Senescence of renal cells: molecular basis and clinical implications

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Introduction

Age-associated changes of the kidney are important not only because normal ageing alters renal function but also because of the high frequency of end-stage renal disease in the elderly (ERA–EDTA Registry Report 2000). Old kidneys perform poorly when transplanted, and donor age is a major determinant of graft survival [1]. It has been proposed that interactions between ageing and diseases may contribute to these problems. Understanding the mechanisms of declining organ function with age may be instructive concerning the mechanisms of decline in disease states, since stress might accelerate ageing changes. Kidney ageing is also of interest as a general model for organ ageing, because renal function can be assessed with relative ease in clinical practice and has been quantified in longitudinal studies [2]. The molecular basis of ageing changes in organs is not known, but organ ageing may reflect aspects of cellular senescence which are understood better now than a few years ago.

Here, I will review recent progress in defining the molecular changes and pathways involved in cellular senescence, their relative contribution in cells from different species, in particular human and mouse, and their relevance to renal ageing and disease.

Terms and definitions

‘Cellular senescence’ describes a phenotype of permanent and irreversible growth arrest shown by mammalian cells in culture. Originally described in human fibroblasts by Hayflick and Moorhead [3], this term was used synonymously with ‘replicative senescence’. However, in recent years, the concept of cellular senescence has been expanded to include other forms of permanent, irreversible cell-cycle arrest. The reason for this extension comes partly from the observation that mouse embryonic fibroblasts in culture do not use replicative senescence to cease replication, but share other senescence features with human fibroblasts such as altered morphology, greater heterogeneity, expression of senescence-associated β-galactosidase (SA-β-GAL) and accumulation of lipofuscin granules. This had been referred to as ‘premature senescence’, ‘stress-induced senescence’ and most recently ‘stimulation and stress-induced senescent-like’ arrest (STASIS) [4]. The term ‘renal senescence’ reflects the structural and functional phenotype associated with aged kidneys.

The phenotype of renal senescence

The phenotype of human renal senescence can be described as a phenotype of loss: the loss of mass, particularly in cortex [5,6], and the loss of function, as reflected by increased renal vascular resistance, reduced renal plasma flow and increased filtration fraction [6,7]. The glomerular filtration rate (GFR), as described by multiple equations such as Cockcroft–Gault [8] and MDRD [9], is not an ideal measurement to assess this malfunction. Although studies in selected populations [2,10], excluding all renal diseases, hypertension and
heart failure, demonstrated mean loss of GFR of 0.75 ml/min per year [2], they also found that one-third [2] to two-thirds [10] of the elderly had perfectly normal GFRs.

The principal histological features of renal senescence are glomerulosclerosis, tubular atrophy, interstitial fibrosis and fibrous thickening of the intima of arteries [11]. Even though it is unclear whether these changes are primary or secondary events, they certainly reflect degeneration and thereby loss of functioning cells or units. In the presence of age-related diseases, such as hypertension and heart failure, these changes can be accelerated.

Mechanisms of cellular senescence and their relevance for renal senescence

Replicative senescence and telomeres

Telomeres are special DNA–protein complexes at the ends of eukaryotic chromosomes. Their function is to protect from chromosomal fusion. Telomerase is an enzyme that synthesizes telomeric DNA. Since most human cells do not express telomerase, chromosome ends cannot replicate completely and telomeres shorten with each cell generation. This eventually results in individual chromosomes with critically short telomeres [12] that are no longer protected or ‘capped’ by specific telomere binding proteins such as TRF2 [12,13] and have lost their function. Short dysfunctional telomeres are sensed as DNA strand break and lead to the activation of p53 and possibly p16\(^{INK4a}\), which in turn induce cell-cycle arrest. Telomere shortening is the cause of replicative senescence. But it is important to note that replication does not seem to be the only cause of telomere shortening [4,14,15]. Replicative senescence has been extensively studied in human fibroblasts in culture [16]. These fibroblasts arrest after 50–70 generations with short telomeres [16,17]. Transfection of telomerase into primary human cells [18] overcomes senescence and leads to immortalization, a state of ongoing replication that still retains many characteristic properties of normal primary fibroblasts and is distinct from cellular transformation and malignancy. In contrast to human cells, current evidence indicates that replicative senescence does not exist in laboratory rodents [19,20]. Mouse telomeres are much longer than human telomeres (40–150 vs 5–20 kb), and most mouse somatic cells express telomerase. Mouse embryonic fibroblasts in culture grow rapidly, often at a faster rate than what would be expected in vivo. However, they arrest after only 15–30 generations and do not show telomere attrition. This phenotype caused by culture stresses and not by replication is termed STASIS.

It has been shown that telomeres become shorter in human kidneys with age and that the rate of loss is greater in cortex than in medulla [21]. Long telomeres, continued telomerase expression and a lack of critical telomere shortening with ageing in rat and mouse kidneys have also been found [22,23]. The species differences with ageing support the conclusion that mouse and rat cells in vivo, like mouse embryonic fibroblasts in vitro, do not use telomere shortening as replication counter, but human cells do (Figure 1). The

![Cellular senescence in renal cells in vivo](https://academic.oup.com/ndt/article-abstract/18/12/2474/1815464)
fact that mouse and rat kidneys have telomeres that remain long even in ageing must be taken into account when using mouse and rat models to explain human diseases, as long telomeres and continued telomerase expression may protect against the consequences of injury. Mice deficient in telomerase activity show progressive telomere loss with increased susceptibility to liver injury [24] and renal dysfunction [25]. The role of telomere function in injury and repair could be relevant to the high susceptibility of aged human kidneys to disease due to a decreased capability to withstand stress.

Cellular senescence reached by other mechanisms

In addition to replication, the exposure of primary mammalian cells to certain types of stress can trigger a permanent and irreversible proliferation arrest that results in a senescence-like phenotype (STASIS). It can be reached by DNA damage, oxidative stress, Ras induction and epigenetic alterations.

The cell-cycle regulators and tumour suppressors p16\(^{INK4a}\) and p19\(^{ARF}\) (and its human equivalent p14\(^{ARF}\)) have been associated with this non-replication-dependent growth arrest [26–28] and are of additional interest to us also because of the differences between species. The two genes share two exons by alternative splicing, and they also share involvement in the induction and epigenetic alterations.

Cellular senescence reached by other mechanisms

Cellular senescence in vivo

Two markers of cellular senescence in vitro have already been shown to be important in vivo in other organs. SA-\(\beta\)-GAL probably reflects an increased lysosome content of senescent cells [36,37], and lipofuscin, the well-known ‘ageing pigment’, has recently suggested to be more than an ‘innocent’ bystander but rather a contributor to the ageing process [38]. The data revealed a striking association between SA-\(\beta\)-GAL and lipofuscin and ageing for rat kidney [22]. Both markers were present in tubular cells and increased exponentially with age. Lipofuscin showed a strong association with atrophic tubules. Aged human kidneys showed a similar pattern for both lipofuscin and SA-\(\beta\)-GAL, but the increases were less steep (A. Melk, unpublished data). Old mouse kidneys showed the least amount of lipofuscin and SA-\(\beta\)-GAL when compared to the other species (A. Melk, unpublished data). Lipofuscin and SA-\(\beta\)-GAL may both be manifestations of impaired homeostasis of cell organelles with age.

Implications for nephrology and transplantation

In renal senescence, some cells drop out completely, some persist in damaged form (atrophic tubular cells) and others remain as senescent cells that show limited function and are unable to proliferate. The potential importance of persisting cells with such features in vivo might be that they compromise the function and integrity of the tissue, since ordinarily the organized tissue rather than the individual cell accounts for function. The results are compatible with the hypothesis that some mechanisms contributing to senescence of somatic cells in vitro also play a role in ageing phenotypes in the kidney in vivo. Measurements of senescence changes could emerge as better markers for function or biological age, adding to existing assessments such as histological findings and chronological age. We might find that the renal cells displaying features of senescence such as increased p16\(^{INK4a}\) expression and shortened telomeres do not affect kidney function under normal conditions. This is supported by the clinical observation that end-stage renal disease remains uncommon in the elderly and that the majority of the elderly that do not have other age-related diseases have normal renal function [6,7]. However, it seems likely that processes of renal senescence result in a decreased ability of the aged nephron to cope with disease stresses and that the measurement of markers such as p16\(^{INK4a}\) or telomere
length might help to explain the performance of a kidney under stress. Despite the species differences, the rodent model is still instructive and will permit us to dissect important molecular events and their contribution to ageing and chronic renal diseases.

A special case for a possible interaction of underlying renal senescence and imposed stresses might be the poor performance of transplanted kidneys from older donors, particularly cadaveric donors. Senescent cells could contribute to the diminished ability of the old donor kidney to withstand peritransplant (brain death, preservation, inflammation) as well as post-transplant (rejection, hypertension) stresses, resulting in a higher incidence of allograft nephropathy and poorer graft survival. In a vascularized, fully MHC mismatched mouse transplant model, it was found that acute rejection in kidneys from 18-month-old donors not only induced p16\textsuperscript{INK4a} but also led to the rapid development of tubular atrophy and loss of tubular cells, despite similar host immune response and donor immunogenicity when compared to 3-month-old donors [39,40]. A steep increase in p16\textsuperscript{INK4a} in human allograft nephropathy biopsies compared to the implantation biopsy was also observed (Figure 2) (A. Melk, unpublished data). Others have found increased p21\textsuperscript{CIP1/WAF1} expression in human allograft nephropathy [41] and chronic liver allograft rejection [42]. Telomere shortening has been observed in a rat model of chronic rejection [43], even though these results have to be seen in the context of data showing that normal rodent cells with telomerase expression do not use the telomere mechanism in kidney ageing [22,23].

Based on the findings in transplanted kidneys, one has to consider that induction or ‘acceleration’ of features found in cellular senescence in vitro might be a common pathway for other chronic renal diseases. p16\textsuperscript{INK4a} expression and/or telomere shortening could be causal in the development of tubular atrophy and interstitial fibrosis. In addition, age-related diseases might accelerate the changes contributing to the phenotype of renal senescence. The observation that telomeres are shorter in intimal cells from arteries exposed to higher blood pressure [44] would suggest an important role for hypertension.

In conclusion, the observation that cells with features of senescent cells in culture can be identified in kidney and seem to be involved in certain disease states is exciting. Further studies have to focus on exploring the causal relationship between the occurrence of senescent cells and organ damage. Such findings will extend the understanding of normal kidney ageing and the pathogenesis of chronic renal diseases.

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**References**


Fig. 2. p16\textsuperscript{INK4a} staining in a case of allograft nephropathy. (A) Zero biopsy from a 19-year-old donor with no p16\textsuperscript{INK4a} staining. (B) One-year follow-up biopsy diagnosed with allograft nephropathy showing nuclear and cytoplasmic staining for p16\textsuperscript{INK4a} in tubular cells. The amount of nuclear staining is compatible with what was found in normal kidneys from individuals in their eighties.