions, and systemic diseases (18). Combined, these factors result in a higher incidence of AKI in the aging population. Since the population over the age of 65 yr will more than double globally by 2025 (102), we can expect an important rise in the number of elderly patients with AKI. The current means of treating AKI remain limited, and there are no specific strategies to adapt treatment options to the aging patient. A better understanding of how the old kidney differs in its susceptibility and response to injury is a necessary first step toward providing preventative strategies and therapeutic treatments that are tailored toward the elderly.

Capacity for Renal Epithelial Cell Proliferation Declines with Aging

Each human kidney produces ~70 liters of glomerular filtrate/day containing over 1 lb of sodium chloride. Because this amount is almost entirely reabsorbed along the renal tubule, the kidneys of a 65-year-old person will have successfully reabsorbed over 1,600 m3 and almost 15 tons of sodium chloride. Despite this high work load, the adult kidney nephron
has a remarkably low rate of cellular turnover. At a given point in time, <1% of tubular cells are proliferating under normal conditions, and this declines further with aging (Schmitt R, unpublished observations and Refs. 11 and 5). This decline in cellular proliferation with aging is likely to result in a progressive loss of epithelial cells, and thus may contribute to the age-dependent macroscopic loss of renal parenchyma (2, 29).

In response to acute damage, the kidney normally has the ability to initiate a burst of cellular proliferation to repopulate and restore injured tubules (Fig. 1) (6). This can lead to full functional recovery even after extensive tubule denudation (23). The proliferative burst appears to decline with age, as indicated by diminished proliferation in aged mouse kidneys after I/R injury (81). The mechanism for this decrement in proliferation following injury is currently poorly understood. It is possible that the systemic milieu changes with aging, failing to deliver the appropriate antiapoptotic and/or proproliferative signals in response to the injurious stimulus. While there is evidence for such an interplay in organs such as skeletal muscle (see Function of Stem and Progenitor Cells Declines with Aging), it is also likely that there are cell-autonomous factors that play a role in this process.

If rodent proximal tubules are microdissected and grown in cell culture systems, they still show a very distinct age-dependent decline in proliferative potential (81). The underlying mechanism is not clear, but recent advances in our understanding of cellular senescence have provided insight into the molecular processes that might be involved. The term cellular senescence originally referred to the cessation of cell division after extended serial passage in culture. In contrast to other forms of cell cycle arrest, senescence is accompanied by specific functional and morphological changes and is believed to be irreversible (reviewed in Ref. 26). In a series of seminal papers, Halloran and colleagues (26, 52–54, 56) have shown that some changes that are associated with this classic in vitro form of senescence can also be found in the aging kidney where increased expression of p16INK4A and shortening of telomeres was demonstrated. Telomeres are repetitive DNA sequences at both ends of chromosomes that minimize the loss of genetic information during cell division. With each cell cycle, telomere repeats are lost because the DNA-polymerase does not replicate linear chromosomes to their very ends. Since most adult human cells lack the enzyme telomerase, which is responsible for de novo synthesis of telomere repeats, this process eventually leads to critical telomere shortening, resulting in cellular senescence via a pathway that appears to involve ataxia telangiectasia mutated (ATM)-mediated activation of p53/p21 (26, 52). Recent data showing a correlation of p53/p21 and renal cellular senescence are consistent with the suggested importance of this final pathway (38).

Fig. 1. Simplified sequence of events in young tubular epithelium after exposure to acute stress. Schematic depicts proximal tubular cells before injury (A), at the peak of injury (B) and during successful repair (C). Following stress, such as ischemia-reperfusion, epithelial cells in the proximal tubule can undergo 3 distinct fates: they either survive, they undergo apoptosis, or, if the damage is extensive, they undergo necrosis (B). Dead cells lift off the basement membrane and leave denuded areas behind. Surviving cells typically undergo dedifferentiation (loss of brush border, cell flattening, and redistribution of polarized proteins) that can be accompanied by the expression of proteins such as vimentin and Fsp-1 that normally characterize mesenchymal cells. Cells that survive the initial injury are faced with derangements in their cellular metabolism that contribute to the generation of reactive oxygen species (ROS). Depending on the balance of pro- and antioxidants, ROS can lead to mitochondrial injury and DNA damage. In conjunction with ROS that derive from infiltrating inflammatory cells, this can trigger activation of the intrinsic pathway of apoptosis. Antiapoptotic factors (e.g., Bcl2) counteract this mechanism. The surviving epithelial cell synthesizes reparative growth factors that act in an autocrine loop, while additional growth factors derive from extratubular cells (e.g., MSC, M2 macrophages). Growth factor signaling results in migration, de- and redifferentiation, and cell cycling (C). As they near confluence, the surviving cells redifferentiate and contribute to complete recovery of the tubular epithelial lining (A).
Telomere shortening is not the only mechanism that can lead to cellular senescence. For example, DNA from human kidneys demonstrates significant telomere loss with aging, whereas cells from rat kidneys express higher levels of telomerase and have considerably longer telomere lengths (53). Despite this, aging rat kidneys still display typical signs of senescence, such as high activity of senescence-associated β-galactosidase (52). In this instance, senescence may occur by a mechanism similar to the in vitro process of “stress-induced premature senescence” (SIPS). Exposure of cells to a variety of noxious stimuli such as X-rays, oxidative stress, and UV irradiation can lead to SIPS. This form of senescence is independent of telomere length but coincides in many cell types with an overexpression of the cyclin-dependent kinase (CDK) inhibitor p16INK4A (reviewed in Ref. 107). By inhibiting CDK4 and CDK6, p16INK4A keeps the retinoblastoma protein (pRb) in its active, hypophosphorylated state and thus prevents progression through the cell cycle.

p16INK4A is upregulated in epithelial and interstitial cells of the aging rodent and human kidney (52). Moreover, renal p16INK4A expression has been shown to increase in an age-independent fashion in chronic allograft rejection, ischemic injury, and glomerular disease (15, 16, 57, 86). It is possible that these forms of injury represent the in vivo equivalent of SIPS. Most recently, Melk et al. (55) have demonstrated that p16INK4A deficiency improves renal graft outcome by promoting higher rates of proliferation and better regeneration. The observation that both aging and cellular stress result in the upregulation of p16INK4A and subsequent cellular senescence suggests that p16INK4A may be an ideal therapeutic target for regenerative improvement in the aging kidney.

Another member of the CDK inhibitor family which acts as an activator of the senescence pathway is p21, also known as CIP1 or WAF1. The pleiotropic effects of p21 in renal physiology include proliferative arrest, apoptosis and cellular hypertrophy depending on the pathological context, and the subcellular localization of the molecule (60, 69). p21 is markedly upregulated after renal I/R, and in this situation p21 deficiency has been described to exacerbate injury (51). Although the absence of p21 should confer an enhanced proliferative potential and thus would theoretically be advantageous for regeneration, it seems that uninhibited proliferation as a result of p21 deficiency is maladaptive in the setting of acute injury. Interestingly, recent data point to an age-dependent reversal of this effect. Basal renal p21 levels have been shown to increase in aged rats (24), and Juncos et al. (37) have found that the lack of p21 is protective against renal I/R injury in the aged animal. Thus it is possible that the prevention of increased p21 expression in the aged p21-deficient animals leads to a net proliferative rate more closely approximating that seen in healthy young animals.

It has been suggested that these senescence-inducing mechanisms act to prevent the development of malignancy in the aging organism, but that this protection comes at the cost of lost proliferative potential (43). Given the large number of regulators that are involved in cell cycle control and senescence, it is likely that more age-modulated pathways will be discovered in the future. However, caution will be necessary in transferring experimental data from short-lived rodents to long-lived humans, where cellular senescence might be regulated by alternative mechanisms.

Susceptibility to Apoptosis is Increased in the Aging Kidney

Apoptosis, or programmed cell death, is important for both normal development and for the removal of nonfunctional and/or precancerous cells from the adult organism. However, apoptosis may be maladaptive under some conditions. In the kidney, acute injury induced by I/R results in massive apoptosis, which exacerbates the tubular damage (12, 31, 64) (Fig. 1).

In the aging rodent, basal rates of apoptosis are increased in many organs including the kidney (49, 71, 93) and are thought to result from a shift in the balance of pro- and antiapoptotic factors, coinciding with altered regulatory mechanisms of the caspase cascade (36). Caspases are a large family of cysteine proteases that mediate the cleavage of a broad range of cellular proteins and thereby induce apoptotic cell death (109). Inhibition of caspases has been shown to confer functional protection in different forms of AKI (30, 32). In studies of aging rats, it was found that the kidney, lung, liver, and spleen displayed increased expression of caspases -3 and -9 (71, 109) as well as the caspase-9 activator cytochrome c (45). Similarly, expression of the proapoptotic Bax protein was shown to be enhanced, whereas expression of antiapoptotic Bcl-2 was reduced (Fig. 2).

Interestingly, these changes were significantly less dramatic when rats were kept on a low-calorie diet from a young age (45). In general, calorie restriction (CR) is the only intervention that has been conclusively shown to slow the aging phenomena in organs from a wide variety of organisms. One mechanism by which CR is thought to counteract apoptosis is decreasing the level of reactive oxygen species (ROS), as reviewed in detail elsewhere (36). The presence of ROS is known to induce cumulative mitochondrial DNA and membrane damage with aging, which in turn can trigger activation of the intrinsic pathway of apoptosis. In the kidney, CR also reduced the age-dependent increase in Fas expression, a cell surface receptor that belongs to the death receptor family and is known to induce apoptosis through the extrinsic pathway (73).

In addition to these basal changes in apoptotic rates during aging, Qiao et al. (71) used aging rats to investigate age-related differences in apoptosis in the context of renal I/R. Their study indicated that the number of apoptotic cells was significantly higher in old kidneys after I/R and that this was mainly governed through the intrinsic pathway of apoptosis (Fig. 2). Cumulatively, the existing data indicate an age-related increase in renal cell apoptosis under both baseline and stress conditions. Alterations in both the intrinsic and the extrinsic pathways of apoptosis seem to be involved. This shift toward a higher proportion of cells undergoing apoptosis in the aged kidney contributes to the higher load of cell death following a given insult and is likely to impair the concerted repair process.

Function of Stem and Progenitor Cells Declines with Aging

Aging can significantly alter stem cell number, regenerative capacity, and function (4, 72). Although direct experimental data regarding the impact of these age-dependent changes on stem cells is lacking for the kidney, the role of multipotent stem or progenitor cells in repair and maintenance of the kidney has been a matter of intensive research during the last several years.
Extrarenal circulating stem cells. In 2000 it was first published that y-chromosome-positive cells can be found in the tubular epithelial lining of female kidneys that were transplanted into male recipients (68). These findings of extrarenal cells that home to the injured kidney challenged the traditional view of renal repair. Numerous animal studies have since tested these findings by using lineage tracing of transplanted bone marrow cells. The focus of most studies has been on two unique cell types of the bone marrow, the hematopoietic stem cell, and the marrow stromal cell (MSC). Some authors have provided evidence that each of these cells can differentiate into a variety of renal cell types, including tubular cells, endothelial cells, mesangial cells, and podocytes (34, 47, 59, 68, 70, 105). It remains unclear whether these observations are due to transdifferentiation of the injected cells, fusion of the injected cells with endogenous kidney cells, or a combination of both events, and whether this process occurs in numbers that are sufficient to contribute significantly to organ repair (reviewed in Ref. 10). However, it has been more consistently shown that injection of the MSC fraction of bone marrow cells confers a protective and regenerative effect for endogenous cells from a variety of organs including the kidney (5, 59, 67, 82, 95). The underlying mechanism for the organ-protective effect of MSCs appears to be an endocrine pathway by which MSCs secrete various growth factors and cytokines, including IGF-1, VEGF, and HGF, that could be effective in renal repair (5, 82, 95, 96).

According to the majority of published studies, the number of MSCs remains relatively stable with aging, but changes in their morphology and function occur (39, 83). For example, the pattern of MSC-secreted factors changes with aging, resulting in less TGF-β and BMP2/4 production, while the secretion of IL-6 increases (83). This coincides with a decreased capacity for proliferation and differentiation (88). Moreover, in myocardial infarction experiments MSC from aged donor rats showed less functional benefit than cells from young animals (108). Schatteman et al. (80) took this notion a step further and showed that old bone marrow cells were not only less regenerative but actually actively inhibited skin wound healing when injected into young animals, suggesting that they secrete anti-reparative factors.

Another important bone marrow-derived cell type that is likely to participate in organ repair is the endothelial progenitor cell (EPC). EPCs are released from the bone marrow in response to various stressor stimuli, such as renal I/R (66). Once in the circulation, they can be recruited to replace damaged endothelial cells in most vascular beds including the kidney (33, 77). Increasing age coincides with lower levels and functional impairment of circulating EPC (92, 94). In a recent study,
Chang et al. (13) elegantly demonstrated that there is an age-dependent impairment of EPC trafficking which correlates with decreased hypoxia-inducible factor 1α stabilization in ischemic tissues. This loss of normal EPC function might directly contribute to the age-dependent impairment of ischemia-induced repair and neovascularization, as suggested by bone marrow transplantation experiments in which young to old transplantation significantly improved angiogenesis (90).

**Intrarenal stem cells.** With the growing body of evidence for the existence of multipotent stem cells not only in organs with high cell turnover but also in the brain, skeletal muscle, and heart, there have been many attempts to identify and locate progenitor cells in the kidney. The growing number of suggested locations include the renal papilla (63), the proximal tubule (44, 50), Bowman's capsule (79) and the interstitium (9). Presently, there are no definitive data that renal progenitor cells outside of the tubular cells themselves play a major role in the normal maintenance of the glomerulus or tubular epithelium, nor have there been any investigations of the impact of aging on renal progenitor cells. However, a well-studied organ that may provide insight into stem cell aging and repair is the skeletal muscle.

Similar to the kidney, skeletal muscles lose their regenerative potential and develop tissue fibrosis with aging. The primary skeletal muscle stem cell is the satellite cell, which is normally quiescent but can be activated to proliferate and give rise to new muscle cells in response to injury (72). The age-related decrease in skeletal muscle repair capacity can partly be attributed to a decrease in satellite cell numbers but also appears to involve an impairment of specific signaling pathways (72). Interestingly, transplantation of old muscle into young animals or parabiotic experiments in which a young and an old mouse share their blood circulation has been shown to restore the regenerative capacity of satellite cells, suggesting that systemic factors may govern the age-dependent loss of regenerative capacity (19).

One such systemic factor may be the Wnt signaling pathway, which is best known for its role in embryological development and in cancer biology. It has been demonstrated that Wnt activity is increased in old mice, resulting in reduced progenitor cell proliferation and increased fibrosis (8). The effects of Wnt activation during aging may normally be counteracted by the antiaging protein klotho (48). Klotho is highly expressed in the kidney and plays a major role in renal mineral metabolism (reviewed in Ref. 97). Klotho-deficient mice show many signs of accelerated aging and suffer from advanced stem cell senescence that might result from unopposed Wnt signaling (48). The relative importance of Klotho and Wnt signaling during the process of kidney aging has yet to be examined, but these may ultimately serve as therapeutic targets to enhance repair and limit fibrosis in older patients.

**Renal Growth Factor Profiles Change with Aging**

Growth factors play a key role during renal development and repair. They are crucial in regulating cellular proliferation, apoptosis, migration, and tubulogenesis (41) (Fig. 1). Some growth factors are synthesized by the kidney itself, and others are produced at distant sites by specific cell types such as MSC (see Extrarenal circulating stem cells). After acute injury, the damaged renal epithelium upregulates the expression of several proregenerative growth factors, including epidermal growth factor (EGF), hepatocyte growth factor (HGF), and insulin-like growth factor-1 (IGF-1) (23). It has been demonstrated that expression levels of many growth factors decline with aging and that their respective receptor transduction pathways are often downregulated. For example, renal EGF expression and circulating IGF-1 levels decrease in an age-dependent fashion (17, 85). At the same time, the responsiveness of EGF and IGF-1 receptors declines (98, 103). Another important growth factor that is attenuated in the old kidney is vascular endothelial growth factor (VEGF), which exerts its mitogenic and antiapoptotic effects primarily on endothelial cells. Since VEGF plays a crucial role in renal vascular repair and EPC recruitment after AKI, it is likely that reduced VEGF expression in the aging rat kidney contributes to repair defects (3, 40).

The decline in VEGF expression coincides with an increased expression of thrombospondin-1 (TSP-1), a potent antagonist of VEGF effects. The resulting imbalance between pro-angiogenic and antiangiogenic factors may partly explain the age-dependent progressive rarefaction of peritubular capillaries and the deficiency of adequate oxygen supply and vascular remodeling during renal repair (40, 91, 93).

While EGF, IGF-1, and VEGF levels decline, there is an age-dependent increase in expression of the profibrotic protein transforming growth factor-β1 (TGF-β) in the kidney (18, 24, 93) (Fig. 2). TGF-β is a multifunctional peptide that plays an important role in many chronic renal diseases. It modulates the renal repair process by triggering epithelial apoptosis, epithelial-mesenchymal transition, and progressive fibrosis. In IR injury TGF-β is expressed by regenerating tubules and by infiltrating immune cells. Neutralization of TGF-β improves the vascular remodeling process and decreases interstitial expansion (87). Downstream effects of TGF-β are mediated through a number of key signaling pathways including the Smad family of transcription factors that regulates the transcription of many proteins including integrin-linked kinase (ILK). ILK is thought to be a key player in the fibrogenic effects of TGF-β. It is therefore of note that ILK expression is significantly increased with aging in the rat kidney under physiological conditions and after unilateral ureteral obstruction (46). Activation of ILK induces epithelial cells to deposit excess extracellular matrix and to undergo dedifferentiation. These prosclerotic processes are maladaptive in the repair response. Taken together, age-related changes in the levels of TGF-β, EGF, IGF-1, and VEGF result in a complex shift of the microenvironmental milieu that is likely to affect tissue homoeostasis under normal conditions and might become critical when coordinated repair is needed in the case of injury.

**How “Immunosenescence” Might Affect Renal Repair**

There is a growing body of evidence supporting the prominent role that immunocompetent cells and their mediators play in “nonimmune” renal diseases such as AKI (76). Many of the classic inflammatory mechanisms that involve the innate and the adaptive immune system have been shown to be active following I/R, cisplatin nephrotoxicity, and other forms of AKI (7) (Fig. 1). This immune activation leads to a significant exacerbation of the initial injury, as shown by experiments in which depletion of circulating macrophages confers significant attenuation of I/R injury (21, 35, 61). The aging immune

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system undergoes a variety of important changes that have been termed immunosenescence (28). In general, these changes are associated with an increased susceptibility to infectious diseases, cancer, and autoimmune disorders. They comprise a permanent low-grade activation of the inflammatory system, dysfunctionality of T cells, defective natural killer cells, and atrophy of the thymus (28). It is likely that these measurable changes represent only a small part of the imbalance between the inflammatory and anti-inflammatory network (27).

In the aging rodent kidney, this imbalance is reflected by an increase in the influx of inflammatory cells under baseline conditions, coinciding with an increased expression of ICAM-1 and VCAM-1 (76, 93, 110). If an old kidney is transplanted into either a young or old recipient, it is more immunogenic and attracts a higher number of inflammatory cells than grafts from younger donors (62, 74). It has been suggested that this is due to an enhanced expression of endothelial PECAM-1 as well as differences in how antigens are processed and presented by resident and infiltrating cells (75).

Immune cells appear to be more versatile than originally thought, and, depending on environmental signals, they can alter their protein expression patterns, resulting in their transition into different subsets with very different functions. Macrophages, for example, are functionally heterogeneous and can change from pro- to anti-inflammatory cells (89). In the kidney, the proinflammatory M1 macrophages are deleterious during the early phase of I/R injury (35) whereas they may eventually transition to an anti-inflammatory phenotype and subsequently exert important reparative functions (101). Macrophages change their phenotype significantly with aging, and their functional adaptability seems to decrease (42). When peritoneal macrophages from young donors are applied to dermal wounds, they accelerate healing, but this effect is diminished if the macrophages are obtained from old donors (20), possibly due to a decline in the production of growth factors such as VEGF and EGF (89). These findings of an age-induced defect in trophic functions and functional adaptability might also contribute to impaired healing in the old kidney. Further exploration of the underlying mechanisms and the specific roles of distinct subsets of macrophages and other immune cells might help to identify new therapeutic options for enhancing repair and preventing fibrosis in the aging kidney.

Conclusions

The process of aging is likely to result in complex alterations in how the kidney copes with normal homeostasis as well as acute and chronic injury. Although remarkable progress has been made in the understanding of fundamental mechanisms that are involved in renal injury and repair, our understanding of how these mechanisms are altered during aging remains limited. Distinct processes that can explain why the old kidney regenerates less successfully than the young kidney include a diminished proliferative reserve, which might in part depend on altered progenitor cell function; an increased tendency for apoptosis; alterations in growth factor profiles; and important changes in immune responses. It will be crucial in the future to better characterize these underlying mechanisms to develop new therapeutic strategies that are suitable for specific needs of the aging patient population.

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REFERENCES


69. Price PM, Megyesi J, Safirstein RL. Invited Review.


