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Renal Aging. Causes and consequences

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Abstract

Individuals aged >65 years are the fastest expanding population demographic throughout the developed world. Consequently, more aged patients are receiving diagnoses of impaired renal function and 'nephrosclerosis' - age associated histological changes in the kidneys. Recent studies have shown that the aged kidney undergoes a range of structural changes and has altered transcriptomic, haemodynamic and physiologic behaviour at rest and in response to renal insults. These changes impair the ability of the kidney to withstand and recover from injury, contributing to the high susceptibility of the aged population to acute kidney injury, and their increased propensity to develop subsequent progressive chronic kidney disease. This review examines these features of the aged kidney, and explores the various proven and putative pathways contributing to the changes seen with aging in both experimental animal models and in man. The potential for further study to increase understanding of the aged kidney, and to lead to novel therapeutic strategies is discussed.

Introduction

The Centre for Disease Control predicts that 72 million Americans will be aged 65 years or older by 2030, accounting for approximately 20% of the U.S. population.[1] Eurostat predicts that 28% of Europeans will be aged over 65 by 2060.[2] These increasing numbers of elderly individuals will inevitably lead to increasing diagnoses of age related kidney impairment.

In renal aging a complex interplay of genetics, environmental change and cellular dysfunction leads to characteristic structural and functional changes.[3] This review summarises our current understanding of the factors driving age-associated changes in the kidney.

Clinical features of renal aging in man

Structural changes of aging

With age there is a decline in total nephron size and number, tubulointerstitial changes, glomerular basement membrane thickening and increased glomerulosclerosis (Figure 1).[4,5] This age-related histological appearance is frequently described as "nephrosclerosis" and describes a combination of 2 or more histological features: any global glomerulosclerosis, tubular atrophy, interstitial fibrosis > 5% and any arteriosclerosis. A study of healthy kidney donors demonstrated nephrosclerosis in only 2.7% of biopsies from donors aged less than 30 years, 58% from 60-69 year olds and 73% from donors

aged greater than 70.[6] Cadaver studies estimate that the upper limit of normal glomerulosclerosis in aging exceeds 10%.[7]

Nephrosclerosis remains a poorly understood observation, and its importance within an aging kidney is far from clear. We know that nephrosclerosis correlates with aging and mild hypertension in healthy living donor kidneys.[8] Importantly however, age related decline in measured GFR does not correlate with the presence or absence of nephrosclerosis.[9] In fact, nephrosclerosis does not correlated with urine albumin excretion, family history of end-stage renal disease, body mass index, serum cholesterol, glucose, or uric acid.[10] It remains unclear then, whether nephrosclerotic changes have any contribution to the functional changes seen in aging, or are perhaps distinct and unrelated.[11]

The Aging-CKD spectrum

Our understanding of the pathways underlying renal aging is incomplete and derived from studies of healthy aging kidneys and extrapolation from experimental and clinical studies of CKD.

It is important to note the distinction between these conditions, with the mechanisms of progressive genetic, immune or toxin mediated injury seen in CKD distinct from the gradual, prevalent changes seen in the aging kidney. Throughout this review we will focus on the changes seen in the 'healthy' aged kidney, though due to the paucity of experimental and clinical data

available in aging kidneys at times reference will be made to mechanisms in progressive CKD which may also be of relevance to the uninjured but aged kidney. Processes discussed below such as cellular senescence, fibrosis, vascular rarefaction and glomerular loss are common to both aging and CKD despite differences in causation and natural history. Similarities are also seen in the behaviour of the chronically damaged and the aged kidney including their heightened susceptibility to further injury and deficient repair.[13]

Declining Glomerular Filtration Rate (GFR)

Population GFR declines with age with longitudinal studies differing in their reported rates of decline.[14,15] While the MDRD study suggested renal function declined at a rate of 3.8ml/min/year/1.73m^2, rates as low as 0.4 ml/min/year/1.73m^2 in the Netherlands have been described.[16–19] A Japanese cohort study suggests the rate of GFR decline increases with advancing age.[20]

Studies of robustly phenotyped Kuna Indians with minimal prevalence of hypertension and cardiovascular disease demonstrate comparable declines in renal function over time, suggesting that there is a true age related decline, rather than the cumulative effects of cardiovascular disease.[21] How a significant minority of individuals apparently remain free of nephrosclerosis and GFR loss remains poorly understood and merits further study.

Decreased Tubular Function

Aging is characterised by progressive tubular dysfunction, decreased sodium reabsorption, potassium excretion and urine concentrating capacity potentially contributing to an increased susceptibility to AKI.[22–24] Elderly patients demonstrate decreased trans-tubular potassium gradients and fail to increase distal tubule potassium excretion when hyperkalaemic or in response to fludrocortisone.[25] Decreased potassium excretion correlates with decreasing GFR, and may reflect a degree of reduced sodium and chloride delivery to the distal convoluted tubule.[26]

Vascular Changes

There are important changes to blood vessel structure and function in the aging kidney. There is increased extracellular matrix (ECM) deposition, increased intimal cell proliferation in pre-glomerular arterioles and increased intrarenal shunting and capillary bypassing predominantly affecting the cortex.[27]

Increased renal sympathetic tone increases vasoconstriction whilst aortic baroreceptor attenuation of sympathetic tone decreases with age.[28,29] Renal vasodilators such as atrial natriuretic peptide, nitric oxide (NO) and amino acids become less effective.[30–32] Human studies demonstrate decreased NO production and platelet responsiveness,[33] with accumulation of the NO synthase inhibitor asymmetric dimethylarginine in elderly individuals.[34] In particular, aging males become increasingly NO dependent to maintain renal plasma flow.[35]

Biological Processes and Mediators Implicated in Experimental Aging

Most rodent experimental models of renal disease are undertaken in young animals, potentially affecting their relevance to the aging kidney. There is limited or no data available regarding the response of the aged rodent kidney to experimental glomerulonephritis, AKI, ureteric obstruction, diabetic nephropathy, 5/6th nephrectomy, adriamycin nephropathy or renal transplantation. Some aspects of renal aging may be studied *in vitro* but others require study *in vivo* in aged mice or other experimental animals (Table 1).

Studies have demonstrated increased susceptibility of the aged kidney to ischemia reperfusion injury (IRI) or toxic AKI.[36,37] Aged mice exhibit increased mortality, AKI severity and chemokine/cytokine responses in a model of uterine sepsis.[38] Furthermore, aged mice exhibited increased mortality, prolonged injury, reduced regeneration, increased scarring and microvascular rarefaction following renal IRI compared to young mice.[39]

The biology of aging is complex involving diverse changes to cells, tissues, organs and the surrounding microenvironment (Figure 2). Many of these processes and mediators are discussed below but the reader should appreciate that this list is not exhaustive.

A) Signalling pathways and oxidative stress in the aging kidney

Falling Klotho levels

Klotho is a transmembrane protein strongly expressed in the kidney and a co-receptor for fibroblast growth factor-23 (FGF-23). Whilst its exact physiological role in aging remains incompletely understood Klotho has a role in modulating diverse aging associated pathways. These include calcium and phosphate metabolism with implications for vascular calcification, hypoxia, cellular regeneration and senescence. Indeed, homozygous transgenic Klotho knockout mice demonstrate arteriosclerosis and vascular changes as part of their aging phenotype.[40] Similarly, FGF-23 knockout mice display high serum phosphate and increased renal phosphate reabsorption in addition to their aging like phenotypes.[41,42] It may be that these vascular changes contribute directly to the aging phenotype we observe.

Klotho's effects on tissue function, autophagy and fibrosis could contribute to abnormal healing and possibly nephrosclerosis.[43,44] Importantly, Klotho deficient mice exhibit reduced lifespan, skin and muscle atrophy, osteoporosis and ectopic calcification.[45] Conversely, mice overexpressing Klotho have a longer mean lifespan.[43]

Klotho decreases epithelial senescence in response to oxidative stress, reduces binding of nuclear factor kappa-B (NFκB) and increases cell survival

in experimental uremia.[46] Klotho also represses insulin and insulin-like growth factor 1 (IGF1) signalling, likely contributing to reduced oxidative stress in mice and *in vitro* models employing Klotho overexpression.[43,45,47] Importantly, Klotho supplementation in a rat UUO model attenuated renal fibrosis.[48]

Increasing Wnt Activation

Mechanisms for the anti-fibrotic effects of Klotho include suppression of fibroblast growth factor and modulation of Wnt signalling.[49–51] Wnt is a conserved signalling pathway activated post injury which promotes pro-fibrotic gene expression.[52] As Klotho levels fall during aging Wnt signalling increases promoting fibrosis and vascular calcification[53] though further experiments are required to clarify causality. Wnt activation promotes renal fibrosis in murine models and is a target for inhibition[54,55] with antagonism of Wnt and its downstream targets ameliorating experimental renal fibrosis.[56,57] The interplay between potentially causative pathways is illustrated by studies demonstrating that renin-angiotensin-aldosterone signalling is Wnt mediated with experimental blockade protecting mice from post-injury fibrosis and proteinuria.[58]

Declining Peroxisome Proliferator-activated Receptor gamma (PPARγ) levels

PPARγ is a nuclear receptor whose activity decreases with age in experimental rodent models, whilst PPARγ agonists increase Klotho expression.[59,60] The PPARγ pathway protects against oxidative stress and improves vascular function *in vitro* and in aging rats[61–63] with PPARγ agonists

protecting human fibroblasts against features of aging and oxidative stress *in vitro*.[64] PPARγ agonism by pioglitazone or baicalin improves age related vascular oxidative stress or renal inflammation respectively, providing a potential therapeutic strategy for elderly patients with reduced PPARγ activity.[60,65]

Angiotensin II

Angiotensin II (AT2) is increased in aged rats compared to young controls,[66] driving increased fibrosis, glomerular cell growth and ECM accumulation,[67] altered mitochondrial redox function and cytoplasmic oxidative stress in the aging kidney.[66,68,69] Angiotensin I receptor activation simulates the pro-fibrotic β-catenin/Wnt pathway mentioned above.[70] Treating aging rats with captopril reduces TGF-β activity and attenuates renal fibrosis.[71,72] AT2 antagonism via ACEi/ARB improves mitochondrial number and function in rats and further studies are warranted.[73]

Oxidative Stress

A balance exists in tissues between reactive oxygen species(ROS) generation and oxidant scavenging and defence mechanisms. When this balance is disturbed, either by increased generation of ROS, decreased detoxification or both, then oxidative stress may occur. It has been hypothesised that oxidative stress leads to tissue damage and contributes to the aging phenotype. Certainly, there is evidence in murine and human studies, of both increased ROS generation and altered oxidant removal in aging.[74–76]

There is a continuous generation of oxidative species through various mechanisms, including mitochondrial oxidative phosphorylation, which increases within the aging kidney.[76,77]Studies in aged rat kidneys support the theory there is also reduced oxidant defence demonstrating decreased antioxidative capacity and reduced levels of Cu/Zn-SOD, catalase and GSH reductase.[78,79] This overall increased oxidative load may contribute to chronic cellular stress and mitochondrial injury[77] as well as apoptosis and possibly inducing tubular cell damage.[80,81]

Contributing to this increased oxidative stress, it has been noted that sirtuins (important antioxidant molecules) are diminished with age. Sirtuins protect against renal inflammation, fibrosis and apoptosis while improving autophagy.[82,83] Thus, defective ability to respond to cell stress in aged kidneys may contribute to the aged phenotype.[84] Mouse models of reduced SIRT-1 expression demonstrate increased apoptosis and fibrosis following UUO.[85] Additional Sirtuin functions include histone deacetylation and regulation of transcription factors controlling cellular stress and survival.[86,87] Altered Sirtuin levels in aging may contribute to aging phenotypes by altering the kidneys capacity to respond to oxidative stress and thus suffer increased oxidative DNA damage. [88,89] Interestingly, angiotensin-II (AT2) downregulates SIRT-3 *in vitro*, suggesting that the damaging effects of raised AT2 levels and low Sirtuin levels may be related in the aging kidney.[90]

B) Cell cycle progression in the aged kidney

Aged animals have reduced proliferative responses after experimental IRI. Tubular epithelial cells in aged mice express higher levels of zinc-alpha (2)-glycoprotein (AZGP1), limiting proliferation following IRI.[91] Whilst reduced proliferation might be expected to delay recovery, AZGP1 knockout mice displayed worsened fibrosis after IRI with AZGP1 administration being protective, implicating control of proliferation as a mechanism limiting fibrosis with aging.[92] Studies in several CKD models demonstrate G2/M arrest in tubular epithelial cells promotes renal fibrosis, but no studies have examined G2/M arrest in aging kidneys.[93]

Cellular senescence, defined as a state of permanent cell cycle arrest, is a key anti-proliferative response to aging and injury. This crucial process shuts down damaged cells, protects against malignant transformation and limits excess fibrosis at both baseline and following injury.[94]

Senescence may occur as a result of repeated cell division and telomere shortening ('replicative senescence') or following factors such as oxidative stress or genotoxic injury ('stress induced premature senescence' [SIPS]) (Figure 3).[95] Increased numbers of senescent cells accumulate in multiple organs including the kidney with advancing age (identified by $p16^{INK4a}$ or senescence-associated β-galactosidase expression).

Cell senescence limits fibroblast proliferation in tissue wounds however there is increasing interest in the role of the Senescence Associated Secretory Phenotype (SASP) in promoting fibrosis.[94] SASP promotes fibrosis and organ

dysfunction in aging via release of factors including Interleukins-6 and 8, Wnt16B and GROα.[96–98] Studies in murine renal transplantation showed that renal $p16^{INK4a}$ deletion reduced pathologic changes and interstitial fibrosis post ischemia reperfusion injury, supporting clinical findings that cellular senescence contributes to adverse long-term allograft outcomes.[99] Cell stress is known to induce SIPS, and consistent with this porcine models have shown that renal $p16^{INK4a}$ expression increases after IRI.[100] Interestingly, $p16^{INK4a}$ knockout mice exposed to experimental renal injury show improved recovery after IRI but worsened fibrosis after UUO.[101,102] These superficially inconsistent findings may reflect the different pathological processes at play, with $p16^{INK4a}$ deficiency leading to less cell death and enhanced regenerative proliferation in AKI, but the lack of $p16^{INK4a}$ induced senescence inducing an exaggerated, maladaptive fibroblast response to ongoing injury in UUO.

Recent seminal studies used transgenic animals to induce specific depletion of $p16^{INK4a}$ expressing senescent cells and demonstrated reduced markers of aging in multiple organs including the kidney and increased overall lifespan.[103] Other work has used Bcl2/xL inhibitors to deplete senescent cells in non-transgenic animals.[104] Whilst these findings open up exciting new therapeutic avenues for the selective targeting of senescent cells to prolong healthy lifespan, further studies focusing upon the aging kidney required.

Telomere Shortening

Telomeres are nucleotide sequences which act as a defensive “cap”: limiting activation of DNA repair pathways, protecting genetic material and minimising

background cellular stress response.[105,106] Although telomere length declines with age, it remains controversial whether this is a primary process or a by-product of aging.[105,107] As telomeres shorten with aging and oxidative stress, chromosome instability ensues, leading to cellular instability, senescence and subsequent apoptosis.[108]

Increased telomere shortening in telomerase deficient mice is associated with increased tubular injury and reduced tubular proliferation after renal IRI with reduced tubular cell autophagy implicated in the limited regenerative response.[109,110] This implies a potential causal role for telomere shortening in some of the vulnerability of aging kidneys to injury and it is noteworthy that experimental elongation of shortened telomeres resulted in partial reversal of aged organ degeneration.[111]

C) Hypoxic Damage and Disordered Repair.

Under physiological conditions, the kidney is supported by a network of resident mononuclear phagocytes and pericytes contributing to tissue homeostasis and vascular stability. Renal oxygen delivery and the functional status of resident and recruited cells in the kidney have been shown to alter in aged and injured experimental animals.

Hypoxia

Whilst the healthy kidney has areas of low oxygen tension, reduced capillary density and increased hypoxia is recognised as a potential driver of CKD, and its role in normal aging is being explored. In experimental CKD, the expected

angiogenic response to hypoxia fails, instead resulting in fibrosis.[112] Increased renal hypoxia has also been demonstrated throughout aged rat kidneys, most prominently in the cortical zones, as detected by use of the hypoxia sensitive marker pimonidazole.[113] Aged rat kidneys demonstrate decreased VEGF globally and increased anti-angiogenic thrombospondin-1, resulting in capillary loss with increased glomerular sclerosis.[114] Recently reported techniques to quantify subtle changes in the renal vasculature have potential to yield new information on the evolution of renal circulatory changes and hypoxia with advancing age.[115]

Leukocytes

Changes in leukocyte function promoting inflammatory activation occur with aging, though whether this is a cause or effect of aging remains unclear.[116] Increased inflammatory signalling and macrophage infiltration,[117] with alterations in inflammasome components such as NOD-like receptor P3 (NLRP3), NLRC4, pro-caspase-1, NFκB and cytokines including IL-1β and IL-18 occur in aging.[118] Aged murine macrophages demonstrate impaired autophagy and reduced nitrate release and phagocytosis.[119] Healthy aged mice have increased glomerular macrophage numbers with increased macrophage infiltration evident post injury, with renal IRI models showing an increased influx of macrophage and T lymphocytes.[39,120] Additionally, aged mice show defective upregulation of the cytoprotective enzyme hemoxygenase-1 after IRI, with pharmacological macrophage hemoxygenase-1 induction protecting against subsequent IRI.[36] Finally, aged macrophages express reduced anti-inflammatory IL-10 during tissue repair in

non-renal injury models.[121] Given the importance of IL-10, and the negative prognostic role of macrophage infiltrates in human renal disease, these aging-associated changes potentially contribute to the increased rates of injury and maladaptive repair seen in aged kidneys.

Further evidence for the importance of the aging immune system in renal aging comes from young-old bone marrow transplant (BMT) studies demonstrating that aged animals receiving BMTs from young mice exhibited reduced renal fibrosis and cellular senescence.[122]

Pericytes

Although important for microvascular health pericytes are also recognised as key cells in renal fibrosis.[123,124] In aged mice renal pericytes decline in number and adopt a pro-fibrotic phenotype,[125] implicating them in aging related fibrotic changes. Pericyte-endothelial detachment under pathological conditions and their differentiation into myofibroblasts promotes microvascular rarefaction, hypoxia and fibrosis.[126,127] Proposed mediators of this pericyte-endothelial cross talk include VEGF and PDGF[128] and blocking this pericyte-endothelial interaction attenuates microvascular damage and interstitial fibrosis.[129,130]

Disordered Repair.

The normal enzymatic equilibrium is disturbed in aging and the balance of metalloproteinases (MMP) shifts towards fibrosis potentially via upregulation of tissue inhibitor of metalloproteinase-1 and increased leukocyte recruitment,[51] a pattern likely to result in increased collagen deposition.

Longitudinal studies of aging mice show increased Collagen I, III and TGF-β1[51] whilst aging rat kidneys exhibit increased ECM deposition and TGF-β3 expression and decreased MMP1 activity suggesting altered collagen production and processing.[131] Further non-inflammatory pathways may contribute to histological changes seen, including pathways driven by Wnt and AT2 as mentioned.[55]

The Aging Human Kidney

The clinical implications of renal aging in man extend beyond changes in glomerular and tubular function. Although data generated by animal studies implicate multiple pathways of potential importance for human renal aging (Figure 4), data supporting their involvement in man is currently sparse, with further studies required.

A) Signalling pathways and oxidative stress in the aging kidney

Falling Klotho levels

Klotho and FGF-23 are present in human kidneys.[132] Klotho levels decline with age, and are implicated in accelerated age-related CKD and atherosclerosis.[133,134] Conversely, patients with increased functional Klotho expression are reported to have increased lifespan.[135] As Klotho falls, FGF-23 levels increase, and alter phosphate and calcium homeostasis. Clinical studies in dialysis and CKD patients show that higher FGF-23 levels associate with increased mortality.[136]

Increasing Wnt activation

Whilst direct evidence of Wnt activation in human aging is lacking, several Wnt antagonists are now undergoing Phase I clinical trials for cancer therapy in man.[137] If effective, these agents offer new therapeutic options for aging associated or fibrotic renal disease.

Declining PPARγ levels

Agonists of PPARγ are used clinically as anti-diabetic agents. Retrospective reviews of renal outcomes in clinical practice suggest that augmented PPARγ activity opposes proteinuria in these patients.[138] A meta-analysis of PPARγ use has also demonstrated that they associate with reduced rates of cerebrovascular disease, supporting a role in delaying age-associated pathology.[139] There is a need for prospective trials assessing their effects on renal function.

Angiotensin II

Despite decreased plasma renin activity in the elderly serum angiotensin II levels do not fall and hypersensitivity to angiotensin II develops in the renal vasculature.[140,141] Whilst ACEi and ARB drugs are in widespread use, there is a lack of human data on the impact of AT2 blocking treatments on normal renal aging and outcomes at present.

Oxidative Stress

As discussed, oxidative stress represents a disruption of the balance of oxidant handling in tissues. In man longitudinal studies demonstrate increased oxidative stress in normal aging and CKD.[74,142] Research has focused on advanced glycation end products as drivers of oxidative stress in aging. These molecules accumulate with age and are associated with increased arterial stiffness, inflammation, oxidative stress and declining renal function.[143] One pharmacological attempt to modify anti-oxidant status in patients with diabetic nephropathy showed no impact on proteinuria despite increased circulating antioxidant levels.[144] Whether an alternative, longer term treatment approach in the healthy aged population might have efficacy remains untested.

B) Cell cycle progression in the aged kidney

The presence of increased numbers of senescent cells has been noted in chronic allograft nephropathy and have been proposed as drivers of the progressive fibrosis seen.[145] Recent advances in our understanding of the roles of aging and stress in inducing the detrimental SASP phenotype adds to the importance of senescence cells found in both aged and disease affected human renal biopsies.[146–148] In humans, senescence is maximal in the medulla, potentially reflecting increased oxidative and cellular stress and relative hypoxia resulting from the vascular changes discussed previously.[149]

Telomeres

Telomeres shorten in human kidneys at a rate of 0.25% length per year.[150] While telomere shortening provides an elegant explanation of cellular aging, currently no data exists to link shorter telomeres to any histological or functional measure of renal aging. Shorter telomeres associate with CKD and worse cardiovascular outcomes and are shorter in diabetic nephropathy where they associate with rates of disease progression.[151,152] Furthermore, studies of hemodialysis patients show increased rates of telomere attrition suggesting they shorten in response to the physiological stress.[153] Although intriguing, the importance of telomere shortening in human aging remains to be elucidated.

C) Hypoxia, inflammation and nephrosclerosis in the aged kidney

Due to the inherent risks of renal biopsy, samples of healthy aged kidney are seldom available for assessment of levels of nephrosclerosis, and there are no time course studies available to chart the temporal relationships of the histological findings in the aged kidney. Ongoing progress in imaging technology should enable serial non-invasive assessment of renal perfusion, vascular resistance, hypoxia, inflammation and atrophy in healthy young and aged volunteers.

Renal Hypoxia

The clinical use of BOLD MRI imaging has demonstrated a lower pO_2 in older kidneys compared to younger subjects.[154] As intrarenal vascular disease contributes to increased glomerular sclerosis in aged biopsies it is possible

that subclinical disease leads to hypoxia before marked macroscopic changes occur.[155]

Inflammation

Inflammation is increased within the aging kidney in man, with pro-inflammatory cytokines detectable in the serum correlating with age related renal disease.[156,157]

Future Research

Reviewing the current evidence base in clinical and experimental renal aging it is clear that more work is required to understand which pathways are dispensable and which represent 'master regulators' of the aging phenotype. Studies in aged animals should allow characterisation of both the importance and interdependence of factors predisposing aged kidneys to injury, fibrosis and maladaptive repair, with subsequent validation in man. Due to the time and cost constraints inherent in using aged animals, establishing whether models of genetically accelerated aging such as the BubR1 progeroid mouse represent useful models of renal aging will be of value.[158] BubR1 mice have a shortened lifespan and exhibit a variety of age related phenotypes, including sarcopenia, cataracts, fat loss, cardiac arrhythmias, arterial wall stiffening and impaired wound healing. Specific to kidney research, BubR1 deficient mice also demonstrate higher senescence-associated beta-galactosidase activity in kidney sections than aged matched controls.[159] Whether they truly manifest a renal aging phenotype is yet to be determined.

Circulating factors

Heterochronic parabiosis with aged and young mice sharing a common circulation has provided evidence in non-renal models that circulating factors may modulate features of aging including impaired regeneration and increased fibrosis.[160–162] Proposed factors include β2-microglobulin and growth differentiation factor 11 and reversal of changes in the brain, cardiac and skeletal muscle has been shown.[163–165] Debate continues as to the significance of individual factors.[166–170] Whether such factors impact the function of the aged kidney remains completely unknown.

Novel experimental species

Undertaking studies of experimental renal disease in aged mice is challenging and other organisms may be of use. Zebrafish have been used as a model for AKI and nephron regeneration and exhibit aging associated abnormalities.[171–173] Thus the use of genetically manipulated zebrafish in renal aging studies may be informative.

Novel therapeutic strategies

Many pathways implicated in the aging process are the target of interventions to improve the aging phenotype in experimental mice (Figure 5). Klotho agonists are under investigation via repurposing of established agents including PPARγ agonists, ACEI and ARB drugs. The importance of maintaining a normal renal microvasculature and pericyte pool is increasingly understood[174] and developing strategies to quantify microvascular function and to promote endothelial and pericyte health is a pressing clinical need.[115]

Drugs targeting cellular senescence ('senolytics') include siRNA therapies, the experimental agent navitoclax and the licenced drugs dasatinib and quercetin.[175] In experiments these agents demonstrate selective toxicity to senescent cells, and their potential utility in animal models and man merits further study.

Genetics

Genome wide association studies (GWAS) have identified upregulation of several genes with aging. Whilst cumulative damage may well influence much of the elderly genetic milieu, candidate genes have declared themselves as being consistently highly expressed in aged kidneys.[176–178] Despite the utility of GWAS in identifying disease specific pathways, it has proved difficult to discover any canonical aging pathways with GWAS.[179]

The most promising genes encode for modulators of the glomerular filtration barrier, fibrosis and inflammatory mediators although difficulty arises when identified candidate genes do not match the experimental observations or models.[180,181] Transcriptomic analysis identified 427 genes strongly associated with renal aging, including mortalin-2, a heat shock protein which may counteract cell senescence and IGF receptor, a target of Klotho.[182–184]

GWAS remains however, a promising tool as whole genome analyses of GWAS data suggest that over 80 % of the heritability of aging is explained by common genetic variants.[185] Future GWAS will continue to generate

meaningful results as more advanced statistical techniques develop, and researchers increase statistical power by increasing samples number, combining studies using meta-analytical techniques, multicentre collaborations and including more extreme phenotypes in the data.[185–188]

Epigenetics

Epigenetics is the study of genome changes that do not alter DNA sequence. Epigenetic changes in aging include methylation and deacetylation of histone lysine residues, chromatin changes and increased transcriptional noise.[179,189] Interestingly, similar changes in DNA methylation and histones are associated with CKD disease progression.[190–192] The role of microRNA expression in modifying gene expression and nephrosclerosis is of interest,[193] with data in other organs suggesting an influence on aging.[194]

Conclusion

Renal aging is complex and remains incompletely understood. Decreased protective factors, hypoxia and microenvironmental stress drive increasingly disordered inflammation and renal fibrosis. The resulting fibrosis, senescence and microvascular rarefaction exacerbate damage and promote progression. The future of treating renal aging likely lies in understanding the key initiating events and the common downstream pathways present in kidney aging that may be shared with CKD. This knowledge should allow the development of therapies capable of arresting the key mechanisms early to preserve kidney function throughout life.

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REFERENCES

1. Centers for Disease Control and Prevention: The State of Aging and Healthy in America 2013. Atlanta, GA:
2. European Commission: The 2015 Ageing Report. *Eur. Econ.* 3: 424, 2015
3. Zhou XJ, Rakheja D, Yu X, Saxena R, Vaziri ND, Silva FG: The aging kidney. *Kidney Int.* 74: 710–20, 2008
4. Newbold KM, Sandison A, Howie AJ: Comparison of size of juxtamedullary and outer cortical glomeruli in normal adult kidney. *Virchows Arch. A. Pathol. Anat. Histopathol.* 420: 127–9, 1992
5. Nyengaard JR, Bendtsen TF: Glomerular number and size in relation to age, kidney weight, and body surface in normal man. *Anat. Rec.* 232: 194–201, 1992
6. Rule AD, Amer H, Cornell LD, Taler SJ, Cosio FG, Kremers WK, Textor SC, Stegall MD: The association between age and nephrosclerosis on renal biopsy among healthy adults. *Ann. Intern. Med.* 152: 561–7, 2010
7. Chan KW, Leung CY, Chan CW: Age-related glomerular sclerosis: baseline values in Hong Kong. *Pathology* 22: 177–80, 1990
8. Denic A, Alexander MP, Kaushik V, Lerman LO, Lieske JC, Stegall MD, Larson JJ, Kremers WK, Vrtiska TJ, Chakkera HA, Poggio ED, Rule AD: Detection and Clinical Patterns of Nephron Hypertrophy and Nephrosclerosis Among Apparently Healthy Adults. *Am. J. Kidney Dis.* 68: 58–67, 2016
9. Rule AD, Cornell LD, Poggio ED: Senile nephrosclerosis--does it explain the decline in glomerular filtration rate with aging? *Nephron. Physiol.* 119 Suppl : p6–11, 2011
10. Rule AD, Amer H, Cornell LD, Taler SJ, Cosio FG, Kremers WK, Textor SC, Stegall MD: The association between age and nephrosclerosis on renal biopsy among healthy adults. *Ann. Intern. Med.* 152: 561–7, 2010
11. Meyrier A: Nephrosclerosis: a term in quest of a disease. *Nephron* 129: 276–82, 2015
12. Meyrier A: Nephrosclerosis: update on a centenarian. *Nephrol. Dial. Transplant* 2014
13. Ferenbach DA, Bonventre J V: Mechanisms of maladaptive repair after AKI leading to accelerated kidney ageing and CKD. *Nat. Rev. Nephrol.* 11: 264–76, 2015
14. Rowe JW, Andres R, Tobin JD, Norris AH, Shock NW: The effect of age on creatinine clearance in men: a cross-sectional and longitudinal study. *J. Gerontol.* 31: 155–63, 1976
15. Lindeman RD, Tobin J, Shock NW: Longitudinal studies on the rate of decline in renal function with age. *J. Am. Geriatr. Soc.* 33: 278–85, 1985
16. Baba M, Shimbo T, Horio M, Ando M, Yasuda Y, Komatsu Y, Masuda K, Matsuo S, Maruyama S: Longitudinal Study of the Decline in Renal Function in Healthy Subjects. *PLoS One* 10: e0129036, 2015
17. Cohen E, Nardi Y, Krause I, Goldberg E, Milo G, Garty M, Krause I: A longitudinal assessment of the natural rate of decline in renal function with age. *J. Nephrol.* 2014
18. Wetzels JFM, Kiemeney LALM, Swinkels DW, Willems HL, den Heijer M: Age- and gender-specific reference values of estimated GFR in Caucasians: the Nijmegen Biomedical Study. *Kidney Int.* 72: 632–7, 2007
19. Sarnak MJ, Poindexter A, Wang S-R, Beck GJ, Kusek JW, Marcovina SM, Greene T, Levey AS: Serum C-reactive protein and leptin as predictors of kidney disease progression in the Modification of Diet in Renal Disease Study. *Kidney Int.* 62: 2208–15, 2002
20. IMAI E, HORIO M, YAMAGATA K, ISEKI K, HARA S, URA N, KIYOHARA Y, MAKINO H, HISHIDA A, MATSUO S: Slower Decline of Glomerular Filtration Rate in the

Japanese General Population: A Longitudinal 10-Year Follow-Up Study. *Hypertens. Res.* 31: 433–441, 2008

21. Hollenberg NK, Rivera A, Meinking T, Martinez G, McCullough M, Passan D, Preston M, Taplin D, Vicaria-Clement M: Age, renal perfusion and function in island-dwelling indigenous Kuna Amerinds of Panama. *Nephron* 82: 131–8, 1999

22. Sands JM: Urine concentrating and diluting ability during aging. *J. Gerontol. A. Biol. Sci. Med. Sci.* 67: 1352–7, 2012

23. Mimran A, Ribstein J, Jover B: Aging and sodium homeostasis. *Kidney Int. Suppl.* 37: S107–13, 1992

24. Michelis MF: Hyperkalemia in the elderly. *Am. J. Kidney Dis.* 16: 296–9, 1990

25. Mc Greevy C, Horan J, Jones D, Biswas K, O'Meara YM, Mulkerrin EC: A study of tubular potassium secretory capacity in older patients with hyperkalaemia. *J. Nutr. Health Aging* 12: 152–5, 2008

26. Musso C, Liakopoulos V, Stefanidis I, De Miguel R, Imperiali N, Algranati L: Correlation between creatinine clearance and transtubular potassium concentration gradient in old people and chronic renal disease patients. *Saudi J. Kidney Dis. Transpl.* 18: 551–5, 2007

27. Takazakura E, Sawabu N, Handa A, Takada A, Shinoda A, Takeuchi J: Intrarenal vascular changes with age and disease. *Kidney Int.* 2: 224–230, 1972

28. Hajduczok G, Chapleau MW, Johnson SL, Abboud FM: Increase in sympathetic activity with age. I. Role of impairment of arterial baroreflexes. *Am. J. Physiol.* 260: H1113–20, 1991

29. Jerkić M, Vojvodić S, López-Novoa JM: The mechanism of increased renal susceptibility to toxic substances in the elderly. Part I. The role of increased vasoconstriction. *Int. Urol. Nephrol.* 32: 539–47, 2001

30. Mulkerrin EC, Brain A, Hampton D, Penney MD, Sykes DA, Williams JD, Coles GA, Woodhouse KW: Reduced renal hemodynamic response to atrial natriuretic peptide in elderly volunteers. *Am. J. Kidney Dis.* 22: 538–44, 1993

31. Fuiano G, Sund S, Mazza G, Rosa M, Caglioti A, Gallo G, Natale G, Andreucci M, Memoli B, De Nicola L, Conte G: Renal hemodynamic response to maximal vasodilating stimulus in healthy older subjects. *Kidney Int.* 59: 1052–1058, 2001

32. Fliser D, Zeier M, Nowack R, Ritz E: Renal functional reserve in healthy elderly subjects. *J. Am. Soc. Nephrol.* 3: 1371–7, 1993

33. Sverdlov AL, Ngo DTM, Chan WPA, Chirkov YY, Horowitz JD: Aging of the nitric oxide system: are we as old as our NO? *J. Am. Heart Assoc.* 3: 2014

34. Kielstein JT, Bode-Böger SM, Frölich JC, Ritz E, Haller H, Fliser D: Asymmetric dimethylarginine, blood pressure, and renal perfusion in elderly subjects. *Circulation* 107: 1891–5, 2003

35. Ahmed SB, Fisher NDL, Hollenberg NK: Gender and the renal nitric oxide synthase system in healthy humans. *Clin. J. Am. Soc. Nephrol.* 2: 926–31, 2007

36. Ferenbach DA, Nkejabega NCJ, McKay J, Choudhary AK, Vernon MA, Beesley MF, Clay S, Conway BC, Marson LP, Kluth DC, Hughes J: The induction of macrophage hemeoxygenase-1 is protective during acute kidney injury in aging mice. *Kidney Int.* 79: 966–76, 2011

37. Nath K a, Grande JP, Farrugia G, Croatt AJ, Belcher JD, Hebbel RP, Vercellotti GM, Katusic ZS: Age sensitizes the kidney to heme protein-induced acute kidney injury. *Am. J. Physiol. Renal Physiol.* 304: F317–25, 2013

38. Maddens B, Vandendriessche B, Demon D, Vanholder R, Chiers K, Cauwels A, Meyer E: Severity of sepsis-induced acute kidney injury in a novel mouse model is age

dependent*. *Crit. Care Med.* 40: 2638–2646, 2012

39. Clements ME, Chaber CJ, Ledbetter SR, Zuk A: Increased cellular senescence and vascular rarefaction exacerbate the progression of kidney fibrosis in aged mice following transient ischemic injury. *PLoS One* 8: e70464, 2013

40. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, Iwasaki H, Iida A, Shiraki-Iida T, Nishikawa S, Nagai R, Nabeshima YI: Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature* 390: 45–51, 1997

41. Shimada T, Kakitani M, Yamazaki Y, Hasegawa H, Takeuchi Y, Fujita T, Fukumoto S, Tomizuka K, Yamashita T: Targeted ablation of Fgf23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. *J. Clin. Invest.* 113: 561–8, 2004

42. Razzaque MS, Sitara D, Taguchi T, St-Arnaud R, Lanske B: Premature aging-like phenotype in fibroblast growth factor 23 null mice is a vitamin D mediated process.

43. Kurosu H, Yamamoto M, Clark JD, Pastor J V, Nandi A, Gurnani P, McGuinness OP, Chikuda H, Yamaguchi M, Kawaguchi H, Shimomura I, Takayama Y, Herz J, Kahn CR, Rosenblatt KP, Kuro-o M: Suppression of aging in mice by the hormone Klotho. *Science* 309: 1829–33, 2005

44. John GB, Cheng C-Y, Kuro-o M: Role of Klotho in aging, phosphate metabolism, and CKD. *Am. J. Kidney Dis.* 58: 127–34, 2011

45. Kuro-o M: Klotho as a regulator of oxidative stress and senescence. *Biol. Chem.* 389: 233–41, 2008

46. Sopjani M, Rinnerthaler M, Kruja J, Dermaku-Sopjani M: Intracellular signaling of the aging suppressor protein Klotho. *Curr. Mol. Med.* 15: 27–37, 2015

47. Yang H, Fogo AB: Cell senescence in the aging kidney. *J. Am. Soc. Nephrol.* 21: 1436–9, 2010

48. Liu Q-F, Ye J-M, Deng Z-Y, Yu L-X, Sun Q, Li S-S: Ameliorating Effect of Klotho on Endoplasmic Reticulum Stress and Renal Fibrosis Induced by Unilateral Ureteral Obstruction. *Iran. J. Kidney Dis.* 9: 291–7, 2015

49. Kuro-o M: Klotho as a regulator of fibroblast growth factor signaling and phosphate/calcium metabolism. *Curr. Opin. Nephrol. Hypertens.* 15: 437–41, 2006

50. Zhou L, Li Y, Zhou D, Tan RJ, Liu Y: Loss of Klotho contributes to kidney injury by derepression of Wnt/β-catenin signaling. *J. Am. Soc. Nephrol.* 24: 771–85, 2013

51. Zhang X, Chen X, Hong Q, Lin H, Zhu H, Liu Q, Wang J, Xie Y, Shang X, Shi S, Lu Y, Yin Z: TIMP-1 promotes age-related renal fibrosis through upregulating ICAM-1 in human TIMP-1 transgenic mice. *J. Gerontol. A. Biol. Sci. Med. Sci.* 61: 1130–43, 2006

52. Tan RJ, Zhou D, Zhou L, Liu Y: Wnt/β-catenin signaling and kidney fibrosis. *Kidney Int. Suppl.* 4: 84–90, 2014

53. Hu MC, Bian A, Neyra J, Zhan M: Klotho, stem cells, and aging. *Clin. Interv. Aging* 10: 1233, 2015

54. Brack AS, Conboy MJ, Roy S, Lee M, Kuo CJ, Keller C, Rando TA: Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. *Science* 317: 807–10, 2007

55. Maarouf OH, Aravamudhan A, Rangarajan D, Kusaba T, Zhang V, Welborn J, Gauvin D, Hou X, Kramann R, Humphreys BD: Paracrine Wnt1 Drives Interstitial Fibrosis without Inflammation by Tubulointerstitial Cross-Talk. *J. Am. Soc. Nephrol.* 2015

56. Hao S, He W, Li Y, Ding H, Hou Y, Nie J, Hou FF, Kahn M, Liu Y: Targeted inhibition of β-catenin/CBP signaling ameliorates renal interstitial fibrosis. *J. Am. Soc. Nephrol.* 22: 1642–53, 2011

57. He W, Kang YS, Dai C, Liu Y: Blockade of Wnt/β-catenin signaling by paricalcitol ameliorates proteinuria and kidney injury. *J. Am. Soc. Nephrol.* 22: 90–103, 2011

58. Zhou L, Li Y, Hao S, Zhou D, Tan RJ, Nie J, Hou FF, Kahn M, Liu Y: Multiple genes of the renin-angiotensin system are novel targets of Wnt/β-catenin signaling. *J. Am. Soc. Nephrol.* 26: 107–20, 2015

59. Iemitsu M, Miyauchi T, Maeda S, Tanabe T, Takanashi M, Irukayama-Tomobe Y, Sakai S, Ohmori H, Matsuda M, Yamaguchi I: Aging-induced decrease in the PPAR-alpha level in hearts is improved by exercise training. *Am. J. Physiol. Heart Circ. Physiol.* 283: H1750–60, 2002

60. Wang P, Li B, Cai G, Huang M, Jiang L, Pu J, Li L, Wu Q, Zuo L, Wang Q, Zhou P: Activation of PPAR-γ by pioglitazone attenuates oxidative stress in aging rat cerebral arteries through upregulating UCP2. *J. Cardiovasc. Pharmacol.* 64: 497–506, 2014

61. Zhang H, Li Y, Fan Y, Wu J, Zhao B, Guan Y, Chien S, Wang N: Klotho is a target gene of PPAR-gamma. *Kidney Int.* 74: 732–9, 2008

62. Zhang R, Zheng F: PPAR-gamma and aging: one link through klotho? *Kidney Int.* 74: 702–4, 2008

63. Sung B, Park S, Yu BP, Chung HY: Modulation of PPAR in aging, inflammation, and calorie restriction. *J. Gerontol. A. Biol. Sci. Med. Sci.* 59: 997–1006, 2004

64. Briganti S, Flori E, Bellei B, Picardo M: Modulation of PPARγ provides new insights in a stress induced premature senescence model. *PLoS One* 9: e104045, 2014

65. Lim HA, Lee EK, Kim JM, Park MH, Kim DH, Choi YJ, Ha YM, Yoon J-H, Choi JS, Yu BP, Chung HY: PPARγ activation by baicalin suppresses NF-κB-mediated inflammation in aged rat kidney. *Biogerontology* 13: 133–45, 2012

66. Sangaralingham SJ, Wang BH, Huang L, Kumfu S, Ichiki T, Krum H, Burnett Jr. JC: Cardiorenal fibrosis and dysfunction in aging: Imbalance in mediators and regulators of collagen. *Peptides* 76: 108–114, 2016

67. Fogo AB: The role of angiotensin II and plasminogen activator inhibitor-1 in progressive glomerulosclerosis. *Am. J. Kidney Dis.* 35: 179–88, 2000

68. Vajapey R, Rini D, Walston J, Abadir P: The impact of age-related dysregulation of the angiotensin system on mitochondrial redox balance. *Front. Physiol.* 5: 439, 2014

69. Benigni A, Cassis P, Remuzzi G: Angiotensin II revisited: new roles in inflammation, immunology and aging. *EMBO Mol. Med.* 2: 247–57, 2010

70. Cuevas CA, Gonzalez AA, Inestrosa NC, Vio CP, Prieto MC: Angiotensin II increases fibronectin and collagen I through the β-catenin-dependent signaling in mouse collecting duct cells. *Am. J. Physiol. Renal Physiol.* 308: F358–65, 2015

71. Ruiz-Torres MP, Bosch RJ, O'Valle F, Del Moral RG, Ramírez C, Masseroli M, Pérez-Caballero C, Iglesias MC, Rodríguez-Puyol M, Rodríguez-Puyol D: Age-related increase in expression of TGF-beta1 in the rat kidney: relationship to morphologic changes. *J. Am. Soc. Nephrol.* 9: 782–91, 1998

72. Cruz CI, Ruiz-Torres P, del Moral RG, Rodríguez-Puyol M, Rodríguez-Puyol D: Age-related progressive renal fibrosis in rats and its prevention with ACE inhibitors and taurine. *Am. J. Physiol. Renal Physiol.* 278: F122–9, 2000

73. de Cavanagh EM V., Piotrkowski B, Basso N, Stella I, Inserra F, Ferder L, Fraga CG: Enalapril and losartan attenuate mitochondrial dysfunction in aged rats. *FASEB J.* 17: 1096–8, 2003

74. Vlassara H, Torreggiani M, Post JB, Zheng F, Uribarri J, Striker GE: Role of oxidants/inflammation in declining renal function in chronic kidney disease and normal aging. *Kidney Int. Suppl.* S3–11, 2009

75. Simão S, Gomes P, Pinto V, Silva E, Amaral JS, Igreja B, Afonso J, Serrão MP, Pinho

MJ, Soares-Da-Silva P: Age-related changes in renal expression of oxidant and antioxidant enzymes and oxidative stress markers in male SHR and WKY rats. *EXG* 46: 468–474, 2011

76. Miyazawa M, Ishii T, Yasuda K, Noda S, Onouchi H, Hartman PS, Ishii N: The role of mitochondrial superoxide anion (O2(-)) on physiological aging in C57BL/6J mice. *J. Radiat. Res.* 50: 73–83, 2009

77. Nath KA: The role of Sirt1 in renal rejuvenation and resistance to stress. *J. Clin. Invest.* 120: 1026–8, 2010

78. Akçetin Z, Erdemli G, Brömme HJ: Experimental study showing a diminished cytosolic antioxidative capacity in kidneys of aged rats. *Urol. Int.* 64: 70–3, 2000

79. Martin R, Fitzl G, Mozet C, Martin H, Welt K, Wieland E: Effect of age and hypoxia/reoxygenation on mRNA expression of antioxidative enzymes in rat liver and kidneys. *Exp. Gerontol.* 37: 1481–7, 2002

80. Qiao X, Chen X, Wu D, Ding R, Wang J, Hong Q, Shi S, Li J, Xie Y, Lu Y, Wang Z: Mitochondrial pathway is responsible for aging-related increase of tubular cell apoptosis in renal ischemia/reperfusion injury. *J. Gerontol. A. Biol. Sci. Med. Sci.* 60: 830–9, 2005

81. Small DM, Bennett NC, Roy S, Gabrielli BG, Johnson DW, Gobe GC: Oxidative stress and cell senescence combine to cause maximal renal tubular epithelial cell dysfunction and loss in an in vitro model of kidney disease. *Nephron. Exp. Nephrol.* 122: 123–30, 2012

82. Kitada M, Kume S, Takeda-Watanabe A, Kanasaki K, Koya D: Sirtuins and renal diseases: relationship with aging and diabetic nephropathy. *Clin. Sci.* 124: 153–164, 2013

83. Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA, Mayo MW: Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J.* 23: 2369–80, 2004

84. Kume S, Uzu T, Horiike K, Chin-Kanasaki M, Isshiki K, Araki S-I, Sugimoto T, Haneda M, Kashiwagi A, Koya D: Calorie restriction enhances cell adaptation to hypoxia through Sirt1-dependent mitochondrial autophagy in mouse aged kidney. *J. Clin. Invest.* 120: 1043–55, 2010

85. He W, Wang Y, Zhang M-Z, You L, Davis LS, Fan H, Yang H-C, Fogo AB, Zent R, Harris RC, Breyer MD, Hao C-M: Sirt1 activation protects the mouse renal medulla from oxidative injury. *J. Clin. Invest.* 120: 1056–68, 2010

86. Sauve AA, Wolberger C, Schramm VL, Boeke JD: The biochemistry of sirtuins. *Annu. Rev. Biochem.* 75: 435–65, 2006

87. Longo VD, Kennedy BK: Sirtuins in aging and age-related disease. *Cell* 126: 257–68, 2006

88. Radak Z, Koltai E, Taylor AW, Higuchi M, Kumagai S, Ohno H, Goto S, Boldogh I: Redox-regulating sirtuins in aging, caloric restriction, and exercise. *Free Radic. Biol. Med.* 58: 87–97, 2013

89. Park S, Mori R, Shimokawa I: Do sirtuins promote mammalian longevity? A critical review on its relevance to the longevity effect induced by calorie restriction. *Mol. Cells* 35: 474–80, 2013

90. Benigni A, Corna D, Zoja C, Sonzogni A, Latini R, Salio M, Conti S, Rottoli D, Longaretti L, Cassis P, Morigi M, Coffman TM, Remuzzi G: Disruption of the Ang II type 1 receptor promotes longevity in mice. *J. Clin. Invest.* 119: 524–530, 2009

91. Schmitt R, Marlier A, Cantley LG: Zag expression during aging suppresses proliferation after kidney injury. *J. Am. Soc. Nephrol.* 19: 2375–83, 2008

92. Sörensen-Zender I, Bhayana S, Susnik N, Rolli V, Batkai S, Arpita B, Bahram S, Sen

P, Teng B, Lindner R, Schiffer M, Thum T, Melk A, Haller H, Schmitt R: Zinc-α2-Glycoprotein Exerts Antifibrotic Effects in Kidney and Heart. *J. Am. Soc. Nephrol.* 2015

93. Yang L, Besschetnova TY, Brooks CR, Shah J V, Bonventre J V: Epithelial cell cycle arrest in G2/M mediates kidney fibrosis after injury. *Nat. Med.* 16: 535–43, 1p following 143, 2010

94. Jun J-I, Lau LF: The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. *Nat. Cell Biol.* 12: 676–85, 2010

95. van Deursen JM: The role of senescent cells in ageing. *Nature* 509: 439–46, 2014

96. Yang G, Rosen DG, Zhang Z, Bast RC, Mills GB, Colacino JA, Mercado-Uribe I, Liu J: The chemokine growth-regulated oncogene 1 (Gro-1) links RAS signaling to the senescence of stromal fibroblasts and ovarian tumorigenesis. *Proc. Natl. Acad. Sci. U. S. A.* 103: 16472–7, 2006

97. Kuilman T, Michaloglou C, Vredeveld LCW, Douma S, van Doorn R, Desmet CJ, Aarden LA, Mooi WJ, Peeper DS: Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. *Cell* 133: 1019–31, 2008

98. Binet R, Ythier D, Robles AI, Collado M, Larrieu D, Fonti C, Brambilla E, Brambilla C, Serrano M, Harris CC, Pedeux R: WNT16B is a new marker of cellular senescence that regulates p53 activity and the phosphoinositide 3-kinase/AKT pathway. *Cancer Res.* 69: 9183–91, 2009

99. Braun H, Schmidt BMW, Raiss M, Baisantry A, Mircea-Constantin D, Wang S, Gross M-L, Serrano M, Schmitt R, Melk A: Cellular senescence limits regenerative capacity and allograft survival. *J. Am. Soc. Nephrol.* 23: 1467–73, 2012

100. Chkhotua AB, Abendroth D, Froeba G, Schelzig H: Up-regulation of cell cycle regulatory genes after renal ischemia/reperfusion: differential expression of p16(INK4a), p21(WAF1/CIP1) and p27(Kip1) cyclin-dependent kinase inhibitor genes depending on reperfusion time. *Transpl. Int.* 19: 72–7, 2006

101. Lee DH, Wolstein JM, Pudasaini B, Plotkin M: INK4a deletion results in improved kidney regeneration and decreased capillary rarefaction after ischemia-reperfusion injury. *Am. J. Physiol. Renal Physiol.* 302: F183–91, 2012

102. Wolstein JM, Lee DH, Michaud J, Buot V, Stefanchik B, Plotkin MD: INK4a knockout mice exhibit increased fibrosis under normal conditions and in response to unilateral ureteral obstruction. *Am. J. Physiol. Renal Physiol.* 299: F1486–95, 2010

103. Baker DJ, Childs BG, Durik M, Wijers ME, Sieben CJ, Zhong J, R AS, Jeganathan KB, Verzosa GC, Pezeshki A, Khazaie K, Miller JD, van Deursen JM: Naturally occurring p16-positive cells shorten healthy lifespan. *Nature* 2016

104. Chang J, Wang Y, Shao L, Laberge RM, Demaria M, Campisi J, Janakiraman K, Sharpless NE, Ding S, Feng W, Luo Y, Wang X, Aykin-Burns N, Krager K, Ponnappan U, Hauer-Jensen M, Meng A, Zhou D: Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. *Nat Med* 22: 78–83, 2016

105. Aubert G, Lansdorp PM: Telomeres and aging. *Physiol. Rev.* 88: 557–79, 2008

106. d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, Saretzki G, Carter NP, Jackson SP: A DNA damage checkpoint response in telomere-initiated senescence. *Nature* 426: 194–8, 2003

107. Mikhelson VM, Gamaley IA: Telomere shortening is a sole mechanism of aging in mammals. *Curr. Aging Sci.* 5: 203–8, 2012

108. Wills LP, Schnellmann RG: Telomeres and telomerase in renal health. *J. Am. Soc. Nephrol.* 22: 39–41, 2011

109. Westhoff JH, Schildhorn C, Jacobi C, Hömme M, Hartner A, Braun H, Kryzer C, Wang C, von Zglinicki T, Kränzlin B, Gretz N, Melk A: Telomere shortening reduces regenerative capacity after acute kidney injury. *J. Am. Soc. Nephrol.* 21: 327–36, 2010

110. Cheng H, Fan X, Lawson WE, Paueksakon P, Harris RC: Telomerase deficiency delays renal recovery in mice after ischemia-reperfusion injury by impairing autophagy. *Kidney Int.* 88: 85–94, 2015

111. Jaskelioff M, Muller FL, Paik J-H, Thomas E, Jiang S, Adams AC, Sahin E, Kost-Alimova M, Protopopov A, Cadiñanos J, Horner JW, Maratos-Flier E, Depinho RA: Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. *Nature* 469: 102–6, 2011

112. Ballermann BJ, Obeidat M: Tipping the balance from angiogenesis to fibrosis in CKD. *Kidney Int. Suppl.* 4: 45–52, 2014

113. Tanaka T, Kato H, Kojima I, Ohse T, Son D, Tawakami T, Yatagawa T, Inagi R, Fujita T, Nangaku M: Hypoxia and Expression of Hypoxia-Inducible Factor in the Aging Kidney. *Journals Gerontol. Ser. A Biol. Sci. Med. Sci.* 61: 795–805, 2006

114. Kang DH, Anderson S, Kim YG, Mazzalli M, Suga S, Jefferson JA, Gordon KL, Oyama TT, Hughes J, Hugo C, Kerjaschki D, Schreiner GF, Johnson RJ: Impaired angiogenesis in the aging kidney: vascular endothelial growth factor and thrombospondin-1 in renal disease. *Am. J. Kidney Dis.* 37: 601–11, 2001

115. Kramann R, Tanaka M, Humphreys BD: Fluorescence Microangiography for Quantitative Assessment of Peritubular Capillary Changes after AKI in Mice. *J. Am. Soc. Nephrol.* 1–8, 2014

116. Sebastián C, Espia M, Serra M, Celada A, Lloberas J: MacrophAging: a cellular and molecular review. *Immunobiology* 210: 121–6, 2005

117. Costa E, Fernandes J, Ribeiro S, Sereno J, Garrido P, Rocha-Pereira P, Coimbra S, Catarino C, Belo L, Bronze-da-Rocha E, Vala H, Alves R, Reis F, Santos-Silva A: Aging is Associated with Impaired Renal Function, INF-gamma Induced Inflammation and with Alterations in Iron Regulatory Proteins Gene Expression. *Aging Dis.* 5: 356–65, 2014

118. Song F, Ma Y, Bai X-Y, Chen X: The Expression Changes of Inflammasomes in the Aging Rat Kidneys. *J. Gerontol. A. Biol. Sci. Med. Sci.* 2015

119. Stranks AJ, Hansen AL, Panse I, Mortensen M, Ferguson DJP, Puleston DJ, Shenderov K, Watson AS, Veldhoen M, Phadwal K, Cerundolo V, Simon AK: Autophagy Controls Acquisition of Aging Features in Macrophages. *J. Innate Immun.* 7: 375–391, 2015

120. Zheng F, Cheng Q-L, Plati A-R, Ye SQ, Berho M, Banerjee A, Potier M, Jaimes EA, Yu H, Guan Y-F, Hao C-M, Striker LJ, Striker GE: The glomerulosclerosis of aging in females: contribution of the proinflammatory mesangial cell phenotype to macrophage infiltration. *Am. J. Pathol.* 165: 1789–98, 2004

121. Zhang B, Bailey WM, Braun KJ, Gensel JC: Age decreases macrophage IL-10 expression: Implications for functional recovery and tissue repair in spinal cord injury. *Exp. Neurol.* 273: 83–91, 2015

122. Yang H-C, Rossini M, Ma L-J, Zuo Y, Ma J, Fogo AB: Cells derived from young bone marrow alleviate renal aging. *J. Am. Soc. Nephrol.* 22: 2028–36, 2011

123. Kramann R, Schneider RK, DiRocco DP, Machado F, Fleig S, Bondzie PA, Henderson JM, Ebert BL, Humphreys BD: Perivascular Gli1+ Progenitors Are Key Contributors to Injury-Induced Organ Fibrosis. *Cell Stem Cell* 16: 51–66, 2014

124. Humphreys BD, Lin S-L, Kobayashi A, Hudson TE, Nowlin BT, Bonventre J V, Valerius MT, McMahon AP, Duffield JS: Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. *Am. J. Pathol.* 176: 85–97, 2010

125. Stefanska A, Eng D, Kaverina N, Duffield JS, Pippin JW, Rabinovitch P, Shankland SJ: Interstitial pericytes decrease in aged mouse kidneys. *Aging (Albany. NY).* 7: 370–82, 2015

126. Gomez IG, Duffield JS: The FOXD1 lineage of kidney perivascular cells and myofibroblasts: functions and responses to injury. *Kidney Int. Suppl.* 4: 26–33, 2014

127. Schrimpf C, Teebken OE, Wilhelmi M, Duffield JS: The role of pericyte detachment in vascular rarefaction. *J. Vasc. Res.* 51: 247–58, 2014

128. Rojas A, Chang F-C, Lin S-L, Duffield JS: The role played by perivascular cells in kidney interstitial injury. *Clin. Nephrol.* 77: 400–8, 2012

129. Lin S-L, Chang F-C, Schrimpf C, Chen Y-T, Wu C-F, Wu V-C, Chiang W-C, Kuhnert F, Kuo CJ, Chen Y-M, Wu K-D, Tsai T-J, Duffield JS: Targeting endothelium-pericyte cross talk by inhibiting VEGF receptor signaling attenuates kidney microvascular rarefaction and fibrosis. *Am. J. Pathol.* 178: 911–23, 2011

130. Chen Y-T, Chang F-C, Wu C-F, Chou Y-H, Hsu H-L, Chiang W-C, Shen J, Chen Y-M, Wu K-D, Tsai T-J, Duffield JS, Lin S-L: Platelet-derived growth factor receptor signaling activates pericyte-myofibroblast transition in obstructive and post-ischemic kidney fibrosis. *Kidney Int.* 80: 1170–81, 2011

131. Gagliano N, Arosio B, Santambrogio D, Balestrieri MR, Padoani G, Tagliabue J, Masson S, Vergani C, Annoni G: Age-Dependent Expression of Fibrosis-Related Genes and Collagen Deposition in Rat Kidney Cortex. *Journals Gerontol. Ser. A Biol. Sci. Med. Sci.* 55: B365–B372, 2000

132. Lim K, Groen A, Molostvov G, Lu T, Lilley KS, Snead D, James S, Wilkinson IB, Ting S, Hsiao L-L, Hiemstra TF, Zehnder D: α-KLOTHO EXPRESSISON IN HUMAN TISSUES. *J. Clin. Endocrinol. Metab.* jc20151800, 2015

133. Keles N, Caliskan M, Dogan B, Keles NN, Kalcik M, Aksu F, Kostek O, Aung SM, Isbilen B, Oguz A: Low Serum Level of Klotho Is an Early Predictor of Atherosclerosis. *Tohoku J. Exp. Med.* 237: 17–23, 2015

134. Xu Y, Sun Z: Molecular Basis of Klotho: From Gene to Function in Aging. *Endocr. Rev.* 36: er20131079, 2015

135. Arking DE, Krebsova A, Macek M, Macek M, Arking A, Mian IS, Fried L, Hamosh A, Dey S, McIntosh I, Dietz HC: Association of human aging with a functional variant of klotho. *Proc. Natl. Acad. Sci. U. S. A.* 99: 856–861, 2002

136. Nitta K, Nagano N, Tsuchiya K: Fibroblast growth factor 23/klotho axis in chronic kidney disease. *Nephron. Clin. Pract.* 128: 1–10, 2014

137. Takebe N, Miele L, Harris PJ, Jeong W, Bando H, Kahn M, Yang SX, Ivy SP: Targeting Notch, Hedgehog, and Wnt pathways in cancer stem cells: clinical update. *Nat. Rev. Clin. Oncol.* 12: 1–20, 2015

138. Speeckaert MM, Vanfraechem C, Speeckaert R, Delanghe JR: Peroxisome proliferator-activated receptor agonists in a battle against the aging kidney. *Ageing Res. Rev.* 14: 1–18, 2014

139. Liu J, Wang LN: Peroxisome proliferator-activated receptor gamma agonists for preventing recurrent stroke and other vascular events in patients with stroke or transient ischaemic attack. *Cochrane Database Syst Rev* 1: CD010693, 2014

140. Duggan J, Kilfeather S, O'Brien E, O'Malley K, Nussberger J: Effects of aging and hypertension on plasma angiotensin II and platelet angiotensin II receptor density. *Am. J. Hypertens.* 5: 687–93, 1992

141. Yoon HE, Choi BS: The renin-angiotensin system and aging in the kidney. *Korean J. Intern. Med.* 29: 291–5, 2014

142. Panickar KS, Jewell DE: The beneficial role of anti-inflammatory dietary ingredients in attenuating markers of chronic low-grade inflammation in aging. *Horm. Mol. Biol. Clin. Investig.* 23: 59–70, 2015

143. Vlassara H, Uribarri J, Ferrucci L, Cai W, Torreggiani M, Post JB, Zheng F, Striker GE: Identifying advanced glycation end products as a major source of oxidants in aging:

implications for the management and/or prevention of reduced renal function in elderly persons. *Semin. Nephrol.* 29: 594–603, 2009

144. Yubero-Serrano EM, Woodward M, Poretsky L, Vlassara H, Striker GE: Effects of sevelamer carbonate on advanced glycation end products and antioxidant/pro-oxidant status in patients with diabetic kidney disease. *Clin. J. Am. Soc. Nephrol.* 10: 759–66, 2015

145. HALLORAN PF, MELK A, BARTH C: Rethinking Chronic Allograft Nephropathy: The Concept of AcceleratedSenescence. *J. Am. Soc. Nephrol.* 10: 167–181, 1999

146. Melk A, Schmidt BMW, Vongwiwatana A, Rayner DC, Halloran PF: Increased expression of senescence-associated cell cycle inhibitor p16/INK4a in deteriorating renal transplants and diseased native kidney. *Am. J. Transplant.* 5: 1375–1382, 2005

147. Verzola D, Gandolfo MT, Gaetani G, Ferraris A, Mangerini R, Ferrario F, Villaggio B, Gianiorio F, Tosetti F, Weiss U, Traverso P, Mji M, Deferrari G, Garibotto G: Accelerated senescence in the kidneys of patients with type 2 diabetic nephropathy. *Am. J. Physiol. Renal Physiol.* 295: F1563–73, 2008

148. Sis B, Tasanarong A, Khoshjou F, Dadras F, Solez K, Halloran PF: Accelerated expression of senescence associated cell cycle inhibitor p16INK4A in kidneys with glomerular disease. *Kidney Int.* 71: 218–26, 2007

149. Melk A, Schmidt BMW, Takeuchi O, Sawitzki B, Rayner DC, Halloran PF: Expression of p16INK4a and other cell cycle regulator and senescence associated genes in aging human kidney. *Kidney Int.* 65: 510–20, 2004

150. Melk A, Ramassar V, Helms LM, Moore R, Rayner D, Solez K, Halloran PF: Telomere shortening in kidneys with age. *J. Am. Soc. Nephrol.* 11: 444–53, 2000

151. Raschenberger J, Kollerits B, Titze S, Köttgen A, Bärthlein B, Ekici AB, Forer L, Schönherr S, Weissensteiner H, Haun M, Wanner C, Eckardt K-U, Kronenberg F: Association of relative telomere length with cardiovascular disease in a large chronic kidney disease cohort: The GCKD study. *Atherosclerosis* 242: 529–534, 2015

152. Raschenberger J, Kollerits B, Ritchie J, Lane B, Kalra PA, Ritz E, Kronenberg F: Association of relative telomere length with progression of chronic kidney disease in two cohorts: effect modification by smoking and diabetes. *Sci. Rep.* 5: 11887, 2015

153. Boxall MC, Goodship THJ, Brown AL, Ward MC, von Zglinicki T: Telomere shortening and haemodialysis. *Blood Purif.* 24: 185–9, 2006

154. Epstein FH, Prasad P: Effects of furosemide on medullary oxygenation in younger and older subjects. *Kidney Int.* 57: 2080–3, 2000

155. Kasiske BL: Relationship between vascular disease and age-associated changes in the human kidney. *Kidney Int.* 31: 1153–9, 1987

156. Sarkar D, Fisher PB: Molecular mechanisms of aging-associated inflammation. *Cancer Lett.* 236: 13–23, 2006

157. Costello-White R, Ryff CD, Coe CL: Aging and low-grade inflammation reduce renal function in middle-aged and older adults in Japan and the USA. *Age (Dordr).* 37: 9808, 2015

158. Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, van de Sluis B, Kirkland JL, van Deursen JM: Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature* 479: 232–236, 2011

159. Baker DJ, Jeganathan KB, Cameron JD, Thompson M, Juneja S, Kopecka A, Kumar R, Jenkins RB, de Groen PC, Roche P, van Deursen JM: BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. *Nat. Genet.* 36: 744–749, 2004

160. Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA: Rejuvenation of aged progenitor cells by exposure to a young systemic environment.

Nature 433: 760–4, 2005

161. Ruckh JM, Zhao J-W, Shadrach JL, van Wijngaarden P, Rao TN, Wagers AJ, Franklin RJM: Rejuvenation of regeneration in the aging central nervous system. *Cell Stem Cell* 10: 96–103, 2012

162. Villeda SA, Luo J, Mosher KI, Zou B, Britschgi M, Bieri G, Stan TM, Fainberg N, Ding Z, Eggel A, Lucin KM, Czirr E, Park J-S, Couillard-Després S, Aigner L, Li G, Peskind ER, Kaye JA, Quinn JF, Galasko DR, Xie XS, Rando TA, Wyss-Coray T: The ageing systemic milieu negatively regulates neurogenesis and cognitive function. *Nature* 477: 90–4, 2011

163. Smith LK, He Y, Park J-S, Bieri G, Snethlage CE, Lin K, Gontier G, Wabl R, Plambeck KE, Udeochu J, Wheatley EG, Bouchard J, Eggel A, Narasimha R, Grant JL, Luo J, Wyss-Coray T, Villeda SA: β2-microglobulin is a systemic pro-aging factor that impairs cognitive function and neurogenesis. *Nat. Med.* 21: 932–7, 2015

164. Katsimpardi L, Litterman NK, Schein PA, Miller CM, Loffredo FS, Wojtkiewicz GR, Chen JW, Lee RT, Wagers AJ, Rubin LL: Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. *Science* 344: 630–4, 2014

165. Sinha M, Jang YC, Oh J, Khong D, Wu EY, Manohar R, Miller C, Regalado SG, Loffredo FS, Pancoast JR, Hirshman MF, Lebowitz J, Shadrach JL, Cerletti M, Kim M-J, Serwold T, Goodyear LJ, Rosner B, Lee RT, Wagers AJ: Restoring systemic GDF11 levels reverses age-related dysfunction in mouse skeletal muscle. *Science* 344: 649–52, 2014

166. Loffredo FS, Steinhauser ML, Jay SM, Gannon J, Pancoast JR, Yalamanchi P, Sinha M, Dall'Osso C, Khong D, Shadrach JL, Miller CM, Singer BS, Stewart A, Psychogios N, Gerszten RE, Hartigan AJ, Kim M-J, Serwold T, Wagers AJ, Lee RT: Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. *Cell* 153: 828–39, 2013

167. Smith SC, Zhang X, Zhang X, Gross P, Starosta T, Mohsin S, Franti M, Gupta P, Hayes D, Myzithras M, Kahn J, Tanner J, Weldon SM, Khalil A, Guo X, Sabri A, Chen X, MacDonnell S, Houser SR: GDF11 Does Not Rescue Aging-Related Pathological Hypertrophy. *Circ. Res.* CIRCRESAHA.115.307527, 2015

168. Rodgers BD, Eldridge JA: Reduced Circulating GDF11 Is Unlikely Responsible for Age-dependent Changes in Mouse Heart, Muscle, and Brain. *Endocrinology* en20151628, 2015

169. Brun CE, Rudnicki MA: GDF11 and the Mythical Fountain of Youth. *Cell Metab.* 22: 54–6, 2015

170. Egerman MA, Cadena SM, Gilbert JA, Meyer A, Nelson HN, Swalley SE, Mallozzi C, Jacobi C, Jennings LL, Clay I, Laurent G, Ma S, Brachat S, Lach-Trifilieff E, Shavlakadze T, Trendelenburg A-U, Brack AS, Glass DJ: GDF11 Increases with Age and Inhibits Skeletal Muscle Regeneration. *Cell Metab.* 22: 164–74, 2015

171. Diep CQ, Ma D, Deo RC, Holm TM, Naylor RW, Arora N, Wingert RA, Bollig F, Djordjevic G, Lichman B, Zhu H, Ikenaga T, Ono F, Englert C, Cowan CA, Hukriede NA, Handin RI, Davidson AJ: Identification of adult nephron progenitors capable of kidney regeneration in zebrafish. *Nature* 470: 95–100, 2011

172. Sander V, Davidson AJ: Kidney injury and regeneration in zebrafish. *Semin. Nephrol.* 34: 437–44, 2014

173. McKee RA, Wingert RA: Zebrafish Renal Pathology: Emerging Models of Acute Kidney Injury. *Curr. Pathobiol. Rep.* 3: 171–181

174. Schrimpf C, Xin C, Campanholle G, Gill SE, Stallcup W, Lin S-L, Davis GE, Gharib S a., Humphreys BD, Duffield JS: Pericyte TIMP3 and ADAMTS1 Modulate Vascular Stability after Kidney Injury. *J. Am. Soc. Nephrol.* 23: 868–883, 2012

175. Zhu Y, Tchkonia T, Pirtskhalava T, Gower A, Ding H, Giorgadze N, Palmer AK, Ikeno

Y, Borden G, Lenburg M, O'Hara SP, LaRusso NF, Miller JD, Roos CM, Verzosa GC, LeBrasseur NK, Wren JD, Farr JN, Khosla S, Stout MB, McGowan SJ, Fuhrmann-Stroissnigg H, Gurkar AU, Zhao J, Colangelo D, Dorronsoro A, Ling YY, Barghouthy AS, Navarro DC, Sano T, Robbins PD, Niedernhofer LJ, Kirkland JL: The Achilles' Heel of Senescent Cells: From Transcriptome to Senolytic Drugs. *Aging Cell* n/a–n/a, 2015

176. Pattaro C, Köttgen A, Teumer A, Garnaas M, Böger CA, Fuchsberger C, Olden M, Chen M-H, Tin A, Taliun D, Li M, Gao X, Gorski M, Yang Q, Hundertmark C, Foster MC, O'Seaghdha CM, Glazer N, Isaacs A, Liu C-T, Smith A V, O'Connell JR, Struchalin M, Tanaka T, Li G, Johnson AD, Gierman HJ, Feitosa M, Hwang S-J, Atkinson EJ, Lohman K, Cornelis MC, Johansson Å, Tönjes A, Dehghan A, Chouraki V, Holliday EG, Sorice R, Kutalik Z, Lehtimäki T, Esko T, Deshmukh H, Ulivi S, Chu AY, Murgia F, Trompet S, Imboden M, Kollerits B, Pistis G, Harris TB, Launer LJ, Aspelund T, Eiriksdottir G, Mitchell BD, Boerwinkle E, Schmidt H, Cavalieri M, Rao M, Hu FB, Demirkan A, Oostra BA, de Andrade M, Turner ST, Ding J, Andrews JS, Freedman BI, Koenig W, Illig T, Döring A, Wichmann H-E, Kolcic I, Zemunik T, Boban M, Minelli C, Wheeler HE, Igl W, Zaboli G, Wild SH, Wright AF, Campbell H, Ellinghaus D, Nöthlings U, Jacobs G, Biffar R, Endlich K, Ernst F, Homuth G, Kroemer HK, Nauck M, Stracke S, Völker U, Völzke H, Kovacs P, Stumvoll M, Mägi R, Hofman A, Uitterlinden AG, Rivadeneira F, Aulchenko YS, Polasek O, Hastie N, Vitart V, Helmer C, Wang JJ, Ruggiero D, Bergmann S, Kähönen M, Viikari J, Nikopensius T, Province M, Ketkar S, Colhoun H, Doney A, Robino A, Giulianini F, Krämer BK, Portas L, Ford I, Buckley BM, Adam M, Thun G-A, Paulweber B, Haun M, Sala C, Metzger M, Mitchell P, Ciullo M, Kim SK, Vollenweider P, Raitakari O, Metspalu A, Palmer C, Gasparini P, Pirastu M, Jukema JW, Probst-Hensch NM, Kronenberg F, Toniolo D, Gudnason V, Shuldiner AR, Coresh J, Schmidt R, Ferrucci L, Siscovick DS, van Duijn CM, Borecki I, Kardia SLR, Liu Y, Curhan GC, Rudan I, Gyllensten U, Wilson JF, Franke A, Pramstaller PP, Rettig R, Prokopenko I, Witteman JCM, Hayward C, Ridker P, Parsa A, Bochud M, Heid IM, Goessling W, Chasman DI, Kao WHL, Fox CS: Genome-wide association and functional follow-up reveals new loci for kidney function. *PLoS Genet.* 8: e1002584, 2012

177. Köttgen A, Glazer NL, Dehghan A, Hwang S-J, Katz R, Li M, Yang Q, Gudnason V, Launer LJ, Harris TB, Smith A V, Arking DE, Astor BC, Boerwinkle E, Ehret GB, Ruczinski I, Scharpf RB, Chen Y-DI, de Boer IH, Haritunians T, Lumley T, Sarnak M, Siscovick D, Benjamin EJ, Levy D, Upadhyay A, Aulchenko YS, Hofman A, Rivadeneira F, Uitterlinden AG, van Duijn CM, Chasman DI, Paré G, Ridker PM, Kao WHL, Witteman JC, Coresh J, Shlipak MG, Fox CS: Multiple loci associated with indices of renal function and chronic kidney disease. *Nat. Genet.* 41: 712–7, 2009

178. Gorski M, Tin A, Garnaas M, McMahon GM, Chu AY, Tayo BO, Pattaro C, Teumer A, Chasman DI, Chalmers J, Hamet P, Tremblay J, Woodward M, Aspelund T, Eiriksdottir G, Gudnason V, Harris TB, Launer LJ, Smith A V, Mitchell BD, O'Connell JR, Shuldiner AR, Coresh J, Li M, Freudenberger P, Hofer E, Schmidt H, Schmidt R, Holliday EG, Mitchell P, Wang JJ, de Boer IH, Li G, Siscovick DS, Kutalik Z, Corre T, Vollenweider P, Waeber G, Gupta J, Kanetsky PA, Hwang S-J, Olden M, Yang Q, de Andrade M, Atkinson EJ, Kardia SLR, Turner ST, Stafford JM, Ding J, Liu Y, Barlassina C, Cusi D, Salvi E, Staessen JA, Ridker PM, Grallert H, Meisinger C, Müller-Nurasyid M, Krämer BK, Kramer H, Rosas SE, Nolte IM, Penninx BW, Snieder H, Fabiola Del Greco M, Franke A, Nöthlings U, Lieb W, Bakker SJL, Gansevoort RT, van der Harst P, Dehghan A, Franco OH, Hofman A, Rivadeneira F, Sedaghat S, Uitterlinden AG, Coassin S, Haun M, Kollerits B, Kronenberg F, Paulweber B, Aumann N, Endlich K, Pietzner M, Völker U, Rettig R, Chouraki V, Helmer C, Lambert J-C, Metzger M, Stengel B, Lehtimäki T, Lyytikäinen L-P, Raitakari O, Johnson A, Parsa A, Bochud M, Heid IM, Goessling W, Köttgen A, Kao WHL, Fox CS, Böger CA: Genome-wide association study of kidney function decline in individuals of European descent. *Kidney Int.* 87: 1017–29, 2015

179. Johnson SC, Dong X, Vijg J, Suh Y: Genetic evidence for common pathways in human age-related diseases. *Aging Cell* 14: 809–817, 2015

180. Tampe B, Zeisberg M: Contribution of genetics and epigenetics to progression of kidney fibrosis. *Nephrol. Dial. Transplant* 29 Suppl 4: iv72–9, 2014

181. Noordmans GA, Hillebrands J-L, van Goor H, Korstanje R: A roadmap for the genetic analysis of renal aging. *Aging Cell* 14: 725–33, 2015

182. Wolf I, Levanon-Cohen S, Bose S, Ligumsky H, Sredni B, Kanety H, Kuro-o M, Karlan B, Kaufman B, Koeffler HP, Rubinek T: Klotho: a tumor suppressor and a modulator of the IGF-1 and FGF pathways in human breast cancer. *Oncogene* 27: 7094–105, 2008

183. Bartke A: Long-lived Klotho mice: new insights into the roles of IGF-1 and insulin in aging. *Trends Endocrinol. Metab.* 17: 33–5, 2006

184. Rodwell GEJ, Sonu R, Zahn JM, Lund J, Wilhelmy J, Wang L, Xiao W, Mindrinos M, Crane E, Segal E, Myers BD, Brooks JD, Davis RW, Higgins J, Owen AB, Kim SK: A Transcriptional Profile of Aging in the Human Kidney. *PLoS Biol.* 2: e427, 2004

185. Broer L, van Duijn CM: GWAS and Meta-Analysis in Aging/Longevity. pp 107–125, 2015

186. Plomin R, Haworth CMA, Davis OSP: Common disorders are quantitative traits. *Nat. Rev. Genet.* 10: 872–8, 2009

187. Tan Q, Zhao JH, Li S, Kruse TA, Christensen K: Power assessment for genetic association study of human longevity using offspring of long-lived subjects. *Eur. J. Epidemiol.* 25: 501–6, 2010

188. Shi H, Belbin O, Medway C, Brown K, Kalsheker N, Carrasquillo M, Proitsi P, Powell J, Lovestone S, Goate A, Younkin S, Passmore P, Morgan K, Craig D, Johnston J, Mcguinness B, Todd S, Kehoe PG, Hooper NM, Vardy ERLC, Mann DM, Smith AD, Wilcock G, Warden D: Genetic variants influencing human aging from late-onset Alzheimer's disease (LOAD) genome-wide association studies (GWAS) the Genetic and Environmental Risk for Alzheimer's Disease (GERAD1) Consortium. *Neurobiol Aging* 3318: 1849–5, 2012

189. Rando TA, Chang HY: Aging, rejuvenation, and epigenetic reprogramming: resetting the aging clock. *Cell* 148: 46–57, 2012

190. Dwivedi RS, Herman JG, McCaffrey TA, Raj DSC: Beyond genetics: epigenetic code in chronic kidney disease. *Kidney Int.* 79: 23–32, 2011

191. Reddy MA, Natarajan R: Epigenetics in diabetic kidney disease. *J. Am. Soc. Nephrol.* 22: 2182–5, 2011

192. Smyth LJ, McKay GJ, Maxwell AP, McKnight AJ: DNA hypermethylation and DNA hypomethylation is present at different loci in chronic kidney disease. *Epigenetics* 9: 366–76, 2014

193. Trionfini P, Benigni A, Remuzzi G: MicroRNAs in kidney physiology and disease. *Nat. Rev. Nephrol.* 11: 23–33, 2015

194. Ben-Avraham D: Epigenetics of aging. *Adv. Exp. Med. Biol.* 847: 179–91, 2015

195. Canaud G, Bonventre J V: Cell cycle arrest and the evolution of chronic kidney disease from acute kidney injury. *Nephrol. Dial. Transplant* 30: 575–583, 2015

196. Hariri RJ, Hajjar DP, Coletti D, Alonso DR, Weksler ME, Rabellino E: Aging and arteriosclerosis. Cell cycle kinetics of young and old arterial smooth muscle cells. *Am. J. Pathol.* 131: 132–6, 1988

197. Rong S, Zhao X, Jin X, Zhang Z, Chen L, Zhu Y, Yuan W: Vascular Calcification in Chronic Kidney Disease is Induced by Bone Morphogenetic Protein-2 via a Mechanism Involving the Wnt/β-Catenin Pathway. *Cell. Physiol. Biochem.* 34: 2049–60, 2014

198. Yonekura Y, Fujii H, Nakai K, Kono K, Goto S, Shinohara M, Nishi S: Anti-oxidative Effect of the β-blocker Carvedilol on Diabetic Nephropathy in Non-obese Type 2

Diabetic Rats. *Clin. Exp. Pharmacol. Physiol.* 2015

199. Bianchessi V, Badi I, Bertolotti M, Nigro P, D'Alessandra Y, Capogrossi MC, Zanobini M, Pompilio G, Raucci A, Lauri A: The mitochondrial lncRNA ASncmtRNA-2 is induced in aging and replicative senescence in Endothelial Cells. *J. Mol. Cell. Cardiol.* 81: 62–70, 2015

200. London G, Covic A, Goldsmith D, Wiecek A, Suleymanlar G, Ortiz A, Massy Z, Lindholm B, Martinez-Castelao A, Fliser D, Agarwal R, Jager KJ, Dekker FW, Blankestijn PJ, Zoccali C: Arterial aging and arterial disease: interplay between central hemodynamics, cardiac work, and organ flow—implications for CKD and cardiovascular disease. *Kidney Int. Suppl.* 1: 10–12, 2011

201. Zeng Y, Wang P-H, Zhang M, Du J-R: Aging-related renal injury and inflammation are associated with downregulation of Klotho and induction of RIG-I/NF-κB signaling pathway in senescence-accelerated mice. *Aging Clin. Exp. Res.* 2015

202. Ren S, Duffield JS: Pericytes in kidney fibrosis. *Curr Opin Nephrol Hypertens* 22: 471–480, 2013

Figure 1 - Functional and structural changes in the aging kidney

With increasing age there are alterations in the function of the kidney. These are accompanied by both macroscopic and microscopic changes and result in an alteration in the response of the kidney to diverse insults.

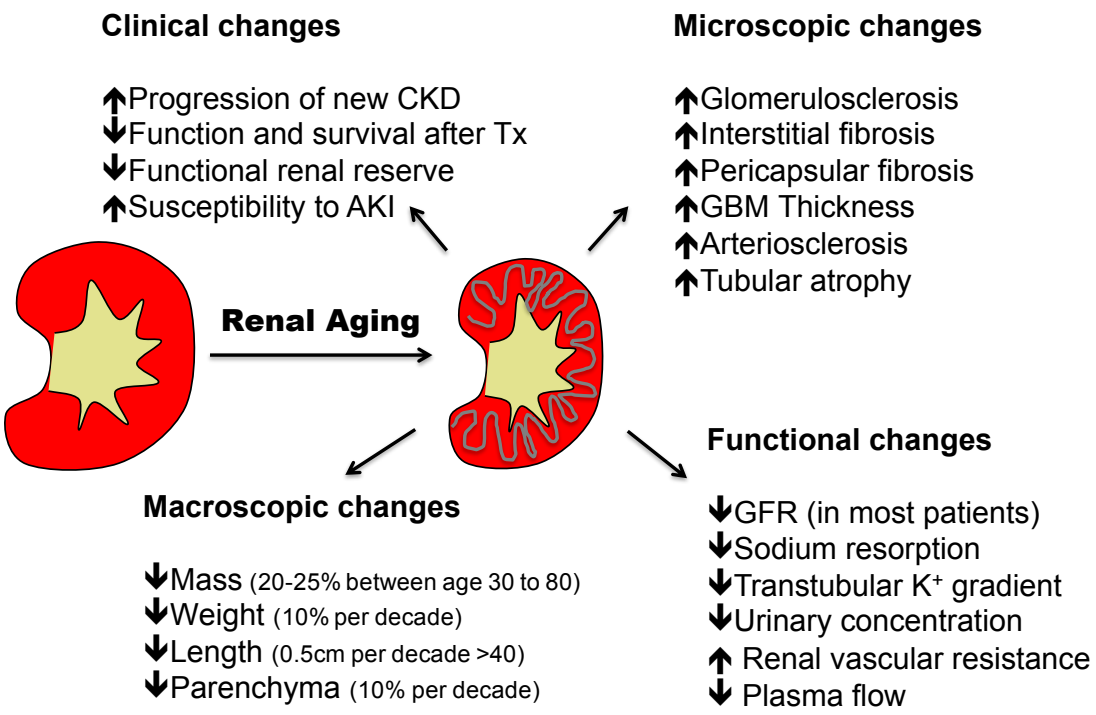

Figure 2 – Alterations in cellular and physiological pathways in the aging kidney

Diverse physiological, cellular and gene expression alterations occur in the aging kidney, impacting on homeostasis, function and the response to renal injury

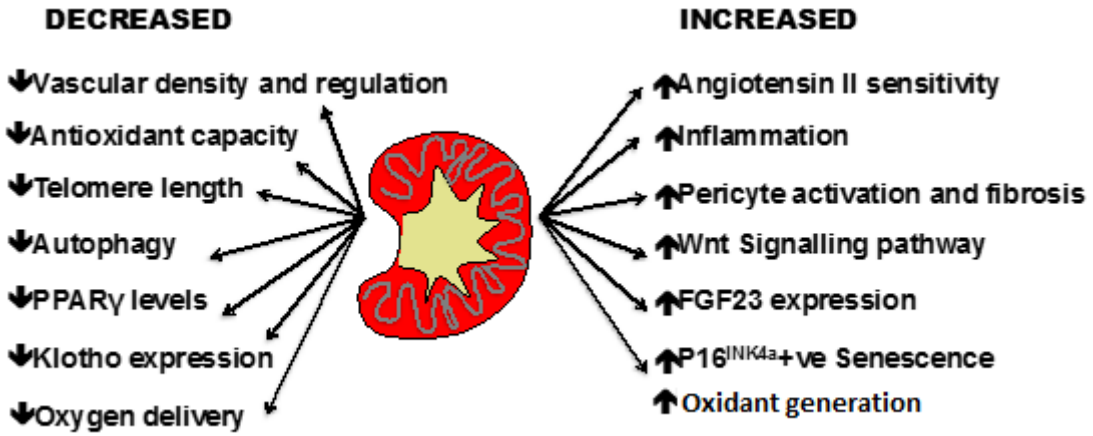

Figure 3 - Cell cycle progression in the aged kidney

Cell cycle arrest in G1/S phase becomes more prevalent with age and results in p16^{INK4a} positive senescent cells expressing multiple cytokines promoting autocrine and paracrine changes in aged kidneys. Whilst studies are lacking in aged animals, increased G2/M cell-cycle arrest in response to injury promotes maladaptive repair in murine kidney injury with raised G2/M counts correlating with fibrosis.[93,195] G2/M cell-cycle arrest may have variable effects in different cell types, being profibrotic in renal tubular cells, but preventing intimal hyperplasia in young smooth muscle cells.[196]

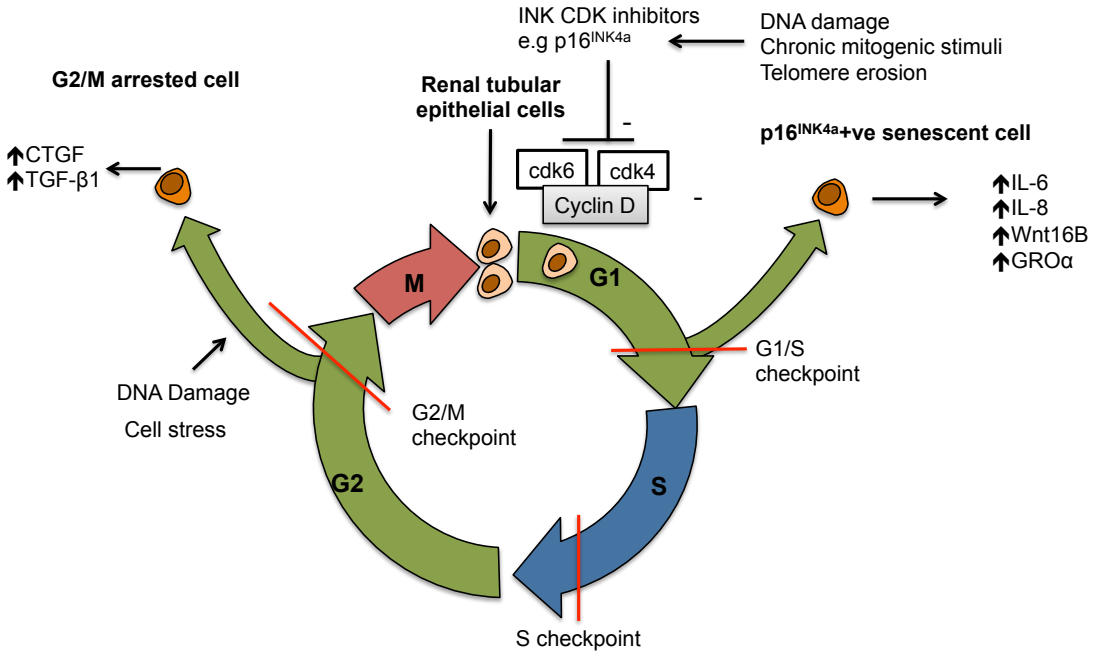

Figure 4 - Age related pathways contributing to altered renal outcomes in the elderly

Multiple pathways interact to produce the changes of renal aging and ↓GFR. Black text indicates implicated upstream effectors of aging, whilst red text reports the functional and histological changes found in the aged kidney

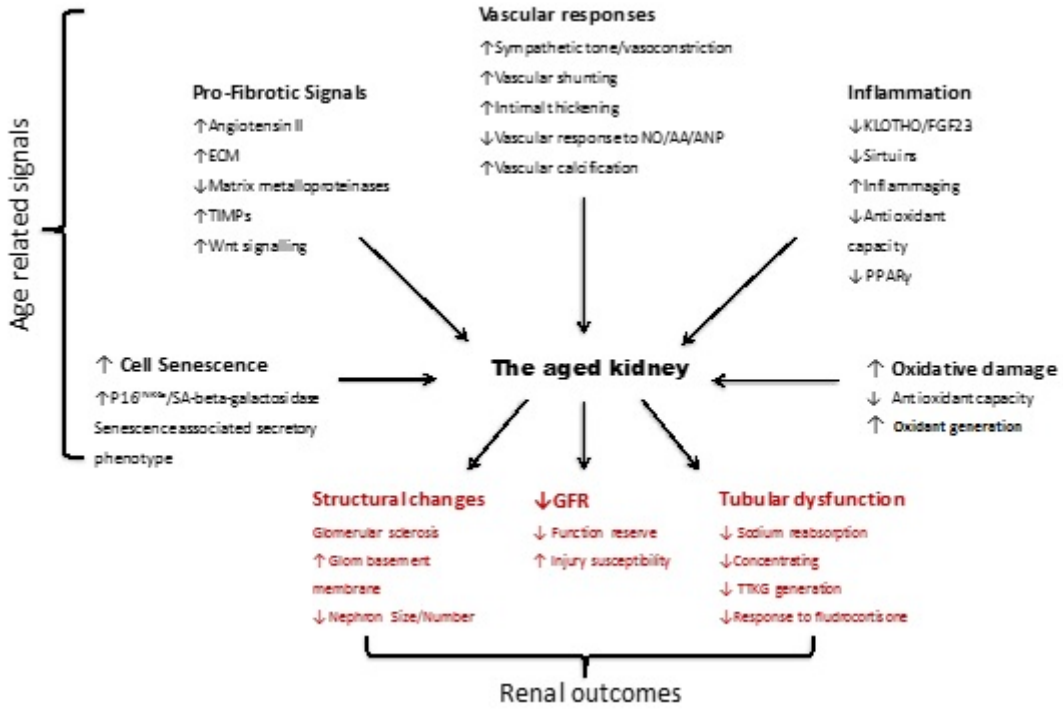

Figure 5 - Potential pathways and therapeutic targets for the treatment of renal aging

Proposed aging associated pathways (left side), and potential interventions to address these, coded by current use in patients (green), experimental use in models of renal disease/aging (orange) or potential for future study in the kidney (red).

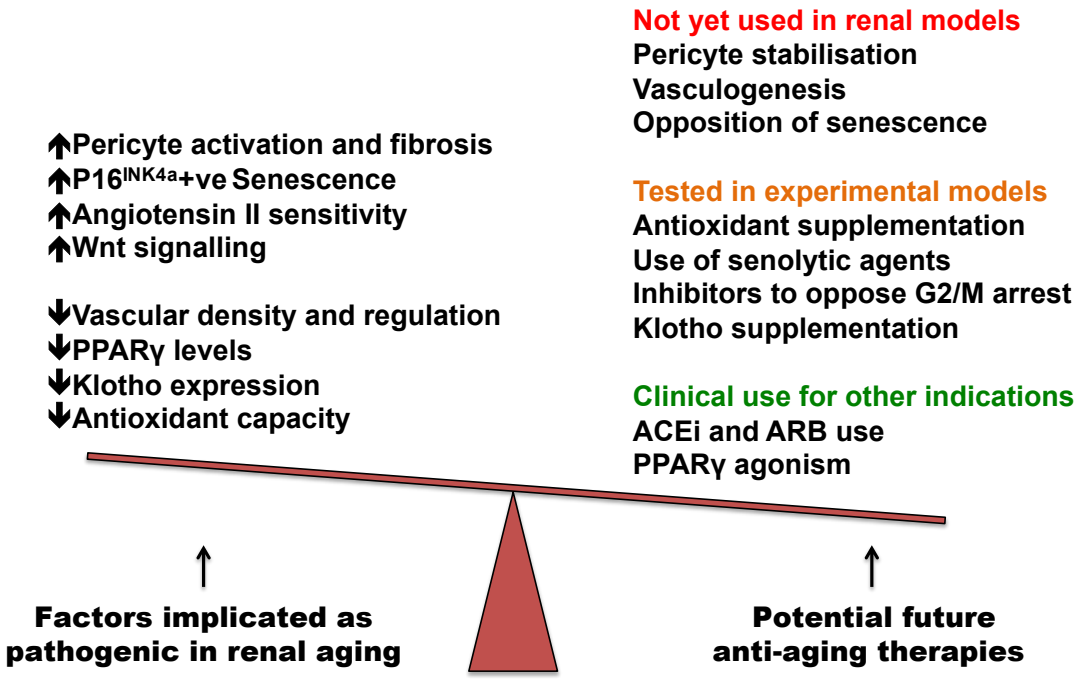

Table 1 - Studies of putative aging pathways *in vitro*, *in vivo* and in man

Changes in activity of various signalling pathways and mechanisms implicated in the response of kidney to increasing age. Column 1 indicates cellular changes observed *in vitro*, column 2 reports effects seen in experimental models of renal aging and injury, and column 3 shows any reported effects in human aging and renal disease.

Aging factor	*In vitro* studies	Experimental studies	Human studies
Telomere shortening	Shown in cells to reduce with length of passage. Critical shortening leads to senescence[106].	Reduced in mice with age[108]. Impaired regeneration after IRI[109]	Reduced with age, with oxidative stress, CKD and HD[150,152]. Risk factor for CVD[151].
Klotho signaling	Klotho opposes signaling of IGF-1 and insulin[43] in cell lines in vitro.	Klotho deficiency decreases lifespan.[45] Overexpression reduces IGF-1 and Wnt signaling and increases lifespan[43]	Reduced with age[132]. Reduction associated with calcification and vascular disease[136]
Wnt signaling	Promotes profibrotic genes e.g. Snail, PAI1, MMP7[52].	Levels increase with injury and in response to falling Klotho with aging[53]. Mediates renal RAAS signalling[58]	Increases seen in CKD & linked to organ fibrosis[197]
PPARγ levels	Reduces oxidative stress/senescence in human fibroblasts[64]	Reduced activity with age[59,60]. Agonists reduce renal inflammation/injury[65]	Studies of PPARγ agonists suggest reduction in rates of proteinuria in diabetics[138]
Antioxidant capacity		Aged rats have reduced renal antioxidant capacity, and enhanced renal injury[79]. Reduced oxidative stress lessens renal injury[198]	Higher levels of oxidative stress in human aging and in CKD[74]. AGE accumulates with age[142].
Fibrosis	ATII promotes fibrosis of glomerular cells and promotes reduction of SIRT-3[90]	Collagen I, III and TGF-β upregulated in aging mice[51] and rats[66]. G2/M arrest is implicated in post injury renal fibrosis[93].	Nephrosclerosis a feature of aging and of hypertensive renal disease[11,12]. Fibrosis and ATII hypersensitivity seen in aged kidneys[141]
Senescence/ G1 Arrest	Human and animal cells undergo senescence in vitro in response to stress or prolonged culture.[95] p16INK4a KO epithelial cells convert to mesenchyme more readily[102]	p16INK4a and SA-beta-gal are markers for senescent cells and increased in aged animals and post-injury. G2/M arrest seen in scarred kidneys in response to injury[93].	Increased numbers of senescent renal cells correlate with increased injury and reduced transplant function[146,147].
Vascular changes	Aged mice aortas have increased G2/M phase cell cycle arrest in vitro[199]	Reduced renal capillary density in aged mice[125] & in response to severe IRI[115]	Increased renal vascular tone and vascular stiffening with age[200]. Loss of efficacy of vasodilators[201]
Pericyte behavior	Pericytes (but not myofibroblasts) stabilize endothelial cell cultures in vitro[174]	Reduction of interstitial pericytes with aging[125]. Increased myofibroblasts in response to UUO and IRI injury[202]	Comparative studies in aged humans (±CKD) have not been undertaken