

Cell Senescence in the Aging Kidney

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ABSTRACT

Pedigree genetics and environment modulate the biological process of aging. The permanent and irreversible growth arrest of cell senescence is a central paradigm of aging. Various pathophysiologic pressures such as oxidative stress and mitochondrial injury can also induce senescence. Senescent cells secrete altered levels of growth factors, show increased susceptibility to apoptosis, and associate with delayed repair and regeneration in the aging kidney. Here we discuss new progress in understanding renal aging, focusing on mechanisms of cell senescence and possible interventions to modulate age-related organ damage.

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What characterizes the aging kidney? Aging in most species associates with impaired adaptive and homeostatic mechanisms that leave an individual susceptible to environmental or internal stress followed by increasing rates of disease and death.¹ The typical histologic features of renal senescence are decreasing cortical mass with corresponding increases in glomerulosclerosis, interstitial fibrosis, tubular atrophy, and arteriosclerosis.² The changes in renal function in elderly individuals include increased renal vascular resistance, reduced renal plasma flow, and increased filtration fraction³ that usually accelerate after age 50 to 60.⁴

Aging-related sclerosis in the kidney varies markedly among ethnic groups, with more injury seen in white compared with Japanese individuals and even higher rates of sclerosis in aging black individuals.⁵ Although some studies demonstrated mean loss of GFR at 0.75 ml/min per year in aging people, 33 to 66% of the elderly maintain perfectly normal GFRs with only minimal histologic changes.⁶

Some features of senescence in the aging kidney, such as the appearance of senescence-associated β -galactosidase (SA- β -gal) and P16INK4a, are present

even without morphologic changes, suggesting that some aspects of cell senescence are common in the aging kidney. Thus, even when the aging kidney has inconspicuous histologic changes, accelerated age-related disease may develop in response to new pathologic stimuli and stresses.

WHAT IS KNOWN ABOUT CELL SENESCENCE?

Aging is a programmed biological process regulated by genes sequentially toggled on or off as signals to the nervous, endocrine, and immune systems; these systems are responsible for maintaining homeostasis and activating responses for host defense. Transcriptional differences between young and old individuals in genome-wide analyses showed an accumulation of small changes in the expression of many genes rather than large changes in expression of a small number of genes.² Numerous mechanisms of injury thus contribute to age-related organ dysfunction, including increased oxygen radicals and fibrogenic mediators, mitochondrial injury, loss of telomeres,

and imbalances in cell repair and proliferation *versus* apoptosis and cell death (Figure 1). These mechanisms, however, are not unique to aging. Rather, they are common to many progressive forms of injury. Cellular senescence is one key element that is tightly linked to aging-related diseases. We therefore focus on mechanisms and modulators of this senescent process in aging.

Cellular senescence was first used to describe the phenotype of permanent and irreversible growth arrest in *in vitro* studies of human fibroblasts.⁷ Senescent cells remain viable but show altered morphology, greater heterogeneity, expression of SA- β -gal, accumulation of lipofuscin granules, and lack of response to mitogenic stimuli. This kind of cellular senescence is also known as replicative senescence. It is accompanied in humans by loss of telomeres and DNA repeats at the ends of chromosomes as a result of lack of telomerase activity.⁸ In contrast, mouse and rat telomeres are relatively long and show little shortening during their lifespan, although the morphology of senescence and aging still develops.

Cellular senescence can also be induced rapidly in response to various physiologic stressors independent of the number of cell divisions, so-called stress-induced premature senescence (SIPS).⁹ SIPS is stimulated through the p16/retinoblastoma or ARF/

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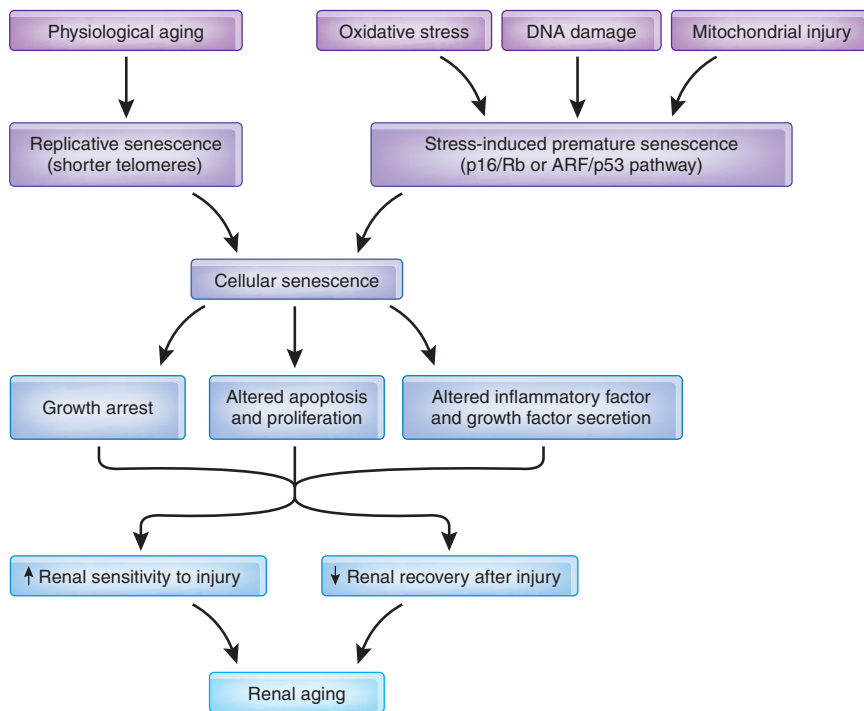


Figure 1. Cell senescence is not only a marker of renal aging but also a participant. The physiologic aging process induces cell replicative senescence, whereas some pathologic stresses, such as oxidative stress and mitochondrial injury, can induce SIPS through the p16/retinoblastoma pathway or ARF/p53 pathway. Senescent cells have arrested growth and imbalance of apoptosis/proliferation and secrete altered levels of growth factors and therefore have increased sensitivity to injury and decreased repair after injury.

p53 signaling pathway. P16INK4a inhibits the activity of the cyclin-dependent kinases 4 and 6, thereby leading to hypophosphorylation of the retinoblastoma gene and irreversible cell-cycle arrest. p19ARF, by binding to the murine double minute 2 oncogene protein, prevents ubiquitination and degradation of p53. This results in cell-cycle arrest mediated by p53 through p21CIP1/WAF1.¹⁰ In both aging mice and humans, increased expression of the cell-cycle regulator p16INK4a is strongly associated with histologic changes and inversely correlates with cell replication.¹⁰

SIPS can be induced by oxidative stress, DNA damage, Ras induction, and lack of nutrients.¹⁰ Data also suggested that chronic oxidant stress contributes to telomere shortening and thus may result in senescent changes.¹¹ Oxidative free radicals (OFR) can induce age-related atrophy through increased apoptosis or DNA mutations. Mitochondria are the primary source of OFRs and are highly susceptible to oxidant-induced damage

because of their proximity to OFRs and the inability of mitochondrial DNA to undergo repair.¹² Using null mice, several longevity-related factors, such as p66SHC and the forkhead family of transcription factors known as FoxOs, that primarily protect against mitochondrial injury in aging have been identified.

Klotho and sirtuin 1 are also recognized as key modulators of aging. Klotho is a newly identified antiaging factor with effects on insulin/IGF-1 signaling, OFRs, and phosphate/calcium homeostasis.^{13,14} Mice genetically deficient of *klotho* develop accelerated aging-related disease, including stroke, arteriosclerosis, and osteoporosis. In contrast, overexpression of the gene encoding *klotho* extends lifespan in the mouse.¹⁵ Sirtuin 1 is a class III histone deacetylase that deacetylates not only histone substrates but also many key transcription factors and co-factors, such as p53, FoxOs, peroxisome proliferator-activated receptor γ (PPAR- γ) co-acti-

vator-1 α , and NF- κ B,^{16–19} thereby affecting crucial cellular pathways involved in response to stress, metabolism, and possibly elongation of lifespan. NF- κ B activity may be a key link to age-related nephropathy and atherosclerosis.²⁰

Senescence is a fundamental cellular program that parallels programmed cell death, known as apoptosis; however, senescent endothelial cells show increased susceptibility to apoptosis.²¹ Furthermore, factors involved in senescence signaling, such as p53, are also involved in the regulation of apoptosis through interaction with the Bcl-2 family of proteins.²² The functional decline in the potential to repair and regenerate is often considered a hallmark of the aging phenotype and may be aggravated by this excess apoptosis.

Senescent cells also secrete altered levels of TGF- β , EGF, IGF-1, and vascular endothelial growth factor, resulting in a complex shift within the microenvironmental milieu, thereby leading to a diminished capacity of the aging kidney to cope or withstand normal and abnormal stresses.²³ The high numbers of cells expressing p16INK4a and telomere shortening could further restrict cellular proliferation, thereby delaying renal recovery from injury such as ischemia in the aging kidney. In fact, increased levels of zinc-alpha (2)-glycoprotein in aging proximal tubules reduce cellular proliferation and modulate the aging phenotype.²⁴

HOW CAN CELL SENESCENCE IN THE AGING KIDNEY BE MODIFIED?

Direct pharmacologic activation of telomerase and reduction of p16INK4a are potential key therapeutic targets in aging kidneys but with serious possible adverse effects. The enzyme telomerase, which maintains telomeres, is oncogenic. Indirect enhancement of telomeres by statins, for example, upregulates the expression of telomere repeat-binding factor, an important protein for telomere capping, and can prevent senescence in some cells.²⁵

Because telomere and cell-cycle proteins are affected by oxidative stress, an-

tioxidant drugs may also be used indirectly to reduce cell senescence. Statins delay senescence of endothelial cells by reducing overproduction of intracellular OFRs and inhibiting nuclear export of telomerase reverse transcriptase.²⁶ Calorie restriction conclusively slows the aging process in organs from a wide variety of organisms, probably by decreasing the level of OFR and the associated, cumulative, mitochondrial DNA and membrane damage of aging.²³ PPAR- γ agonists protected against renal injury in aging by reducing proteinuria, improving GFR, decreasing sclerosis, and alleviating cell senescence. These drugs regulate p66SHC phosphorylation, an integration point for many signaling pathways that affect mitochondrial function and longevity, by reducing protein kinase C- β . PPAR- γ agonists also increase klotho and decrease systemic and renal oxidative stress, all pathways associated with alleviating cell senescence in the kidney.²⁷

Reducing the number of renal senescent cells by replacing them with non-senescent cells can also decrease aging-related injury. The number of stem cells remains relatively stable with aging, but their morphology and function are altered.²⁸ For example, aging stem cells secrete less TGF- β and bone morphogenetic proteins 2/4 and more IL-6.²⁹ Aging mice that were reconstituted with young bone marrow have less injury and less p16INK4a expression in the kidney compared with mice that received old bone marrow.^{30,31} This decreased structural injury parallels decreasing SA- β -gal staining, a marker of senescent cells. These SA- β -gal⁺ cells do not co-localize with the bone marrow-derived cells, indicating that no or few bone marrow-derived cells show markers of senescence; however, there are fewer SA- β -gal⁺ cells adjacent to the bone marrow-derived cells in mice that received young bone marrow *versus* those that received old bone marrow. In contrast, transplantation of old bone marrow into young mice did not induce senescent renal changes.³¹ Transplantation of old muscle into young animals restored the regenerative capacity of satellite cells,³² suggesting that systemic fac-

tors may govern age-dependent loss of regenerative capacity. One such systemic factor may be the Wnt signaling pathway, which shows increased activity in older mice, resulting in reduced progenitor cell proliferation and increased fibrosis. The effects of Wnt activation during aging may be counteracted by the antiaging protein klotho.³³

CONCLUSIONS

Old kidneys are functional but fragile. Decreasing renal function with aging is usual but not inevitable. Manipulation of cell senescence may be beneficial in aging-related kidney disease as our ability to probe this problem better starts to emerge.

DISCLOSURES

None.

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