

Targeting kidney cell senescence: a new paradigm for the treatment of chronic kidney disease?

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Abstract

Cellular senescence is a condition where cells undergo a permanent cell cycle arrest, accompanied by a unique set of functional and morphological changes. While initial studies of senescence have largely focused on its role as a barrier to extended cell division and tumorigenesis, in recent years cell senescence has emerged as an important driver of aging and age-related disease in different tissues, including the kidney. Accelerated cell senescence may decrease kidney repair capacity because of cell cycle arrest; in addition cell senescence promotes glomerulosclerosis and tubulointerstitial fibrosis via the production and release of proinflammatory and matrix-degrading molecules. Although almost all glomerular and tubular cells may undergo senescent changes, the glomerular podocytes and proximal tubule cells are the most commonly affected cells. In these days, treating cell senescence is moving his steps from the preclinical to the clinical stage. While cell senescence appears to be well accepted as a new mechanism for kidney damage and chronic kidney disease [CKD] progression, there still are many unanswered questions regarding how to detect it in kidney

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biopsies, which is prognostic meaning of individual kidney senescence markers, which the role of different cells involved, as well as the efficacy of the emerging senescence-targeted therapies on the progression and complications of CKD.

Introduction

Primary cells growing in culture don't proliferate forever, but their division rate decreases with time, finally ending. Cell senescence, a condition where cells undergo a permanent cell cycle arrest, accompanied by a unique set of functional and morphological changes, was first described by Hayflick et al. in 1961, when they observed the *in vitro* cultivation of human diploid cell strains [1]. With time, senescent cells remain viable, but they become unresponsive to proliferative stimuli and undergo an increase in volume, acquire a flattened cytoplasmic phenotype along with changes in nuclear structure and gene expression [1,2] with upregulation of pathways to resist apoptosis [3]. Finally, most senescent cells develop a senescence-associated secretory phenotype [SASP], a distinctive secretome consisting of various proinflammatory molecules, metalloproteases and growth factors [4]. This form of cell senescence, which follows a period of propagation of cells in culture, has also been defined as "replicative senescence" [5].

Initially, little was known about the role played by this phenomenon *in vivo*; besides the developmental stages, the study of senescence has focused largely on its role as a barrier to extended cell division and to tumorigenesis [6]. In addition, in recent years the study of cell senescence has focused on a general cellular stress response program. With this regard, cellular senescence has emerged as a major driver of aging and age-related disease in several tissues and organs, including the kidney [2,7]. Both kidney aging and chronic kidney disease [CKD] have common [7] several clinical phenotypes, such a decrease kidney plasma flow and glomerular filtration rate, an increased sensitivity to acute kidney injury [AKI] as well as glomerular sclerosis and interstitial fibrosis [7,8]; these similarities suggest that both the aging process and CKD-associated damage and its progression have in common some molecular mechanism[s]. It has also been suggested that CKD is the resultant of an interaction between age-associated senescence and age-associated disease, such as hypertension and diabetes, which can further decrease the limited ability of the kidney to repair and maintain epithelial functions [7].

Emerging evidence shows that accelerated cell senescence in kidney diseases is detrimental at least for two reasons. First, cell senescence may decrease kidney repair capacity because of cell cycle arrest. Second, senescent cells produce proinflammatory and matrix-degrading molecules [in what is known as the SASP], thus promoting glomerulosclerosis and tubulointerstitial fibrosis [4,8].

Cell senescence initiation

Cell senescence appears in embryonic and postnatal stages, but it has a dramatical upregulation at an older age, as revealed by the expression of p16^{INKA} in different organs [brain, heart, liver, and kidney] in mice [9]. Evolutionarily, cell senescence is a protective mechanism to prevent the transmission of gene defects to the next generations. In addition, by the clearance of senescent cells, a controlled senescence program may promote wound repair and tissue regeneration [10,11]. Cell senescence may also develop as an acute injury-response [10,11]; this kind of “acute senescence” participates in repair mechanisms and limits fibrosis. In sharp contrast, in chronic diseases senescent cells accumulate in response to a variety of stressors to mediate disease progression. Several stressors, such as metabolic stress, telomere shortening [12] oncogenic mutations, inflammation and mitochondrial dysfunction promote cell-cycle arrest via pathways either dependent or independent of the DNA damage response. Differently from the “acute senescence” condition, in chronic diseases senescent cells are scarcely removed by apoptosis or immune clearance.

Many of the stressors inducing cell senescence cause the overexpression of the cyclin-dependent kinase inhibitors p16^{INKA} and/or p21^{cip1} which, by binding to CDK4/6, inhibit cyclin D-CDK4/6 complex and induce cell-cycle arrest at the G1/S cell-cycle checkpoint. As shown in experimental models of CKD, the accumulation of senescent cells later the G2/M phase triggers the SASP phenotype [13], a finding which suggests that inhibition of cell progress through G1/S may reduce the number of cells that are arrested at G2/M.

Cell senescence is accelerated in many different kidney diseases, including hypertensive disease [14] transition of acute kidney injury to CKD [15], IgA nephropathy [16], focal segmental glomerulosclerosis [17], diabetic nephropathy [18] and chronic glomerulonephritis [19]. Such different diseases have in common the upregulation of cell senescence markers [mainly p16^{INKA} and p27] in tubules, which correlates with tubulointerstitial damage, suggesting that kidney cell senescence is not a disease-specific, but a CKD-related mechanism.

How to detect cell senescence in the kidney?

Currently, it is difficult to identify unique markers which allow both the identification and quantification of senescent cells. Available studies are based on the presence/absence of cell-cycle arrest markers; however this may not be sufficient to confirm senescence, as the increase in nuclei positively immunostaining for p16^{INKA} does not always coincide with the upregulation of p16^{INKA} expression in vivo [9]. It has been proposed that quantifying tissue senescence need considering

three approaches: staining of cell-cycle arrest markers, quantifying the effects of senescence-associated protein release, and excluding coexisting cellular proliferation [9].

Actually, a major issue is how to diagnose cell senescence in kidney biopsies. It has been proposed that SA- β -Gal, a lysosomal hydrolase which cleaves terminal β -d-galactose residues [20] can be used for screening for subsequent approaches, since this enzyme activity is upregulated by senescence in several tissues. The activity of SA- β -Gal is a classic marker of senescence in cellular biology [20]. *In vitro*, SA- β -Gal, at high enzyme activity, can injury the basement membranes through hydrolysis of proteoglycans glycosidic bonds [20]. Even as low as 10% senescent cells in tissues appear to be sufficient to cause damage and dysfunction in studies *in vitro* and *in vivo* models [21]. The regulation of SA- β -Gal activity is independent of DNA synthesis and reflects senescence-related changes in cell function [20,21]. SA- β -Gal detection is easy and relatively fast to perform on frozen samples of kidney biopsies. However, SA- β -Gal activity staining decreases with cryopreservation time [22]. Therefore SA- β -Gal staining needs to be standardized and assessed early [commonly 24-48 hours] from the freezing of the sample. In addition, SA- β -Gal is not a completely specific marker of cellular senescence. In patients with diabetic nephropathy, we observed that SA- β -Gal staining in the tubular compartment was directly related to the nuclear + cytoplasmic expression of p16^{INK4A}, but not to nuclear p16^{INK4A}. When we examined the SA- β -Gal and p16^{INK4A} coexpression, some tubule cells showed a complete coexpression of these two markers, while others showed an isolated expression of SA- β -Gal or p16^{INK4A} [18]. This suggests that the aging phenotypes are differently expressed at the same time in the same tissue. In addition, the predictive role of SA- β -Gal on eGFR loss is still not clear, since in early cohort studies in patients with glomerular diseases, telomere shortening activity and p16^{INKA} expression but not SA- β -Gal, were related to subsequent eGFR loss. In a recent study we tested the hypothesis that elevated SA- β -Gal activity in kidney biopsies is a predictor of subsequent eGFR loss in patients with CKD [23]. With this in mind, we retrospectively studied the expression of SA- β -Gal in kidney biopsies obtained in a cohort of incident CKD patients from various etiologies who were followed up for 3 years. The SA- β -Gal signal, which was predominantly observed in tubular cells, was ~2.6-fold higher in biopsies of CKD patients vs. controls [Figure 1].

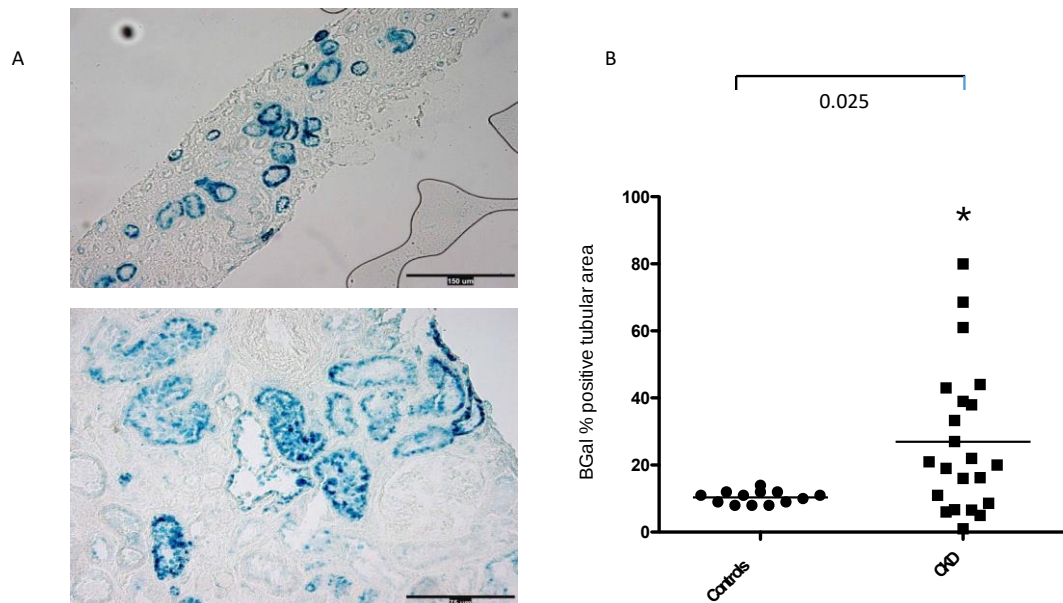


Figure 1 [A,B]. Representative images of SA-β-Gal tubular expression in CKD patients. SA-β-Gal assay was ~2.6-fold higher [$p < 0.025$] in the tubular compartment of biopsies of CKD patients than of controls. Magnification $\times 200$. SA-β-Gal, senescence-associated β-galactosidase; scale bar = 150–75 μm. * = $p < 0.025$ Reproduced from Esposito, P.; Picciotto, D.; Verzola, D.; Garibotto, G.; Parodi, E.L.; Sofia, A.; Costigliolo, F.; Gaggero, G.; Zanetti, V.; Saio, M.; et al. SA-β-Gal in Kidney Tubules as a Predictor of Renal Outcome in Patients with Chronic Kidney Disease. *J. Clin. Med.* **2024**, *13*, 322. <https://doi.org/10.3390/jcm13020322>

At follow up, at univariate analysis, eGFR loss was predicted by SA-β-Gal expression in kidney tubules, but not by baseline proteinuria. About 63% of biopsies had $>10\%$ of tubuli expressing SA-β-Gal, however only tubular SA-β-Gal percent expression $> 30\%$ was associated with faster [>3.5 ml/min/year] CKD progression. These findings suggest that SA-β-Gal activity in kidney biopsies at the time of diagnosis is predictive of subsequent loss of function. However, the study is retrospective and the cohort studied is small and possibly statistically underpowered to detect all the existing clinical associations of tubular cell senescence and results need to be confirmed in multicentric, prospective, controlled studies.

Which kidney cell types are involved in CKD-related cell senescence?

The aging process causes changes in number, structure, and function to all resident glomerular cell types [18, 24]. Markers of senescence expressed in podocytes in human and mouse kidneys with advancing age include SA-β-Gal and lipofuscin staining and an increase in p16, p19, p21, and p53 mRNA and protein levels [26]. It is, however, important to note that molecular markers for aging and senescence are still only partially established for any cell type or organ [26].

In previous studies, we observed that normal kidneys of age 58–67 years show p16^{INK4A} staining in $1.3 \pm 0.63\%$ of glomerular cells, $1.5 \pm 0.27\%$ of tubular cells, and $0.3 \pm 0.6\%$ of vessels [18]. Only scanty interstitial cells were p16^{INK4A} positive [$0.1 \pm 0.2\%$]. In addition to the nuclear staining, 70% of normal kidneys showed tubular cytoplasmic p16^{INK4A} expression. When nuclear p16^{INK4A} expression in glomeruli was analyzed by cell type, podocyte nuclear p16^{INK4A} staining was particularly increased in diabetic kidney disease compared with normal kidneys. Therefore, both the glomerular podocyte and tubular cells are more commonly involved by cell senescence.

It is interesting that podocytes are terminally differentiated and are unable to self-renew if they are lost in the aging process or disease. Glycogen synthase kinase [GSK] seems to play a major role in podocyte cell senescence. GSK, originally characterized as a key transducer of the insulin signaling cascade that controls glycogenesis is a highly conserved, ubiquitously expressed enzyme. GSK3 β rather than GSK3 α is predominantly expressed in podocytes. Combined knockout [KO] of both GSK3 α and GSK3 β in glomerular podocytes in embryonic or adult mice caused severe podocyte damage, glomerulosclerosis, and proteinuria [26]. It is interesting that GSK3 β is overexpressed with aging in glomerular podocytes, correlating with functional and histological signs of kidney aging. Moreover, podocyte-specific ablation of GSK3 β substantially attenuates podocyte senescence and glomerular aging in mice. Thus, GSK3 β appears to play an important role in podocyte senescence by modulating senescence signaling.

Tubular cells are the most common cell type involved in cell senescence across different CKD models and human disease. This is interesting, since chronic tubulointerstitial damage occurs across all types of kidney diseases and is associated to worse outcome. Hypertension and vascular disease are possible stressors accounting for kidney cell senescence [14]. In addition, proteinuria and glucose reabsorption by the proximal tubule have been proposed to trigger to accelerated cell senescence in kidney tubules [2,7,22,28]. The process of continuous glucose reabsorption by proximal tubules is now considered one of the mechanisms of tubulointerstitial damage and CKD progression both in diabetic and in non-diabetic disease [28]. In previous studies we observed also that human proximal tubule cells exposed to high glucose enter into a senescent, nonreplicative state in which they are metabolically active but do not respond to mitogenic stimuli [18]. Cells are characterized by enhanced expression of senescence markers, including SA- β -Gal, and different sets of genes, including negative regulators of the cell cycle [mainly p16^{INKA}]. Interestingly, these changes can be prevented by pre-treatment with phloretin, an inhibitor of SGLT action [28].

A senescent tissue phenotype is common in patients with end-stage renal disease [ESRD]. In CKD the progressive decrease in renal function and/or renal metabolism is associated with tissue

accumulation of uremic toxins, which *per se* may accelerate systemic progression of tissue senescence [29-32]. Therefore, while chronic senescent cells accumulating in damaged kidneys further activate renal fibrogenesis, premature aging may negatively influence life quality and survival of CKD patients [29,30].

Abating cell senescence in the kidney

About fifteen years ago, Baker et al. [33] showed that the clearance of senescent cells in a mouse progeroid model *in vivo* can delay age-related tissue dysfunction. More recently, it was observed that the removal of senescent cells augments lifespan, delays accelerated aging, and restores organ function in blood vessels [34] and kidney [35], without increasing the risk of cancer. These studies have provided evidence of safety and efficacy of senescent cell depletion, by using drugs which target the antiapoptotic pathways which are upregulated in senescent cells [35] or in the transgenic mice with selective sensitivity to pharmacologic agents [36]. More recently Baar et al. [35] observed that FOXO4-DRI [a peptide which blunts the p53-FOXO4 interaction and causes p53 nuclear exclusion and cell-intrinsic apoptosis] allowed a better renal function in both the fast-aging and the naturally aged mice; in addition FOXO4-DRI treatment normalized the percentage of tubular cells lacking LMNB1 and the tubular IL-6 elevation, suggesting that it may target high SASP-expressing senescent cells. Accordingly, targeting the number and/or type of senescent cells to delay the progression of kidney diseases appears to be very intriguing.

There are however other treatments which can protect or cure from accelerated cell senescence. In rats, Wiggins *et al* [37] observed that calorie-restriction prevented age-associated podocyte hypertrophy, improved the levels of several key podocyte genes, and reduced podocyte stress. Sirtuin signaling, which plays a regulatory role in the control of inflammation, apoptosis and metabolic regulation [38] is a candidate pathway for age-associated podocyte damage. Targeting mitochondrial dysfunction is also a promising treatment for accelerated aging. Treatment of old mice with elamipretide, a small synthetic peptide that ameliorates mitochondrial dysfunction, preserved podocyte mitochondrial integrity, lowered the expression of the reactive oxygen species-generating enzyme *Nox4*. Most important, elamipretide reduced foot process effacement, mitochondrial dysfunction and podocyte hypertrophy, and also increased podocyte density [39]. Senotherapeutic drugs induce selective cell death of senescent cells [senolytics] or suppress markers of senescence [senomorphics], in particular the SASP. Drugs belonging to the senolytic category [which selectively clear senescent cells], such as pan-tyrosine kinase inhibitor dasatinib and the flavonoids quercetin and fisetin, that interfere with a subset of cellular kinases [25,40] have been approved by the US Food and Drug Administration for non-kidney diseases. Quercetin and dasatinib reduce fibrosis and tubular

injury in experimental AKI and aging. Senomorphics aim to suppress all or at least several characteristics of the senescence phenotype. Among those, metformin and rapamycin inhibit senescence and SASP by inducing autophagy, thereby reducing the accumulation of damaged organelles. Similarly, Janus kinase inhibitors, such as the ruxolitinib, tofacitinib, and baricitinib, reduce SASP cytokines.

Conclusions

CKD has become a worldwide health problem. CKD incidence and prevalence is high and still increasing, mainly owing to obesity and diabetes, and is associated with growing morbidity and mortality [41]. Overall CKD mortality has increased by ~30% over the last 10 years, making it one of the fastest-rising major causes of death, together with diabetes and dementia [42]. CKD is the 12th most common primary cause of death, accounting for about 1 million deaths per year worldwide [41]. Current management of CKD includes blood pressure control, use of SGLT2i, treatment of albuminuria with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, nutritional intervention, avoidance of potential nephrotoxins and obesity, drug dosing adjustments, and cardiovascular risk reduction. Recent progress in our understanding of CKD pathophysiology together with the development of novel therapeutic agents has led to a renewed attention on the treatment of CKD. A most interesting perspective is offered by downregulating cell senescence to cure CKD and its progression to ESRD. In these days, treating cell senescence is moving his steps from the preclinical to the clinical stage. While cell senescence appears to be well accepted as a new mechanism for kidney damage and CKD progression, there still are many unanswered questions regarding how to detect it in kidney biopsies, which is prognostic meaning of individual senescence markers in the kidney, the role of different cells involved and the best option of treatment. As an example, it has been underlined that removing senescent podocytes from glomeruli may not be the most advisable therapeutic strategy because removal of senescent podocytes causes podocyte depletion, thus causing glomerular injury [25]. Currently, ongoing clinical trials have been developed to targeting cell senescence with senolytics to improve cellular aging markers [NCT01256840], skeletal health [NCT04313634], prevention of cardiovascular diseases [NCT05975528], senescent cell burden, frailty, and adipose-derived mesenchymal stem cell function in individuals with diabetic chronic kidney disease [NCT02848131]. Several other clinical trials are early to come, so in the next years we will know if treating cell senescence will change our paradigms for care of CKD and its evolution to ESRD.

Declarations

Conflict of Interest

The Authors declare that there is no conflict of interest.

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