

# Fibrosis and renal aging

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**Glomerulosclerosis and interstitial fibrosis increase in the aging kidney, and glomerular filtration rate (GFR) decreases with increasing age. Decreases in stem cell number and function contribute to renal aging. High-dose angiotensin receptor blocker (ARB) not only slows the progression of glomerular and vascular sclerosis in aging but can also induce regression of these processes independently of its hemodynamic actions. By using new interventions, such as peroxisome proliferator activator receptor gamma (PPAR $\gamma$ ) agonist, we can manipulate the process of renal aging by regulating stem cells and other mechanisms.**

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Aging is linked to increases in cardiovascular disease, cancer, and chronic kidney disease. The phenotype of renal aging includes loss of kidney parenchymal mass, increased renal vascular resistance, reduced renal plasma flow, and increased filtration fraction. These alterations usually accelerate after the age of 50 to 60 years.<sup>1</sup> The typical histologic features of renal aging are decreased cortical mass with corresponding increases in glomerulosclerosis, interstitial fibrosis, tubular atrophy, and arteriosclerosis.<sup>2</sup> Several mechanisms are involved in renal aging, such as genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, altered intercellular communication, and stem cell exhaustion.<sup>3</sup> Urinary proteome assays and cDNA microarrays of kidneys showed high similarity between aging and chronic kidney disease.<sup>4,5</sup> This review will focus on the potential of regression of fibrosis in the aging kidney and its relation to stem cells, with the discussion of potential new pharmaceutical targets.

## REGRESSION OF RENAL AGING

Although aging appears to be inexorable, lifespan can be experimentally manipulated. Genetic modification and environmental challenges have been shown to extend the lifespan of model organisms. Angiotensin type 1a receptor knockout resulted in marked prolongation of lifespan in mice with less cardiac and renal injury, and less oxidative damage, than in wild-type mice.<sup>6</sup> Further, angiotensin-converting enzyme inhibitor administration prolonged lifespan in rats, and was associated with decreased food intake, body weight reduction, and was correlated with adipose tissue metabolic modulation with increased peroxisome proliferator activator receptor gamma (PPAR $\gamma$ ) expression, adiponectin, and antioxidant enzymes.<sup>7</sup> However, these studies did not address whether injuries occurring in the aging kidney could be reversed.

We have previously shown that existing glomerulosclerosis can be remodeled with high-dose angiotensin receptor blocker (ARB) in the remnant kidney model in rats.<sup>8</sup> Started 8 weeks after 5/6 nephrectomy, this regression occurred over the first 4 weeks of treatment. There was less glomerulosclerosis and less tubulointerstitial fibrosis, with maintenance of complex capillary branching. The renin angiotensin aldosterone system is likewise altered during aging, with reduced plasma renin activity and aldosterone levels, partially compensated for by increases in the density of angiotensin II receptors in aging rats and in older patients.

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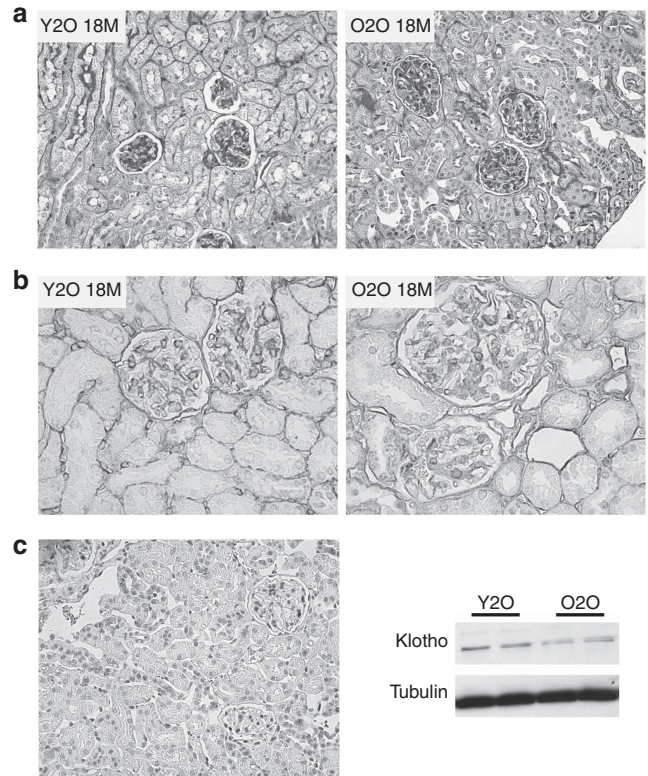
These renin angiotensin aldosterone system changes may contribute to age-related fibrosis in the kidney and the heart. When we studied aging rats with existing sclerosis, that are being treated with high dose of ARB from 18 to 24 months, and are assessed for effects on age-related scarring, ARB therapy not only slowed the progression of glomerular and vascular sclerosis in aging, as assessed with the help of morphology and collagen content, but also induced the regression of these processes.<sup>9</sup> This study suggests that remodeling of renal aging is possible.

### STEM CELL AND RENAL AGING

Aging can significantly alter stem cell number, regenerative capacity, and function.<sup>10</sup> Recent advances in stem cell biology have begun to unlock the molecular mechanisms and related downstream effects behind these reprogramming. For example, DBA/2J mice have more stem cells when they are young than C57BL/6J mice. However, they have faster stem cell turnover and fewer stem cells when old, which appears to be related to a shorter lifespan compared with C57BL/6J mice.<sup>11</sup> The hematopoietic stem cell (HSC) compartment is heterogeneous, consisting of epigenetically fixed subpopulations of HSCs that differ in self-renewal and differentiation capacity. New data show that the proportions of these HSC subsets change during aging, with lymphocytic HSCs being depleted, and myeloid HSCs being enriched, in the aged HSC compartment. Myeloid-biased HSCs, even when isolated from young donors, have most of the characteristics that had been attributed to aged HSCs.<sup>12</sup>

We examined the impact of bone marrow-derived cells on aging-related renal injuries.<sup>13</sup> The bone marrow from young or old mice was transplanted into aged mice. Mice were then followed for 6 months. The mice receiving young bone marrow had minimal progression of proteinuria, and consequently much less proteinuria than the mice receiving old bone marrow by the end of the study. There was also less corresponding glomerulosclerosis and glomerular collagen IV in mice receiving young bone marrow (Figure 1a and b), whereas mice transplanted with old marrow showed a greater influx of macrophages and more senescent cells. There was also increased expression of transforming growth factor-beta and plasminogen activator inhibitor-1 but no excess in platelet-derived growth factor in old mice receiving old marrow. Remarkably, old mice receiving young bone marrow even showed decreases in these profibrotic factors. By double staining, using the Y chromosome as marker in these male-to-female transplant mice, we determined that bone marrow cells did not directly replace parenchymal cells. Rather, our results support the theory that bone marrow-derived cells have paracrine effects on renal parenchymal cells.

One mechanism may be *via* the Wnt signaling pathway, which is best known for its role in embryological development and in cancer biology. Wnt activity is increased in old mice, resulting in reduced progenitor cell proliferation and increased fibrosis.<sup>14</sup> The effects of Wnt activation during aging may normally be counteracted by the antiaging protein



**Figure 1 | Young bone marrow reduces renal injury in aging.** The bone marrow from young mice or old mice was transplanted into aged mice. Six months later, there was more interstitial fibrosis and glomerular mesangial expansion in old-to-old (O2O) compared with young-to-old (Y2O) bone marrow transplant mice ((a) periodic acid-Schiff staining,  $\times 200$ ). Young bone marrow resulted in significantly less glomerular collagen IV deposition than old bone marrow ((b)  $\times 400$ ). Klotho, the antiaging protein, is mainly expressed in distal tubules. Its level was preserved in young bone marrow-transplanted mice, and was decreased in old bone marrow-transplanted mice (c).

klotho.<sup>15</sup> Klotho is highly expressed in the kidney and has a major role in renal mineral metabolism.<sup>16</sup> Klotho-deficient mice show many signs of accelerated aging and suffer from advanced stem cell senescence that might result from unopposed Wnt signaling. In our study, Klotho levels were preserved in young bone marrow-transplanted mice, and were decreased in old bone marrow-transplanted mice (Figure 1c).

However, stem cells alone do not account for the full story. When we transplanted young mice with the bone marrow from either old mice or young mice, the mice receiving old bone marrow showed augmented collagen type IV in the kidney; however, by 6 months both groups showed similar increased profibrotic factors. In old mice that received young bone marrow, there was less SA- $\beta$ -gal staining, a marker of senescent cells, than in mice that received old bone marrow. These SA- $\beta$ -gal<sup>+</sup> cells did not colocalize with the bone marrow-derived cells but were localized adjacent to the bone marrow-derived cells. However, in young mice recipients, cell senescence was similar in those receiving young or old bone marrow. These findings suggest that the cellular microenvironment in these mice influences stem cell function. A finely

tuned relationship between bone marrow-derived stem cells and local cell populations is required for homeostasis. Currently, we have little information about which factors interact with circulating stem cells to help in maintaining stem cell function and number. Thus, further studies are required to pinpoint this relationship and how it changes during aging.

In addition, there is growing evidence for the existence of multipotent stem cells in the kidney. Such stem cells may be located in the renal papilla, the proximal tubule, lining of the Bowman's capsule, and in the interstitium.<sup>17</sup> Cells lining the Bowman's capsule may even serve as niche progenitors for podocytes, and migrate to the glomerular tuft. Although the number of parietal epithelial cells (PECs) and PECs expressing podocyte proteins increase in aging nephropathic mice, they are not sufficient to compensate for the decrease in podocyte number observed during aging.<sup>18</sup> Thus, limitations of both circulating stem cells and local multipotent stem cells may contribute to fibrosis in the aging kidney.

### NEW INTERVENTIONS FOR RENAL AGING

Various interventions, such as resveratrol, calorie restriction, and spermidine, have been linked to an increased lifespan. Notably, administration of rapamycin, an mTOR inhibitor, starting at midlife can extend the life span of mice, suggesting that aging can be delayed or reversed in multiple cell types.<sup>19</sup> In the hematopoietic system, aging is associated with an increase in mTOR activation in stem cells and progenitors.<sup>20</sup> Administration of rapamycin to old mice to inhibit mTOR not only limited the normal age-related increases in hematopoietic stem cells and biomarkers of aging in those cells but also enhanced the performance of the stem cells to become as effective as young stem cells in heterochronic (young-to-old, and *vice versa*) transplantation experiments.

PPAR $\gamma$  may also regulate stem cell function. PPAR $\gamma$  functions as a transcription factor, and may affect numerous genes by binding to PPAR response element motifs. Pioglitazone, a PPAR $\gamma$  agonist, dramatically increased the cardiomyogenic transdifferentiation efficiency of human bone marrow-derived mesenchymal stem cells. This effect was linked to PPAR $\gamma$  activation *in vivo* and *in vitro*. Pioglitazone-pretreated bone marrow-derived mesenchymal stem cell transplantation significantly improved impaired cardiac function in the myocardial infarct model *in vivo* and significantly reduced the size of the infarct.<sup>21</sup> We examined the effect of a PPAR $\gamma$  agonist on renal aging.<sup>22</sup> Mice were treated from the age of 18 months with pioglitazone. They showed less proteinuria and improved GFR, with less glomerulosclerosis and tubulointerstitial fibrosis compared with untreated age-matched control. Cell senescence, as assessed with SA-beta-gal staining, was also decreased. These changes were linked to increased PPAR $\gamma$  activation in tissue, and decreased kidney lipid oxidation, as assessed with the help of urinary isoprostane excretion. There was also a decrease in mitochondrial injury, with decreased tyrosine phosphorylation of p66Shc, and a decrease in mitochondrial

DNA deletion. The *klotho* gene has two upstream noncanonical PPAR $\gamma$ -binding sites. Importantly, we found that pioglitazone treatment in aging also increased renal *klotho* expression by more than 60%, another possible contributor to its effects on senescence and reactive oxygen species. The Pro/Ala PPAR $\gamma$  gene polymorphism, which is related to PPAR $\gamma$  activity and insulin sensitivity, is also associated with human longevity, supporting that similar mechanisms may be relevant in human aging.<sup>23</sup>

### SUMMARY AND PERSPECTIVE

New pathologic mechanisms that have been identified in renal sclerosis provide potential targets to halt what has been considered an inevitable loss of renal function and structure with aging. Animal studies suggest that kidney scarring can be ameliorated, and in some cases reversed. These insights in models of chronic kidney disease have now been extended to the aging kidney. Multifactorial intervention in humans, modifying the cellular aging microenvironment, optimizing the reparative potential of stem cells, decreasing ongoing injury, and augmenting antiaging and repair mechanisms may have beneficial impact on the chronic kidney disease currently seen in our aging population.

### DISCLOSURE

All the authors declared no competing interests.

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