Cellular senescence in renal ageing and disease

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Abstract | The senescence programme is implicated in diverse biological processes, including embryogenesis, tissue regeneration and repair, tumorigenesis, and ageing. Although *in vivo* studies of senescence are in their infancy, evidence suggesting that senescent cells are a heterogeneous cell type is accumulating: senescence can be induced by different stressors, and senescent cells have varying degrees of genomic and epigenomic instability and different cell origins, contributing to their diversity. Two main classes of senescent cells have been identified: acute and chronic senescent cells. Acute senescent cells are generated during coordinated, beneficial biological processes characterized by a defined senescence trigger, transient senescent-cell signalling functions, and eventual senescent-cell clearance. In contrast, chronic senescent cells arise more slowly from cumulative, diverse stresses and are inefficiently eliminated, leading to their accumulation and deleterious effects through a secretory phenotype. Senescent cells have been identified in many tissues and organs, including the kidney. Here, we discuss the emerging roles of senescent cells in renal development, homeostasis, and pathology. We also address how senotherapy, or targeting of senescent cells, might be used to improve renal function with normal ageing, disease, or therapy-induced damage.

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Ageing involves the progressive decline in tissue function over time, which results in loss of homeostasis and ultimately, loss of the organism's fitness[1,2]. Although the mechanisms underlying the ageing process have been studied for almost a century, why and how we age remain largely elusive. The antagonistic pleiotropy theory of ageing hypothesizes that evolutionarily selected traits that ensure fitness early in life can be detrimental at an advanced age[3]. Cellular senescence, a cellular programme characterized by a permanent cell-cycle arrest that alters cellular function (BOX 1) fits into this model, in which acutely senescent cells generated early in life provide an advantage during development[4,5], tissue regeneration[6], and by inhibiting neoplastic transformation[7], but aberrant and chronic accumulation of senescent cells late in life drives various features of ageing, including age-related disease and tissue deterioration[8,9].

Compelling evidence suggests that the distinctive secretome acquired by senescent cells, termed the senescence-associated secretory phenotype (SASP), is a key determinant in the attraction, activation, and differentiation of immune cells that can result in senescent-cell clearance through a process called immune surveillance[10–14]. This closed cycle of acute senescent cell generation, signalling, and elimination is thought to be a transient, highly efficient and beneficial physiological process[15]. However, increased age and/or impaired cell removal by the immune system can lead to an accumulation of chronic senescent cells, which can promote pathologies and reduce health and lifespan[16].

The kidney consists of a variety of cell types that face unique environments, stressors, and challenges. As discussed in detail below, studies of cultured cells, mouse models, and human samples suggest that aspects of the senescence programme are active during the entire lifetime of the kidney, including during development and disease. In this Review, we discuss current understanding of the mechanisms of developmental, regenerative, cancer-associated, and chronic senescence in the kidney, their contribution to each process and areas that would benefit from further research. Finally, we discuss potential therapeutic options for targeting senescent cells, termed senotherapies, to maintain renal function into old age, ameliorate disease progression, and improve success of renal transplantation.

Features of cellular senescence

Identification

Cellular senescence is complex and diverse (BOX 1). It can be induced by a broad spectrum of stressors (FIG. 1) and in many different cell types, tissues and organs. In addition, senescent cells are constantly evolving,

Key points

- Cellular senescence is a multi-faceted programme involved in diverse physiological and pathological processes including embryonic development, regeneration and repair, cancer-protection, ageing, and disease
- Senescent cells that are transiently present (acute senescence) are beneficial, whereas prolonged signalling and aberrant accumulation of senescent cells (chronic senescence) impairs renal function and promotes kidney disease
- Chronic senescent cells accumulate in the kidneys during natural ageing and have been causally linked to age-related decline in renal function
- Senescent cell accumulation also occurs in association with several renal diseases and therapeutic damage, and correlates with disease progression or deterioration in several instances
- Therapeutic interventions that target senescent cells, termed senotherapies, have potential to attenuate age-related renal dysfunction, improve disease outcome, and ensure success of kidney transplantation
- Development of effective and safe senotherapies should greatly benefit from future research aimed at understanding of the location, origin and properties of senescent cells in greater detail

Senescence-associated secretory phenotype (SASP). High amounts of pro-inflammatory and matrix-degrading molecules produced and secreted by senescent cells.

Immune surveillance Mechanism by which senescent cells are detected and eliminated by the immune system.

Mesonephros Transitory embryonic excretory organ derived from the metanephric mesenchyme that forms around embryonic day (E) 9 and degenerates by E15.5 in mice.

which results in substantial variability and heterogeneity of senescent cells, even in the same tissue[15,17]. Such variability, as well as the lack of tools to identify these cells, especially *in vivo*, makes establishing a universally accepted definition of cellular senescence difficult. Many studies have assessed a limited number of senescence features (BOX 2), none of which are specifically unique to senescence. Nonetheless, the consensus that particular phenotypes and signalling pathways are integral parts of the senescence programme is accepted in the field (BOX 2).

Triggers

Specific 'senescence-inducing' signalling events can differ between senescence programmes (FIG. 1). For example, senescence during embryonic development (FIG. 1) is mediated by the cyclin-dependent kinase inhibitor 1 ($p21^{CIP1}$) and does not seem to involve a DNA damage response (DDR)[4]. On the other hand, regenerative, cancer-protective, and chronic senescence programmes often involve induction of cyclin-dependent kinase inhibitor 2A ($p16^{INK4A}$), activation of the DDR and other key molecules, including cellular tumour antigen TP53, $p21^{CIP1}$, and tumour suppressor ARF (known as $p14^{ARF}$ in humans and $p19^{Arf}$ in mice), which converge to inhibit cyclin-dependent kinases (CDKs) and retinoblastoma protein (RB)[18–20] (FIG. 1). These findings suggest that developmental senescence is a distinctly regulated signalling programme and is not the consequence of cumulative cellular stress[4].

Secretory phenotype

The SASP is an important distinguishing characteristic of senescence programmes. SASP factors with developmental functions remain to be identified. During regeneration and repair of cutaneous wounds *in vivo*, the SASP is characterized by production of CYR61 (also known as CCN1) and platelet-derived growth factor (PDGF)-AA, which have central roles in the induction and maintenance of the senescent state[6,21]. Immune surveillance of oncogene-induced senescent cells in the liver is promoted by the monocyte chemoattractant protein 1 (MCP-1, also known as C-C motif chemokine 2)[11,12]. Chronic senescent cells acquired with ageing or following treatment are highly variable depending on the type of stressor, tissue, and species[22], but they consistently induce IL-6 and plasminogen activator inhibitor 1 (PAI-1) *in vivo*[16]. Together, these studies have identified IL-6, IL-1α, PAI-1, and MCP-1 as SASP factors that are frequently induced by senescent cells.

Developmental senescence

Within the past 5 years, several studies have shown that senescence is involved in embryogenesis and tissue remodelling[4,5,23] (FIG. 2). Senescent cells are transiently present in several embryonic structures, including the mesonephros, the endolymphatic sac of the inner ear, and the apical ectodermal ridge of the limbs[4,5]. Developmental senescence, which seemingly fine-tunes organogenesis, is dispensable for successful embryogenesis. $p21^{Cip1}$ (also known as *Cdkn1a*)-knockout mice, which are unable to undergo developmental senescence, develop successfully by activating compensatory mechanisms such as apoptosis[4]. In the mesonephros, senescent cells appear in the mesonephric tubules at embryonic day (E) 12.5–14.5 in mice and at approximately 9 weeks in human embryos[4]. Senescent cell accumulation precedes macrophage infiltration, suggesting that senescent cells attract immune cells to facilitate mesonephros regression and ultimately, the clearance of the remaining senescent cells[4,5] (FIG. 2). Loss of developmental senescence in $p21^{Cip1}$-knockout mice led to compensatory caspase activation, apoptosis, and delayed, but ultimately successful, mesonephros regression[4]. During organogenesis of the female Wolffian duct, however, loss of $p21^{Cip1}$ impaired senescence-mediated tissue regression and morphogenesis of the vagina, resulting in increased incidence of vaginal septa and reduced offspring numbers[4]. Collectively, these studies indicate that senescence functions as a mechanism that complements apoptosis for the elimination of specific groups of cells during the morphogenesis of certain tissues[4,24].

Box 1 | **Cellular senescence: basic principles**

Cellular senescence refers to an irreversible fate of damaged cells that is induced by a variety of stressors, including end of replicative lifespan, oncogenic stimuli, DNA damage, and mechanical stress. Unlike apoptosis, which results in the elimination of damaged cells, senescence is a complex and multi-faceted cellular programme that gives rise to permanently arrested, yet viable and metabolically active cells. Senescent cells obtain distinct phenotypic traits, including chromatin modifications and profound changes in protein secretion, which are referred to as the senescence-associated secretory phenotype (SASP). Although senescent cells are thought to constitute a minor fraction of cells in adult tissues, these cells can contribute to various physiological functions, including wound healing, cancer protection, age-related diseases and organismal ageing.

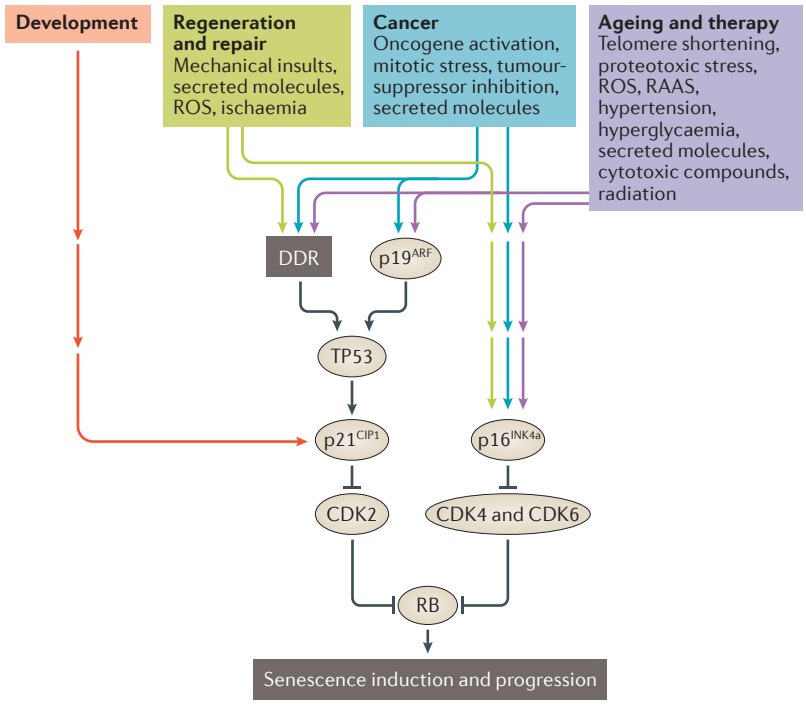

Figure 1 | **Main triggers of senescence.** Developmental senescence is thought to be induced as part of a physiological programme that depends on $p21^{CIP1}$, but not on other cyclin-dependent kinase (CDK) inhibitors such as tumour suppressor ARF ($p19^{ARF}$), $p16^{INK4a}$ or TP53 (REFS 4,5). On the other hand, senescence induced by stress, insults, regeneration, cancer, ageing, diseases and therapy, can vary substantially depending on the stimulus, context or cell type involved. Stress (for example, an insult, cancer, ageing or therapy) usually activates a signalling cascade involving a DNA damage response (DDR) via ATM or ATR kinases, TP53 activation and increased $p21^{Cip1}$ transcription[184,185] and/or induction of $p16^{Ink4a}$ expression via multiple signalling pathways[186]. Notably, murine oncogene-induced senescence mainly relies on $p19^{ARF}$, whereas human cells are mainly dependent on $p16^{INK4a}$ signalling[38,187]. Activation of $p21^{CIP1}$ and $p16^{INK4a}$ results in inhibition of CDK complexes and retinoblastoma protein (RB) phosphorylation[18,19]. RAAS, renin–angiotensin–aldosterone system; ROS, reactive oxygen species.

However, the reasons why senescence is favoured over apoptosis in certain structures are unclear. Perhaps, larger structures, such as the mesonephros, require a coordinated effort of apoptosis and senescence to warrant an efficient, timely and complete regression, whereas smaller structures, such as the pronephros, might not.

Senescence in regeneration

Acute regenerative senescence is induced after an initial insult as part of a healing or repair response (FIG. 2). For example, in the early stages of cutaneous wound healing, senescent cells induce myofibroblast differentiation and promote wound closure through paracrine signalling[6]. Depletion of senescent cells via targeted ablation of $p16^{Ink4a}$-positive cells during wound healing in mice delayed the repair process, but did not affect the total time to full wound closure[6,16], suggesting that senescence in the context of tissue regeneration has a dispensable, fine-tuning role. Interestingly, in the later stages of cutaneous wound healing[21] and after cardiac infarction[25], senescent fibroblasts limit tissue fibrosis and mitigate tissue damage by secreting anti-fibrotic factors, such as matrix metalloproteinases. Prolonged, chronic senescent-cell signalling can, however, lead to detrimental consequences. For example, although senescent hepatic stellate cells reduce fibrosis after liver damage in mice[26,27], impairment of their subsequent removal promotes fibrosis[28].

Renal tubule and interstitial cells become senescent after unilateral ureteral obstruction (UUO)[29]. Preventing senescence through $p16^{Ink4a}$ inactivation increased renal fibrosis after UUO, indicating that senescence is part of an anti-fibrotic mechanism in this context[29]. Acute regenerative senescence was also beneficial immediately after ischaemia–reperfusion injury (IRI) in murine kidneys. Within 2 days of IRI, $p16^{Ink4a}$ and $p21^{Cip1}$ expression was induced in tubule cells[30] and $p21^{Cip1}$-knockout mice had impaired renal recovery, higher renal damage and mortality after IRI[31]. Additionally, mice with proximal tubules lacking autophagy protein 5 (ATG5), a protein involved in the degradation and recycling process of autophagy, which is implicated in senescence induction[32] and SASP production[33], showed impaired renal senescence[30] with increased renal damage and cell death in the acute phase after IRI[30,34,35]. The dual role of senescent cells after IRI is demonstrated in studies of $p16^{Ink4a}$-knockout and *Atg5*-knockout mice, in which detrimental long-term consequences after IRI, such as interstitial fibrosis, tubular atrophy, and impaired kidney function, are attenuated[30,36]. These examples suggest time-dependent contributions of senescent cells to renal injury, with early positive effects, followed by detrimental long-term consequences. How deleterious senescent cells form, evade elimination by the immune system, and exert their tissue-deteriorating properties are important questions to investigate.

Dual role of senescence in cancer

Senescence is a potent tumour-suppressive mechanism that prevents the expansion of damaged and preneoplastic cells (FIG. 2); hyperactivated oncogenes or inhibited expression of tumour-suppressor proteins trigger the senescence programme[37,38]. Conditional inactivation of the tumour-suppressor *Apc* in murine renal epithelial cells induces senescence, a response that inhibits formation of renal carcinomas as demonstrated by the combined inactivation of $p21^{Cip1}$ or $p16^{Ink4a}$/$p19^{Arf}$ and *Apc* resulting in an earlier onset of renal carcinoma[39]. Loss of *Vhl*, a key renal tumour-suppressor gene, also induces senescence in the renal epithelium[40], underscoring the importance of senescence as a cancer-protective mechanism in the kidney.

On the other hand, malignant cancers exploit the secretome of senescent stromal cells to stimulate growth, angiogenesis, epithelial-to-mesenchymal transition (EMT), immune cell evasion, and metastasis[10,41,42]. Strikingly, tumour cells themselves can promote stromal cell senescence via paracrine signalling[42,43]. For example, growth-regulated α protein (GRO1, encoded by *CXCL1*) induced senescence of stromal fibroblasts *in vitro*[43]. In addition, the presence of senescent cells in human and mouse tumours is consistent with a cancer-promoting role of senescent cells[42–44]. Removal of senescent cells using the *INK-ATTAC* mouse model, in which

Box 2 | **Identifying senescent cells**

The identification of senescent cells *in vivo* is challenging, especially considering their diversity and heterogeneity. However, some features are considered in the field to be necessary markers of senescence. The most commonly used marker is senescence-associated β-galactosidase (SA-β-gal) activity at pH 6.0, which reflects the increased lysosomal content of senescent cells[174]. Alternatively, lysosomal structure visualization with lipofuscin can be used under some circumstances[175]. Key characteristics of senescence are the absence of proliferation markers such as Ki67, DNA replication licensing factor MCM2, or incorporation of bromodeoxyuridine or ethynyldeoxyuridine, and increased levels of cyclin-dependent kinase inhibitors such as $p21^{CIP1}$ (encoded by *Cdkn1a*), $p16^{INK4a}$ and $p19^{ARF}$ (encoded by *Cdkn2a* in mouse), $p14^{ARF}$ (encoded by *Cdkn2a* in human), $p27^{KIP1}$ (encoded by *Cdkn1b*) or $p15^{Ink4b}$ (encoded by *Cdkn2b*). The DNA damage response (detected with γ-histone H2AX$^+$ or tumour suppressor p53-binding protein 1$^+$ foci)[176] and senescence-associated heterochromatic foci (SAHF; marked by chromobox protein homologue 3 (also known as HP1-γ), histone H3K9me3 or core histone macro-H2A.1)[177] are other important features of many senescence mechanisms. Canonical factors of the senescence-associated secretory phenotype (SASP) such as IL-6, IL-1α, plasminogen activator inhibitor 1 (PAI-1), C-C motif chemokine 2 (CCL2, also known as MCP-1) (TABLE. 1), as well as other pathophysiologically relevant SASP markers should be assessed routinely. Importantly, none of these markers are specific for senescent cells and, therefore, a panel of markers must be used to demonstrate the presence of senescence. For example, SA-β-gal activity can also be detected in osteoclasts[178], macrophage subpopulations[179] or owing to increased cell confluence *in vitro*[180]; $p19^{ARF}$ and $p16^{INK4a}$ are expressed in immune cells[181] or in tumours with retinoblastoma protein inactivation[182]. Use of reporter animal models such as the mouse models for $p16^{INK4a}$-mediated senescence *p16*-LUC[44,183], *p16*-3MR[6] or *INK-ATTAC*[9,16] can provide further proof of senescence and help to identify the cell type undergoing senescence in tissues.

$p16^{Ink4a}$-positive cells are selectively eliminated from most tissues upon drug treatment, delayed the development of several tumours, including liver and lung carcinomas, lymphomas and sarcomas. However, this senescent cell ablation ultimately did not affect tumour incidence or spectrum at the time of death[16], indicating that naturally occurring senescent cells stimulate tumour progression. Whether the tumour-promoting ability of senescent cells also applies to renal cancers remains to be experimentally tested.

Stress-induced chronic senescence

Acute senescence in development, regeneration, and oncogene-activation is viewed as beneficial whereas chronic senescence due to gradually increasing macromolecular damage and other chronic stresses (cellular 'wear and tear') commonly observed during ageing and disease, is thought to be largely detrimental (FIG. 2; TABLE 1). Establishing whether senescence is beneficial or detrimental in a given context requires the use of model systems that allow experimental manipulation before, during, and after the development of senescent cells. With such studies, determining the time-dependent effects and relative importance of senescent cells will aid in understanding how cellular senescence contributes to disease initiation, development and progression, which will be imperative to develop treatments of various human diseases.

Currently, the mechanisms underlying senescent cell accumulation during ageing and disease and the kinetics of this process are incompletely understood. Age-related senescent cell accumulation coincides with reduced immune system function[45,46], suggesting that impaired immune function allows senescent cells to evade clearance. This accumulation of senescent cells over time in mice shortens lifespan, promotes tissue deterioration, and impairs the function of several organs, including the heart, vasculature, adipose tissue, and kidney[16]. These effects might be mediated through the proinflammatory properties of the SASP[47] or through cell-autonomous effects of reduced stem-cell regenerative capacity[48–51]. Disease-induced stress or damage might also induce senescence, or conversely, senescent cells might initiate or promote disease progression[52–59]. In high fat diet-induced atherosclerosis, senescent cells were found to be key drivers of all stages of the disease[52]. Such studies could serve as a template for research to clarify causality between stress-induced senescence and disease. High fat diet-induced senescence has also been implicated in fat tissue dysfunction underlying type 2 diabetes mellitus, whereas senescence in pancreatic β-cell islets has been associated with type 1 diabetes mellitus[53]. Similarly, mechanical stress caused by hypertension can induce senescence in circulating endothelial progenitor cells[54,55], as well as in the heart and kidney[56]. Exogenous stresses from therapeutic intervention, such as organ transplantation[57,58] or chemotherapy with cytotoxic drugs[59,60] or irradiation[61,62] also stimulate senescence. The kidney encounters stress or damage from many sources. Below, we discuss stress-induced senescence in the kidney in various contexts, including renal ageing, disease, and therapy-induced damage (TABLE 1).

Senescence in renal ageing

Features of renal ageing

The kidney undergoes many structural and functional changes with ageing including glomerulosclerosis, tubular atrophy, interstitial fibrosis, arteriosclerosis, nephron loss and hypertrophy, and tubular diverticula[63–66] (FIG. 3a; TABLE 1). Macroscopic age-related changes include reduced cortical volume, renal cysts and tumours, atherosclerosis of renal arteries, parenchymal calcifications and cortical scars[67–70]. At the functional level, glomerular filtration rate (GFR), the ability to conserve and secrete sodium, and urine concentrating and diluting abilities all decline with age[71,72]. Although GFR gradually decreases over time in most individuals, renal function typically remains within what is considered a normal range[73], and does not necessarily predict development of end-stage renal disease (ESRD)[70]. On the other hand, dependent on the age of onset, rate of accumulation, and genetic and environmental factors, structural and functional changes with ageing can increase renal susceptibility to additional injury or damage, ultimately leading to pathological consequences.

The regenerative potential after acute kidney injury (AKI) or kidney transplantation decreases with age in both mice and humans[74–77]. In addition, kidneys from old donors have reduced function[78] and transplantation success[79,80], poor regenerative capacity after acute rejection, and higher rates of graft functional loss due to chronic degeneration[81,82]. Reduced GFR and nephron numbers in aged kidneys can also cause toxic accumulation of medications that are usually cleared by the kidney[70].

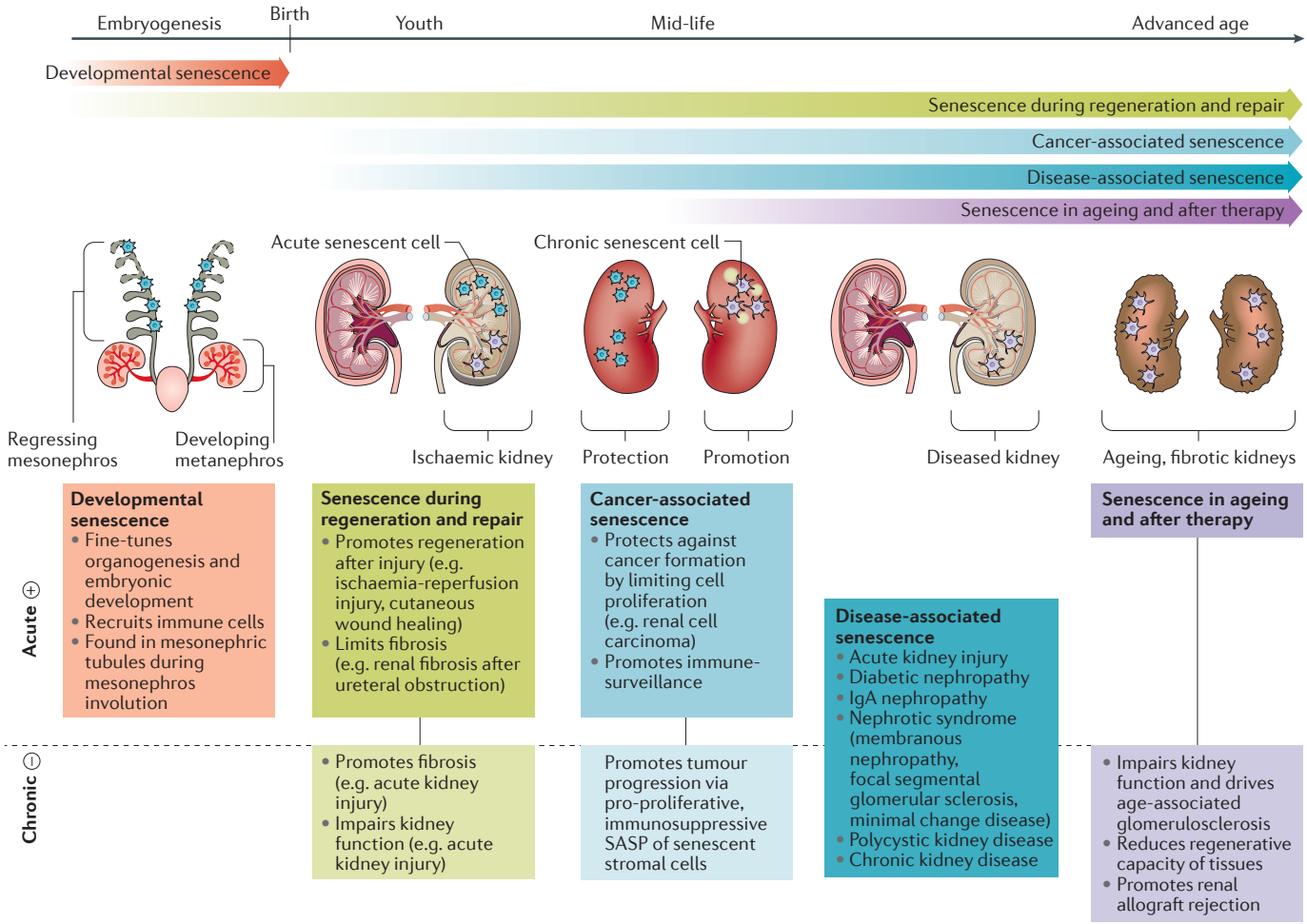

Figure 2 | **Potential roles of senescent cells during the life of the kidney.** Senescent cells can have beneficial roles when acutely present during renal development, repair and protection against cancer. However, their chronic presence has mainly deleterious effects over time in tumour progression, some diseases processes and during ageing. Further studies are required to elicit the contribution of acute and chronic senescent cells to renal disease.

Given that the kidney has a fairly large functional reserve capacity, assessing when age-related changes transition into kidney disease is difficult. Regardless, as for most organs, kidney ageing is a major risk factor for disease.

Glomerulosclerosis

Senescent cells accumulate in the kidney with age and correlate with functional decline and features of age-related deterioration (FIG. 3a; TABLE 1). A 2016 study in which naturally occurring $p16^{INK4a}$-expressing cells were ablated in 1-year-old *INK-ATTAC*-transgenic mice provided compelling evidence that senescent cells contribute to organismal ageing, including aspects of kidney ageing[16]. Clearance of senescent cells markedly extended lifespan irrespective of gender and genetic background. Mice lacking senescent cells had attenuated glomerulosclerosis and retained youthful blood urea nitrogen levels with ageing, indicating that the senescence programme actively contributes to these age-related pathological alterations. Senescence occurred in proximal tubule cells and increased the expression of *Agtr1a*, which encodes type 1A angiotensin II receptor (AT1A), throughout the kidney, thus hyperactivating the local renin–angiotensin–aldosterone system (RAAS)[16].

Nephron atrophy

Other studies have reported an increase of the number of cells with senescence features in cortical tubules, glomeruli, and interstitium of mouse[16,83] and human[84,85] kidneys with age. Nephron atrophy is seemingly a downstream consequence of glomerulosclerosis, and might therefore also be indirectly caused by cellular senescence. However, nephron atrophy might also be directly driven by senescence of tubule cells. The presence of senescent tubule cells might limit the proliferative capacity of the structure if repair were necessary, thus promoting nephron atrophy. Also, tubular senescent cells might impair local nephron functionality, increasing the risk of tubular atrophy.

Interstitial fibrosis

The effect of senescent cells on interstitial fibrosis seems complex. Several studies have shown positive correlations

Functional reserve capacity
Capacity an organ to preserve function, if damage should occur.

Table 1 | **Senescent cells in renal ageing, disease, and therapy-induced damage**

Renal Defect	Organism	Senescence markers used (method)	Cell types affected	Potential effect	Refs
Age-related					
Glomerulosclerosis	Mouse	SA-β-Gal (EM)/*p16^{Ink4a}*, *p19^{Arf}*, *p21^{Cip1}* (qPCR)	Proximal tubules	Detrimental	16
	Human	p16^{INK4A}, TP53 (IHC)	Cortex, tubules, glomeruli, interstitium, and arteries	Detrimental	85
Interstitial fibrosis	Mouse and rat	SA-β-Gal, p16^{INK4a} (IHC/qPCR), p19^{ARF} (qPCR)	Cortical tubules and glomeruli	Detrimental	83
	Human	p16^{INK4A}, TP53, TGFβ1 (IHC)	Cortex, tubules, glomeruli, interstitium and arteries	Detrimental	85
Nephron atrophy	Human	p16^{INK4A}, p27^{KIP1} (IHC)	Cortical tubules and interstitium	Detrimental	84
	Human	p16^{INK4A}, TP53, p14^{ARF}, TGFβ1 (IHC)	Cortex, tubules glomeruli, interstitium and arteries	Detrimental	85
Disease					
Acute kidney injury	Mouse	SA-β-Gal, p21^{CIP1} (W)	Tubules	Detrimental	75
	Mouse	SA-β-Gal/γ-H2A.X (IF)/ *p16^{Ink4a}*, *p19^{Arf} (qPCR)*	Tubules	Detrimental	30
	Mouse	p21^{Cip1} (IHC)/p16^{Ink4a}, p21^{Cip1} (IHC)	Tubules	Beneficial/ detrimental	31,188
IgA nephropathy	Human	SA-β-Gal, p16^{INK4A}, p21^{CIP1} (IHC)	Tubules	Detrimental	89
Diabetic nephropathy	Human	SA-β-Gal, p16^{INK4A} (IHC)	Glomeruli and tubules	Detrimental	90
	Mouse	SA-β-Gal, p21^{CIP1} (IHC)	Glomeruli and tubules	Detrimental	106
Membranous nephropathy, FSGS, and minimal change disease	Human	p16^{INK4A}, p21^{CIP1} (IHC both; p21^{CIP1} in FSGS only)	Glomeruli, tubules and interstitium	Detrimental	88
Autosomal dominant polycystic kidney disease	Human and rat	p21^{CIP1} (IHC, W)	NA; induction of senescence in tubules ameliorated disease	Beneficial	117
Nephronophthisis	Mouse	SA-β-Gal, p16^{INK4a} (W)	Tubules	Beneficial/ detrimental?	121
Chronic kidney disease	Cat	SA-β-Gal	Proximal and distal tubules	Detrimental	126
Therapy-induced					
Cisplatin	Mouse	p21^{CIP1}, p27^{KIP1} (IHC)	Outer medulla	Detrimental?	130
Renal transplantation	Human	SA-β-Gal, p16^{INK4A} (IHC)	Glomeruli, tubules, and interstitium	Detrimental	85

EM, electron microscopy; FSGS, focal segemental glomerulosclerosis; IF, immunofluroscence; IHC, immunohistochemistry; NA, not assessed; qPCR, quantitative PCR; W, western blot.

between senescent cell accumulation and fibrosis in the kidney during ageing[85–88] and disease[36,56,58,75,89,90]. However, few studies have assessed the direct impact of senescent cells in this process. Inactivation of *p16^{Ink4a}* in mice increased renal fibrosis both under normal conditions and in response to UUO[29]. A subsequent study, on the other hand, showed that inactivation of *p16^{Ink4a}* reduced interstitial fibrosis and nephron atrophy in kidney transplantation experiments[36]. This discrepancy might be explained by the dual action of senescent cells in these processes, but testing this hypothesis requires further experimentation.

Although induction of acute senescence after renal transplantation might promote wound closure and limit tissue fibrosis, the presence of chronic senescent cells in graft tissue is detrimental[81]. Therefore, the combined use of donor age and renal p16^{INK4a} levels might provide an excellent prognostic indicator of renal allograft function after transplantation[78,91]. Indeed, kidneys transplanted from old donor mice were more susceptible to stress during transplantation and had higher levels of p16^{INK4a} compared to kidneys from young donors[92]. The presence of chronic senescent cells might negatively impact the initial phase after transplantation, where epithelial cell proliferation is required[93]; grafts from old donor mice demonstrate reduced proliferation of renal tubule cells after transplantation than grafts from young mice[92].

Renal pathologies and senescence

Acute kidney injury

One of the major risk factors for AKI is age, but other factors include infections, glomerulonephritis, sepsis, and toxic compounds such as therapeutics and contrast agents used for imaging[94–96]. AKI is a primary driver of renal damage and chronic kidney disease

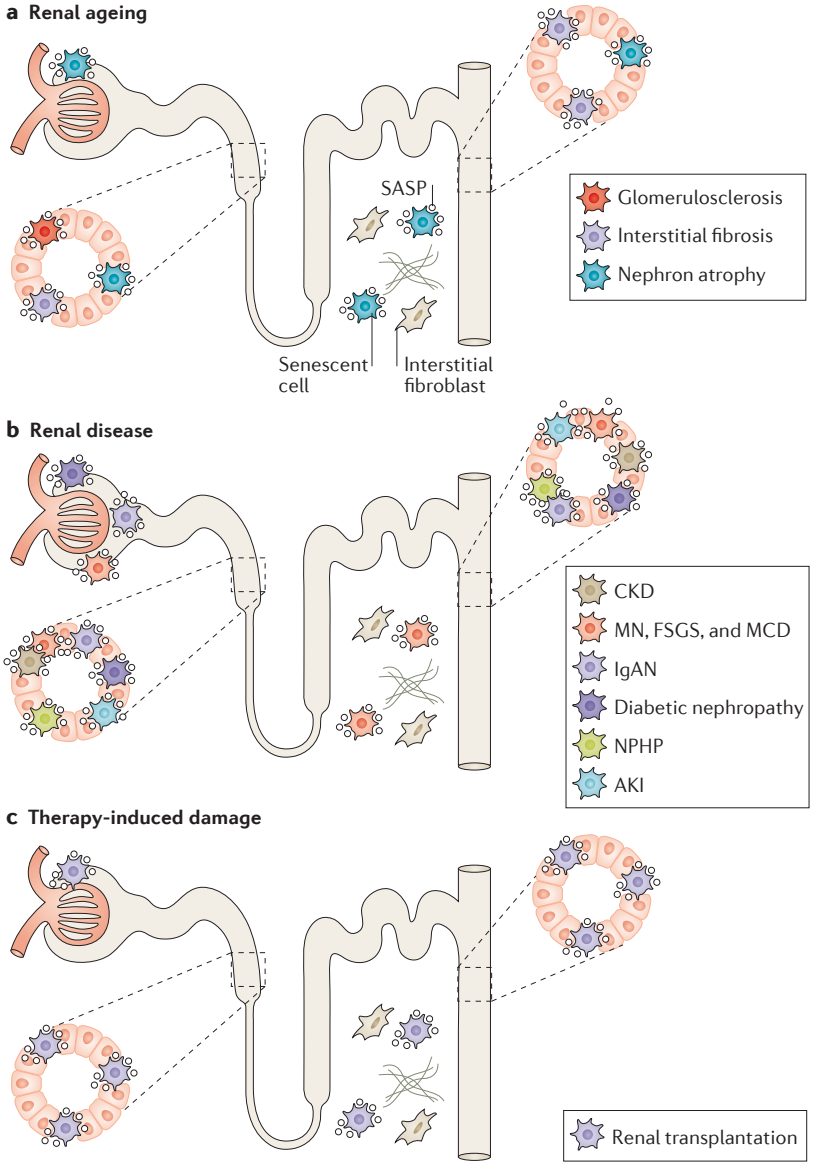

Figure 3 | **Senescent cell accumulation in the kidney associated with ageing, disease, and therapy.** Senescent cells have been identified in a variety of locations within the nephron in association with renal ageing (part **a**), disease (part **b**), and therapy-induced damage (part **c**). **a** | Senescent cells are present in aged kidneys and are associated with glomerulosclerosis, interstitial fibrosis, and nephron atrophy. **b** | $p16^{INK4a}$-positive or senescent cells are also present in kidneys in association with several renal diseases including chronic kidney disease (CKD), membraneous nephropathy (MN), focal segmental glomerular sclerosis (FSGS), minimal change disease (MCD); IgA nephropathy (IgAN), diabetic nephropathy, nephronophthisis (NPHP), and acute kidney injury (AKI). **c** | Senescent cells are also induced by transplant-associated stress. SASP, senescence-associated secretory phenotype.

(CKD) in humans[97], and maladaptive repair after AKI can be highly detrimental for kidney function in the elderly[70,74,75]. Consistent with these findings, renal dysfunction and mortality of rodents after IRI are markedly aggravated with ageing and associated with increased interstitial fibrosis, oxidative stress, inflammation, TP53 and $p21^{CIP1}$ levels, numbers of senescence-associated β-galactosidase (SA-β-gal)-positive tubules, and reduced cell proliferation[75,98] (FIG. 3b). These results suggest that increased levels of basal chronic senescence and reduced regenerative potential of aged kidneys have dramatic consequences on recovery after injury. Senescence in tubules might prevent the proliferation required for repair of damaged cells or aggravate the development of fibrosis.

Glomerulonephritis

IgA nephropathy (IgAN) is triggered by genetic or environmental factors and is acquired independently of age[99]. Disease progression correlates with telomere shortening[100] and several other features of senescence, including increased expression of $p21^{Cip1}$ and $p16^{Ink4a}$, and elevated SA-β-gal activity[89] (FIG. 3b), suggesting a potentially important role of cellular senescence in the progression of IgAN. However, whether senescence in this context is simply associated with tissue damage or contributes to disease progression remains to be determined.

Diabetes and diabetic nephropathy

Aberrant glucose metabolism, as observed in diabetes mellitus, is associated with serious long-term cardiac, vascular, and renal complications and various features of ageing, including sarcopenia, functional disability, frailty, and early mortality in older adults[101]. In addition, diabetes mellitus has been associated with cellular senescence in pancreatic β-cells[53] and adipose tissue[102,103]. In the kidney, impaired glucose metabolism can lead to diabetic nephropathy[104], which is the most common cause of CKD and ESRD[105].

Tubule cells, podocytes, glomerular mesangial and endothelial cells, and vascular endothelial cells from patients with type 2 diabetic nephropathy[90] and a streptozotocin-induced mouse model of type 1 diabetes[106] express $p16^{INK4a}$ and show SA-β-gal activity. Furthermore, several studies have shown a direct link between hyperglycaemia and the induction of senescence *in vitro* in mesangial[107–109] and proximal tubule cells[106], and *in vivo* by normalization of glucose levels in a mouse model of type 1 diabetes mellitus[106]. In addition, $p21^{Cip1}$ and $p27^{Kip1}$ depletion in models of diabetic nephropathy showed reduced proteinuria, glomerular hypertrophy, and tubulointerstitial damage[110,111]. Together, these findings suggest that cellular senescence has a role in the pathogenesis of diabetic nephropathy (FIG. 3b) and that hyperglycaemia is an important driver of senescence in this disease. The systemic impact of diabetic nephropathy has also been associated with cellular senescence, as skin fibroblasts from patients with insulin-dependent diabetic nephropathy were prematurely senescent[112], suggesting a potential link between renal disease and accelerated ageing.

Nephrotic syndrome

Several other glomerular diseases that cause nephrotic syndrome have also been associated with increased levels of $p16^{INK4a}$. Evaluation of renal biopsy samples from patients with membranous nephropathy, focal segmental glomerular sclerosis (FSGS), or minimal

Ciliopathies
Group of diseases that arise from mutations in genes encoding primary cilia-related proteins and that affect several organs such as the eyes, limbs and kidneys.

change disease (MCD) showed a dramatic increase in $p16^{INK4a}$ levels in glomeruli, interstitium, and tubules, independently of age (FIG. 3b). This increase correlated with the degree of interstitial fibrosis and tubular atrophy[88]. In patients with tubulointerstitial fibrosis, inflammation was associated with increased $p16^{INK4a}$ levels in interstitial and tubule cells[88]. Although elevated $p16^{INK4a}$ levels alone are not sufficient to determine if cells are senescent, $p21^{CIP1}$ levels were also increased in biopsy samples from patients with FSGS[88]. Whether additional markers of senescence are present in these diseased tissues and if senescent cells might contribute to disease initiation or progression are two key questions that remain to be addressed.

Polycystic kidney disease

The most common form of polycystic kidney disease (PKD) is autosomal dominant polycystic kidney disease (ADPKD), which leads to ESRD in the fifth to seventh decade of life[113]. PKD belongs to the family of ciliopathies, which primarily affect the ciliated epithelial cells that line the renal tubules[114]. PKD is also characterized by increased cell proliferation leading to tubule expansion[115,116]. Levels of $p21^{CIP1}$ are reduced in kidneys from patients with ADPKD and in a rat model of PKD[117]. In addition, treatment with the CDK inhibitor roscovitine restored $p21^{CIP1}$ levels *in vitro* and *in vivo*, increased SA-β-gal staining *in vitro*, decreased renal tubule cell proliferation and attenuated disease progression in an ADPKD mouse model[117–119]. These findings suggest that senescence hinders ADPKD progression.

In contrast, cellular senescence is induced in nephronophthisis (NPHP), another ciliopathy[120]. *Glis2*-knockout mice, a murine model of NPHP, had fewer proliferating renal cells (marked by the expression of Ki67), increased levels of $p16^{INK4a}$, and increased numbers of SA-β-gal^+ renal tubules compared to levels in wild-type mice[121] (FIG. 3b). Crossing these mice with a non-orthologous model of PKD (conditional *Kif3a*-knockout), dramatically reduced the massive cystic expansion seen in *Kif3a*-deficient mice, with reduced cell proliferation, increased DNA damage and numbers of SA-β-gal^+ tubules[121].

Together, these findings suggest that cellular senescence is involved in multiple forms of PKD and its role in some forms of PKD, such as ADPKD, is still unclear. Interestingly, increased senescence and fibrosis were observed in ADPKD[121], suggesting that finding a balance between mild cystic expansion and fibrosis might be important for amelioration of these diseases.

Chronic kidney disease

Individuals with CKD are at risk of developing other age-related renal pathologies, including glomerulosclerosis[122] and AKI[94], and young patients with CKD frequently have features of early ageing, including vascular ageing, muscle wasting, bone disease, cognitive dysfunction and frailty, highlighting the importance of proper renal function to prevent premature ageing of the kidney and at a systemic level[123,124].

CKD is associated with features of cellular senescence in various animal models. For example, a murine model of adenine-induced nephropathy has increased levels of inflammatory markers of the SASP[125], whereas a feline model of CKD has shortened telomeres and higher levels of SA-β-gal in proximal and distal collecting tubules than levels in young and aged cats[126]. The systemic consequences of CKD are also correlated with cellular senescence. Bone marrow-derived mesenchymal stem cells from rats with CKD were prematurely senescent[127], and lymphocytes from patients with CKD had increased expression of *TP53* and *RB1* and reduced proliferation[127]. Although these correlations suggest a relationship between cellular senescence and CKD, additional studies are required to determine the role of senescence in disease progression and whether it is a cause or consequence of pathogenesis.

Therapy-induced senescence

Collateral damage to normal tissue induced by cancer therapy is a serious health concern for patients, especially survivors of childhood cancers, who have a high risk of developing chronic pathologies, including secondary cancers, cardiovascular disease, renal dysfunction, musculoskeletal problems, and endocrinopathies[128]. For instance, cisplatin, a chemotherapy agent used to treat several solid tumours including testicular and ovarian cancers, causes renal damage and induces a marked increase in $p21^{CIP1}$ in rodent kidneys[129,130] (FIG. 3c; TABLE 1). Furthermore, the incidence of cisplatin-induced nephrotoxicity increases with age in both mice and humans[131]. Although these findings are suggestive of a relationship between chemotherapy and induction of senescence in the kidney, formal evidence is lacking.

Perhaps the most well documented therapy-induced acquisition of renal senescence occurs with transplantation. As mentioned above, cellular senescence in donors of advanced age reduces transplantation success and organ longevity[78,91]. In addition, stresses such as IRI, acute rejection, or hypertension that occur during and after transplantation can also induce senescence[74]. Indeed, renal transplantation promoted substantial telomere shortening and increased expression of $p21^{Cip1}$ and $p16^{Ink4a}$ in rat renal graft tissue[57]. Likewise, biopsy samples from renal transplant recipients showed high $p16^{INK4a}$ levels in renal tubules, tubular atrophy, interstitial fibrosis, and high numbers of glomerular and interstitial cells[58,88] (FIG. 3c; TABLE 1). Furthermore, mice transplanted with kidneys from *Cdkn2a*-null donors had markedly reduced renal damage and significantly better survival than mice transplanted with wild-type kidneys, correlating with increased tubule cell proliferation and markedly reduced senescence, defined by the number of $Ki67^-$,γ-histone $H2AX^+$ cells[36].

Allograft rejection is also associated with cellular senescence and glomerular, tubular and interstitial cells from rejected grafts expressed elevated levels of $p16^{INK4a}$ and $p27^{KIP1}$, which correlated with the grade of chronic allograft nephropathy[84]. In addition, rejected kidneys from a rat model of chronic rejection also had increased

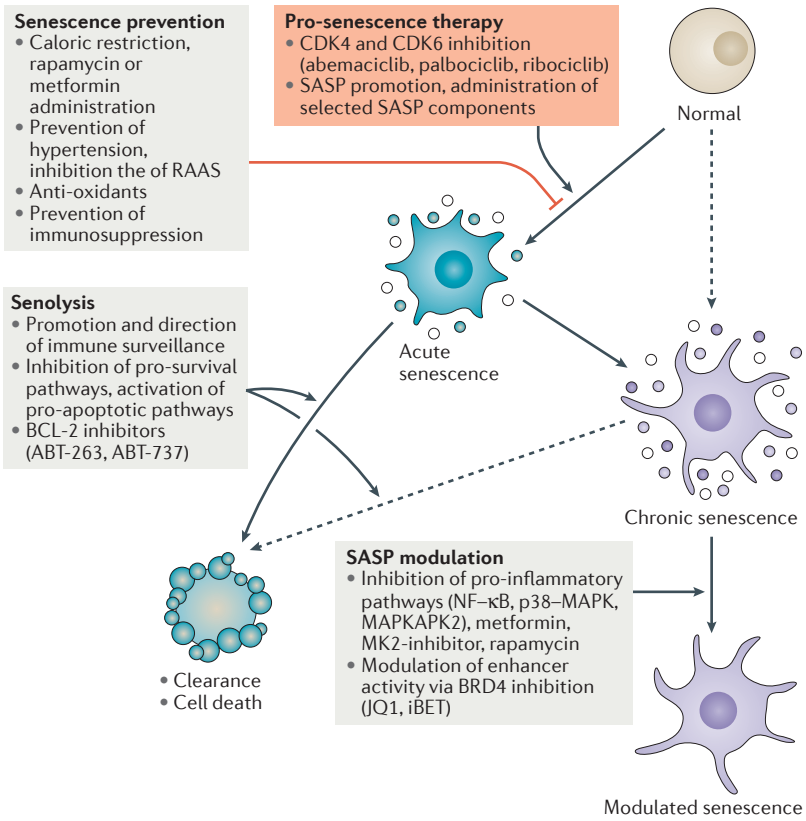

Figure 4 | **Senotherapeutic targeting strategies.** Pro-senescence interventions such as cyclin-dependent kinase (CDK) 4 and CDK6 inhibition act on senescence-inducing pathways. Anti-senescence strategies include prevention, cell lysis and modulation of the senescence-associated secretory phenotype (SASP). Senescence prevention is achieved by mitigating pro-senescent stressors. Several drugs and lifestyle interventions currently considered life extending, such as caloric restriction, fall into this category. Senescent cells can be removed by senolysis by interfering with the anti-apoptotic and pro-survival signalling. Navitoclax, an inhibitor of BCL-2, BCL-xL and BCL-W, selectively induces apoptosis in senescent cells *in vitro* and *in vivo*. Besides removal, SASP modulation through inhibition of proinflammatory pathways such as NFκ-B, p38-MAPK, MAPKAPK2 is another option to interfere with senescent cells. This strategy includes inhibition of proinflammatory pathways or gene expression of SASP factors via bromodomain-containing protein 4 (BRD4) inhibition. RAAS, renin–angiotensin–aldosterone system; iBET, Bromodomain and extra-terminal motif inhibitor; JQ1, potent inhibitor of the BET family.

p16^{Ink4a} and *p21*^{Cip1} levels, and SA-β-gal activity[57]. Based on current knowledge, cellular senescence seems to have mainly negative effects at all stages of kidney transplantation and is ultimately associated with allograft rejection and chronic allograft nephropathy.

Senotherapy

As putative drivers of ageing and disease in various tissues and organs, including the kidney, senescent cells have emerged as promising new targets for a therapeutic intervention known as senotherapy[132] (FIG. 4). However, given the complex roles of senescence in different biological processes and tissues, proper timing and delivery of senotherapy should be carefully considered. Targeted drug delivery to kidneys provides an exciting opportunity for senotherapy in this organ. Such targeted therapies include polyvinylpyrrolidone (PVP)-derivatives[133], low molecular weight protein (LMWP)-carriers[134], ligand-conjugates specific for renal-associated receptors[135], and the targeting peptide $(KKEEE)_3K$[136]. These approaches target tubule cells, which display features of senescence with ageing and pathology. Targeting other renal cell types, such as mesangial cells, which become senescent as a consequence of hypertension[137–139] and diabetic nephropathy[90], is more challenging, but could be accomplished using nanoparticles[140,141] or liposomes[142]. Another promising approach used in IRI mouse models is intravenous administration of oligonucleotides such as small interfering RNAs against TP53 to target proximal tubules, as they are the primary sites of uptake[143].

Preventing senescence

Potential interventions to protect cells against macromolecular damage include healthy diet, exercise and avoidance of lifestyle-related stresses such as cigarette smoking, but they can also include drugs that extend healthy lifespan in rodents, such as metformin and rapamycin. Restricted calorie intake extends health and lifespan in rodents and reduces oxidative stress and the age-associated rise in circulating proinflammatory factors, thereby mitigating known inducers of senescence[144,145]. In addition, kidneys from aged calorie-restricted rats have reduced $p16^{INK4a}$ levels, numbers of SA-β-gal$^+$ cells, glomerular volume, and fibrosis[146]. Calorie restriction decelerates cellular ageing by reducing activation of the mTOR signalling pathway in a process that involves AMP-activated protein kinase (AMPK)[147]. Several drugs that target this pathway are available, including compounds regarded as lifespan-extending, such as the mTOR inhibitor rapamycin or the AMPK activator metformin. Rapamycin protects cells against mTOR-induced cellular senescence[148,149] and phosphate-induced premature ageing[150], whereas metformin prevents the increase in $p16^{INK4a}$ and $p21^{CIP1}$ levels and proinflammatory SASP-related cytokines[151] in cells with irradiation-induced senescence. Metformin also reduces the production of reactive oxygen species in cultured podocytes[152], prevents diabetes-induced renal hypertrophy[153], and protects the kidney from gentamicin and cisplatin-induced renal damage in mice[154,155], suggesting a possible positive role in reducing senescence burden in the kidney. The main challenge when developing interventions that prevent senescence is to ensure that stressed cells at risk of neoplastic transformation are fully capable of activating the senescence programme.

Clearance of senescent cells

Elimination of cells after they have become senescent preserves the tumour-suppressive nature of cellular senescence but mitigates its potentially negative long-term consequences. As studies on senescent cell depletion in *INK-ATTAC* transgenic mice suggest[9,16], agents able to remove senescent cells, termed senolytics, might soon become tools to treat age-related diseases and promote healthy ageing. Senolysis can perhaps be best achieved with strategies similar to those used to kill cancer cells, including activation of the immune system, inhibition of pro-survival pathways or activation

of pro-apoptotic pathways. Identifying the differences in survival pathways and SASP between senescent cell types will certainly aid these efforts. These properties might greatly affect senolytic efficacy. Through these efforts, cell type, tissue or context specific senotherapies might show promise in reducing potential off-target effects. Furthermore, identification of non-senescent cells that rely on pathways targeted by senotherapies will facilitate understanding of any adverse effects of treatment.

In many organs, senescent cells are assumed to be targeted by the immune system, although this process has only been clearly demonstrated in the liver[156]. In the context of developmental, regenerative and cancer-protective senescence, the immune system seems to clear the majority of senescent cells. Understanding how chronic senescent cells resist clearance might be informative for developing immune-based therapies. Although our understanding of the immune surveillance of senescent cells is in its infancy, different mechanisms seem to mediate clearance in different contexts. Whereas the adaptive immune system is essential in recognizing pre-malignant hepatocytes, the innate immune response is particularly important for the physiological removal of senescent cells. Impaired physiological natural killer cell-mediated clearance of activated stellate cells in a liver fibrosis murine model leads to increased liver fibrosis[28]. Furthermore, the SASP of senescent liver cells activates macrophages and induces their polarization towards the secretory M1 type, which ultimately leads to senescent cell clearance[14]. Perhaps pharmacological agents designed to increase susceptibility of senescent cells to immune cell-mediated clearance might represent an effective therapeutic tool.

ABT-263 (navitoclax), an inhibitor of the pro-survival proteins apoptosis regulator BCL-2, apoptosis regulator BCL-xL (encoded by *Bcl2l1*), and apoptosis regulator BCL-W (encoded by *Bcl2l2*), was identified as a first-generation senolytic in mice[157]. Multiple laboratories have independently demonstrated its senolytic activity *in vitro* in the context of irradiation, replicative, and oncogene-induced senescence in a cell type and species-independent manner[52,157,158] and when administered to aged animals[157]. However, whether this drug also targets senescent cells involved in development and regeneration has not been investigated. Interestingly, BCL-xL levels were consistently increased with DNA damage, replication stress, and oncogene-induced senescence *in vitro*[158] but its expression was downregulated during developmental senescence of the mesonephros[4], indicating that different programmes of senescence involve BCL-xL differently. Combinatorial treatment with quercetin, a flavonol with antioxidant properties that inhibits a broad spectrum of protein kinases[159], and dasatinib, an inhibitor of several tyrosine kinases[160], has also been used to target senescent cells *in vivo* and *in vitro*[161] but whether biological effects associated with these compounds are due to senescent cell removal or their impact on a multitude of alternative targets remains to be determined.

Removal of renal senescent cells is potentially beneficial in ageing and in many kidney diseases, but this positive effect has not been experimentally confirmed. Feasibility will need to be assessed for each kidney disease separately. Kidney transplantation is one instance in which senolysis could be highly relevant. Pretreating donors well before explanting the kidney or perfusing the organ after removal with senolytics might reduce the chronic senescent cell burden and lower the risk of transplant rejection. Importantly, during the healing period after implantation, newly generated senescent cells promote cutaneous wound healing[6]; therefore, anti-senescence strategies might need to be avoided in the initial phases after transplantation. However, senescent cells induced by postoperative stresses or that accumulate after immunosuppressant therapy could be removed by senolysis once sufficient healing has occurred.

SASP modulation

The SASP is a key feature of cellular senescence that has profound effects on neighbouring cells. SASP interference can be achieved by inhibiting proinflammatory signalling pathways, such as nuclear factor (NF)-κB or p38 mitogen-activated protein kinase (MAPK) pathways[162,163]; however, these interventions will also affect non-senescent cells[164]. Interestingly, rapamycin and metformin also dampen the SASP *in vitro* through inhibition of mTOR, p38-MAPK, MAPK-activated protein kinase 2 (MAPKAPK-2) and NFκB signalling pathways in human fibroblasts and mouse xenografts[165,166]. Bromodomain-containing protein 4 (BRD4, also known as MCAP) inhibitors modulate the SASP with high specificity[167]. BRD4 binds to acetylated lysines on histones, thereby marking open chromatin regions including active enhancer elements that regulate components of the SASP[167]. BRD4 inhibitors allowed senescent cells to escape immune surveillance in a mouse model of oncogene-induced senescence[167].

SASP modulation would not only reduce the chronic burden of senescent cell-associated inflammation, but would also impair immune-mediated clearance, potentially leading to an excessive accumulation of senescent cells. Thus, the implication of these interventions on senescent-cell accumulation requires further study. In addition, whether the aforementioned interventions attenuate the accumulation of senescent cells generated through different senescence programmes in a similar fashion, or more likely, whether they affect cell types produced through specific senescence programmes induced by a particular stressor, is currently unclear.

Strategies to stimulate senescence

Senescence can be induced by cytotoxic and radiation therapies[168] in cancer cells and it is recognized as a favourable outcome that limits tumour progression[169,170]. These findings have led to the development of compounds that induce senescence in cancer cells. Several inhibitors of CDK4 and CDK6 have been developed for this purpose, including abemaciclib, palbociclib and ribociclib, all of which are being tested for therapeutic efficacy in various cancers, including breast, non-small

cell lung, melanoma, glioma, and metastatic pancreatic tumours[171,172]. Palbociclib was also used in a mouse model of AKI, in which treatment after IRI protected against DNA damage, apoptosis, and subsequent kidney damage[173]; the senescent cell burden and identity were not assessed. An obvious concern with the use of senescence-inducing drugs to combat cancer is the potential for long-term detrimental effects resulting from an accumulation of excessive numbers of senescent cells. Perhaps such negative adverse effects can easily be avoided by combining pro-senescence therapy with senolysis.

Conclusion

Developmental, regenerative, cancer-related, age-related and disease-associated senescent cells have variable effects, mechanisms of induction, secreted factors, and lifespan. In the short-term, senescence has beneficial effects on several physiological and pathological processes. These transient benefits are outweighed, however, by the detrimental long-term consequences if the clearance of senescent cells is inefficient and they accumulate. Unfortunately, no distinguishing characteristics exist to discriminate between the short-term and long-term contributions of senescent cells, and further investigation involving the use of model systems that allow targeting of senescent cells for depletion or modulation is required. In *INK-ATTAC*-transgenic mice, removal of senescent cells extended healthy lifespan and prevented the loss of kidney function associated with age without any detrimental long-term effects, indicating that senescent cell accumulation during normal ageing is primarily detrimental.

Renal tubule cells are frequently affected in ageing and disease. Detailed characterization of these cells in healthy individuals and in patients with renal disease is therefore necessary. Additionally, further characterization of the senescent properties of all renal cell types during ageing and in disease is warranted, as features of cellular senescence have been reported in all renal cell types. A limitation of such studies is that most studies so far have only used a subset of established senescence markers, which highlights the need for further molecular characterization of senescence. Although the contribution of senescent cells to renal ageing and pathology is only beginning to be elucidated, the development of therapeutic tools to support healthy kidney ageing, ameliorate kidney disease, and improve the success of renal transplantation will likely involve modulation of the senescence programme in this vital organ.

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Author contributions

All authors researched the data, discussed the article's content, wrote the text and reviewed or edited the article before submission.

Competing interests statement

The authors declare no competing interests.