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REVIEW

Aging and uremia: Is there cellular and molecular crossover?

William E White, Muhammad M Yaqoob, Steven M Harwood

William E White, Muhammad M Yaqoob, Steven M Harwood, Queen Mary University of London, Translational Medicine and Therapeutics, William Harvey Research Institute, John Vane Science Centre, EC1M 6BQ London, United Kingdom
Muhammad M Yaqoob, Department of Nephrology, Barts Health NHS Trust, The Royal London Hospital, Whitechapel, E1 1BB London, United Kingdom
Author contributions: All authors contributed to this work.

Correspondence to: Steven M Harwood, PhD, Queen Mary University of London, Translational Medicine and Therapeutics, William Harvey Research Institute, John Vane Science Centre, Charterhouse Square, EC1M 6BQ London, United Kingdom. s.m.harwood@qmul.ac.uk
Telephone: +44-020-78822122
Fax: +44-020-78828252
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Abstract

Many observers have noted that the morphological changes that occur in chronic kidney disease (CKD) patients resemble those seen in the geriatric population, with strikingly similar morbidity and mortality profiles and rates of frailty in the two groups, and shared characteristics at a pathophysiological level especially in respect to the changes seen in their vascular and immune systems. However, whilst much has been documented about the shared physical characteristics of aging and uremia, the molecular and cellular similarities between the two have received less attention. In order to bridge this perceived gap we have reviewed published research concerning the common molecular processes seen in aging subjects and CKD patients, with specific attention to altered proteostasis, mitochondrial dysfunction, post-translational protein modification, and senescence and telomere attrition. We have also sought to illustrate how the cell death and survival pathways apoptosis, necroptosis and autophagy are closely interrelated, and how an understanding of these overlapping pathways is helpful in order to appreciate the shared molecular basis behind the pathophysiology of aging and uremia. This analysis revealed many common molecular characteristics and showed similar patterns of cellular dysfunction. We conclude that the accelerated aging seen in patients with CKD is underpinned at the molecular level, and that a greater understanding of these molecular processes might eventually lead to new much needed therapeutic strategies of benefit to patients with renal disease.

Key words: Aging; Uremia; Apoptosis; Autophagy; Senescence; Telomeres; Mitochondria; Post-translational protein modification; Klotho

Core tip: This review presents evidence that suggests that the morphological similarities between uremia and physiological aging are underpinned by similarities at a cellular and molecular level. Several of the classical cellular features of aging such as mitochondrial dysfunction and altered proteostasis have been observed in the cells and tissues of uremic humans and animals, and in *in vitro* models of uremia. There are also many shared features between aging and uremia in terms of

cell death and survival pathways. These commonalities may present new targets for the future management of patients with chronic kidney disease.

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INTRODUCTION

Observation alone suggests that patients with end stage kidney disease (ESKD) are biologically older than their unaffected peers. As a group, ESKD patients have a morbidity and mortality profile similar to that of the geriatric population, and the pathophysiology of the uremic syndrome has interesting parallels with the aging process. Based on these thoughts it has been posited that kidney failure results in accelerated, pathological aging[1]. Indeed there are striking analogies between the effects of aging and uremia on the structure and function of the heart and vasculature, with similar changes seen in pulse contour, pulse wave velocity, and impedance, and similar structural abnormalities with wall thickening, decreased elastin, and increased collagen content[2].

Aging is characterized by a progressive loss of physiological integrity, leading to impaired function and increased vulnerability to death[3]. Dialysis dependent patients of any age have an increased risk of mortality when compared to those with a functioning transplant and healthy controls of the same age[4], and are more susceptible to disease, particularly that of the cardiovascular system: a 25-34-year-old dialysis patient has a relative risk of cardiovascular mortality similar to that of a > 75-year-old in the general population[5]. Furthermore, the prognosis for chronic kidney disease (CKD) patients is still extremely poor and has not improved greatly despite many treatment advances: CKD patients receiving dialysis aged 50 and under are likely to live 30 years less than age-matched people without CKD[5]. Whilst survival rates have slightly improved they have not kept pace with the rises seen in the normal population without CKD, with the result that relative survival in age-specific patients with CKD actually decreased between 1977 and 2007[6]. There is thus a need to identify if CKD is inducing an aging-like cellular and molecular dysfunction, and if so whether any novel potential therapy might be derived from an increased understanding of the pathways that are induced by both CKD and aging.

ESKD confers a greatly increased risk of infectious morbidity and mortality, whilst simultaneously being a chronic inflammatory state, a pattern of immune dysfunction also associated with aging[7]. These abnormalities also seem to be reflected at a cellular level, with preferential loss of cells belonging to the lymphoid cell lineage, and inflammation and expansion of proinflammatory immune cells[8].

There is a high prevalence of the frailty syndrome amongst dialysis patients, a phenotype partly defined by weight loss, muscle weakness, and fatigue, which is associated with adverse outcomes in geriatric patients[9]. In the original study that developed this definition, 6.9% of participants ⩾ 65-year-old were classified as frail; in a more recent study of dialysis patients 44% of those under 40-year-old were found to be frail[10]. Cognitive impairment is also highly prevalent in the dialysis-dependent population and occurs in comparatively young patients[1,11].

Whilst much has already been written about the intriguing similarities that appear to exist between the aging process and CKD[1,8,12,13], comparatively little work has been undertaken looking at the cellular and molecular hallmarks of aging in the context of the known evidence concerning uremia-induced cellular and molecular pathways. Therefore in this review, in order to try and fill this perceived gap in the literature, we have first briefly outlined what the main cell death pathways are and by what means these processes interact with each other, followed by an analysis of published research concerning the mechanisms of aging and uremia-induced cell death and their common molecular pathways and cellular characteristics. Lastly we provide an assessment of how this knowledge may lead to benefits in both nephrology and gerontology.

CELL DEATH AND SURVIVAL PATHWAYS

An outline of cell death

Since the first descriptions of apoptotic cell death appeared more than 40 years ago[14] the study of cell death has become a substantial and important area. The main cell death pathways have been reviewed exhaustively in the literature and it is not the aim of this review to repeat this information. What is pertinent here is how much our understanding of cell death has changed and evolved in recent years. This is because cell death and survival pathways are now being assessed more as molecular processes and less as a series of morphological characteristics. One of the most fundamental changes is that each death pathway is no longer considered in isolation and there is an appreciation that cell death can no longer be considered as a choice between apoptotic, autophagic or necrotic death. Pathways once thought of as discreet have been found to be closely interconnected with others whilst some pathways have needed to be recategorized. In addition several completely novel pathways have been described. An example of reclassification is that necrosis is now subdivided into two distinct forms, one being programmed necrosis that

is usually termed necroptosis or regulated necrosis, and accidental or non-regulated necrosis which is more in line with the original concept of necrosis. Another example of recent developments is that apoptosis has now been split into four different classes whilst a total of 13 functional classes of regulated cell death have been described[15]. So whilst this review is focusing on the most established and described death and survival pathways they must not be considered as being complete. Lastly, the role of autophagy in cell death has been recently challenged[16,17] whilst its role in cell survival[18] asserted.

Uremia induced apoptosis

Although apoptosis and uremia have been studied extensively both separately and together, a clear picture of how uremia induces apoptosis has yet to be established. Instead a large number of studies using experimental models and human subjects have shown that uremia is associated with apoptosis in a wide range of cells and tissues such as skeletal muscle[19,20], myocardium[21], platelets[22,23], monocytes[24], neutrophils[25], lymphocytes[26], leukocytes[27] and vascular endothelial cells[28]. The kidney has also been shown as a target for apoptosis in uremia with both podocytes[29] and proximal tubular cells identified as having increased apoptotic cell death[30]. Furthermore, it has become known that it certain circumstances dialysis itself can be an activator of apoptosis[20,26]. It is unclear if the apoptosis seen in the kidney is the cause or the effect of CKD. However, it does seem probable that acute kidney injury (AKI) induced apoptosis can subsequently lead to the activation of interstitial fibroblasts *via* transforming growth factor beta (TGF-β) resulting in CKD[31,32]. In fact expression of TGF-β has been found to be elevated in nearly all human and experimental forms of CKD[33] and demonstrated to be directly associated with age in healthy human subjects[34].

Uremia induced necroptosis

Uremia induced necroptosis (or programmed necrosis) has yet to feature prominently in the literature although this is possibly due, at least in part to previous cell death descriptions not being classified correctly according to current definitions (see aging induced apoptosis below).

Aging induced apoptosis and necroptosis

The induction of apoptosis in aging in most tissues awaits clarification. However, in skeletal muscle at least there is clear evidence that muscle mass decreases with age[35-37] with apoptosis being known to be elevated in the skeletal muscle of aged subjects[38-41]. It has been suggested that aging increases cell death by caspase independent mechanisms. There is also some evidence that terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining is greater the kidneys of aged in mice[42] but TUNEL staining has been shown not to be specific for apoptosis[43]. It seems plausible that at least some of the examples for age induced apoptosis in the literature instead reflect increases in necroptosis.

Apoptosis and necroptosis crosstalk

It is now appreciated how significantly involved the apoptosis machinery is in other cell death and survival pathways. Many of the described apoptotic death receptors such as tumor necrosis factor receptor 1 and FAS are now also known to be able to induce necroptotic cell death[44,45]. Caspase-8, a key component of receptor mediated apoptosis is now thought to regulate the activation of necroptosis[45]. Inhibitor of apoptosis (IAP) are endogenous caspase inhibitors and therefore play a role in controlling apoptosis. When IAP levels are reduced this leads to caspases being activated which results in apoptotic cell death. Another IAP, X-Chromosome-linked IAP has been shown to be reduced in the muscle of CKD mice and *in vitro* in muscle cells treated with serum obtained from CKD mice[46].

The activation of autophagy is known to breakdown IAPs and lead subsequently to the induction of necroptosis. Furthermore, in conditions where IAPs are suppressed or absent and caspase activity is inhibited can lead to the activation of necroptosis *via* receptor-interacting protein1 (RIP1) and its downstream kinase (RIPK1)[47]. It has been postulated that RIP1 together with RIP3, cIAP, Caspase-8 and cFlip act as essential components of the ripoptosome, a signalling platform that can switch modes between apoptotic and necroptotic cell death[48]. Recent work indicates that it is RIPK3 activity that determines whether cells die by necroptosis, or in its absence, by caspase-8 mediated apoptosis[49] whilst another group have suggested that necroptosis can be induced in the absence of RIPK1 and without the formation of a functioning ripoptosome[50], the complex considered essential for necroptosis to occur.

Autophagy

Autophagy is the dynamic, multistep cellular process wherein portions of cytoplasm, including organelles, are sequestered into double-membrane vesicles (termed autophagosomes) and delivered to lysosomes where they are degraded, with eventual recycling of the resultant macromolecules[51]. By removing excessive and aberrant organelles and proteins, autophagy contributes to cellular homeostasis and protein quality control, and functions as a source of energy for the cell[52]. Autophagy is up-regulated and has a protective function in the face of cellular stressors such as starvation[53] and ischemia[54].

Autophagy and apoptosis crosstalk

It is perhaps not surprising that autophagy and apoptosis exhibit crosstalk as both pathways play such significant roles in development, homeostasis and pathology[55]. Evidence of this crosstalk has been plentiful[56-60] and indicates that the pathways can interact in an additive or antagonistic fashion and that the molecular machinery

for both can combine *via* p27[56], p38[57], p53[58] and beclin-1[59,60]. It is likely that these overlapping pathways are involved in uremia and aging induced dysfunction. For example in autophagy-deficient mice the onset of ischemia/reperfusion injury resulted in greater proximal tubular apoptotic injury with significantly elevations in serum urea and creatinine compared to wild type animals. This indicates that autophagy maintains proximal tubular homeostasis and protects against ischemic injury[61]. In another study using a dietary adenine-induced chronic renal failure model a high phosphate diet was found to increase apoptosis in vascular smooth muscle cells (VSMC) and that this rise could be reduced by autophagy inhibition. However, reducing autophagy was associated with an increase in calcium deposition in VSMC. The study concluded that autophagy might be an endogenous protective mechanism against phosphate-induced vascular calcification[62].

Autophagy and necroptosis

In addition to necroptosis crosstalking with apoptosis *via* IAP (see apoptosis and necroptosis crosstalk) there is also evidence of autophagy and necroptosis crosstalk in a similar fashion. Using a novel chalcone derivative as an anti-cancer agent it was found that Jun N-terminal kinases-mediated autophagy was able to cause IAP degradation followed by necroptosis[63]. It seems likely therefore that there is a therapeutic potential for autophagy to be exploited by anticancer agents to provoke cancer cell death. However, it should be noted that the molecular interactions between the two processes is still largely unknown and indeed there is evidence that autophagy activation can block necroptosis in several cell lines[64,65].

Autophagy in aging

Beyond its function at a cellular and organ level, autophagy has been heavily implicated in the aging process and the determination of life span. Normal and pathological aging are associated with failing proteostasis and reduced autophagic activity[3], and genetic inhibition of autophagy produces degenerative changes in mammalian tissue resembling those seen in aging. Caloric restriction, which has been shown to promote longevity in model organisms, stimulates autophagy, as do some pharmacological interventions and genetic manipulations that increase life span in model organisms, and inhibiting autophagy attenuates this effect[66].

Autophagy in uremia

Much work has been published describing the role of autophagy in the pathophysiology of AKI and CKD, but very little has been published looking at the effects of uremia on autophagy in other tissues. Chen *et al*[67] assessed autophagy activation in leukocytes isolated from peripheral blood samples, which had been taken from stage 5 CKD patients and healthy controls after overnight fasting and 2 h after breakfast. Overnight fasting induced conversion of microtubule-associated protein light chain 3 (LC3) Ⅰ to Ⅱ (as detected by western blot as increased quantities of the latter, and signifying autophagosome formation) in healthy subjects. mRNA levels of autophagy-related gene 5 (*Atg5*) and beclin-1 also increased in fasted healthy subjects but not in CKD patients. Interestingly there was no difference between CKD patients receiving or not receiving hemodialysis. Furthermore, a negative association was found between LC3Ⅱ and left atrium size, Atg5 transcription and left ventricular end-diastolic diameter, and beclin-1 transcription and mitral inflow E- and A-wave sizes. The authors conclude that autophagic activation is impaired in CKD patients and is not reversed with hemodialysis, and that this impairment is related to cardiac abnormalities.

Siedlecki *et al*[68] assessed the effect of rapamycin administration in a murine model of normotensive uremic cardiomyopathy. Treatment of surgically induced renal injury mice with rapamycin blocked the development of cardiac hypertrophy and fibrosis when compared with vehicle-treated animals. The experimenters suggest that this protective effect is mediated by the extracellular signal-regulated kinase and mammalian target of rapamycin (mTOR) pathways. They do not speculate on the possible involvement of autophagy, but rapamycin is known to stimulate autophagy *via* mTOR, and has been shown to have anti-aging effects in mammals[69]. The authors raise the interesting question of whether renal transplant recipients taking rapamycin as an immunosuppressant exhibit reversal of uremia-induced cardiac changes beyond that associated with successful transplantation.

In summary, the principle cell death and survival molecular pathways consisting of apoptosis, necroptosis and autophagy are strongly interrelated and crossover at many points. Whilst our current knowledge on how these interacting pathways are controlled and regulated is far from complete our appreciation of how similar many of the molecular signalling induced by uremia and aging appears to be growing pathways.

SHARED CELLULAR CHARACTERISTICS OF AGING AND UREMIA

Cell senescence, telomere shortening and stem cell exhaustion

Cellular senescence can be defined as stable arrest of the cell cycle coupled to classic phenotypic changes[70]. This was originally described by Hayflick *et al*[71] in serially passaged human fibroblasts, which undergo a certain number of divisions before entering a senescent phase (the "Hayflick limit"). This phenomenon was subsequently shown to be due to telomere shortening[72], but can be triggered by non-telomeric aging-associated stimuli such as DNA damage and excessive mitogenic signaling[3].

Senescent cells accumulate in aged organisms, although senescence *per se* does not cause aging,

having a protective effect by preventing the propagation and causing the removal of damaged and potentially oncogenic cells from tissues. A failure to clear senescent cells and replace these with new ones may, however lead to their accumulation[3]. Senescent cells are known to possess large amounts of proinflammatory cytokines and matrix metalloproteinases (the "senescence-associated secretory phenotype") which may in themselves contribute to aging[73].

Senescent cells have a flattened and enlarged morphology, and express a different set of genes such as p16, p21, p53, and retinoblastoma protein (pRb)[74]. Senescence-associated β-galactosidase (SA-β-gal) is a frequently used biomarker of cell senescence *in vivo* and *in vitro*[75].

Jimenez *et al*[76] looked at markers of senescence in circulating immune cells in uremic pre-dialysis, hemodialysis-dependent and transplanted patients. Abnormal telomere shortening was seen in a subpopulation of lymphocytes in pre-dialysis patients. In hemodialysis patients who dialyzed with cellulosic membranes, a subset of mononuclear cells demonstrated telomere shortening and exhibited increased levels of intracytoplasmic proinflammatory cytokines, which were released in response to substimulatory doses of lipopolysaccharide and bacterial DNA *in vitro*. The authors postulate that these senescent mononuclear cells both result from and contribute to chronic inflammation in such patients. A subpopulation of lymphocytes with shortened telomeres was also found in transplant patients with near normal renal function. It was suggested that these resulted from chronic activation due to major histocompatibility complex incompatibility and immunosuppressive therapy.

Tsirpanlis *et al*[77] measured the activity of telomerase (the enzyme that preserves telomere length and structure and thus prevents senescence[78]) in peripheral blood mononuclear cells in hemodialysis-dependent patients and non-renal failure subjects. Telomerase activity was reduced in hemodialysis patients compared to healthy controls, and was lower in long-term than in short-term dialysis patients. These findings indicate that defence against senescence is reduced in this cell type and associated with chronicity in hemodialysis patients.

Several groups have looked at the role of senescence in the endothelial dysfunction associated with cardiovascular disease in uremia. Adijiang *et al*[79] administered indoxyl sulphate, a uremic toxin, to hypertensive and normotensive rats, and examined their aorta for histological and immunohistochemical evidence of senescence. The indoxyl sulphate-treated animals showed significantly increased aortic calcification and wall thickness, and significantly increased expression of SA-β-gal, p16, p21, p53 and pRb in cells embedded in the calcification area. The same group went on to demonstrate that indoxyl sulphate stimulated senescence of cultured human aortic smooth muscle cells *via* an oxidative stress mechanism[74].

Carracedo *et al*[80] evaluated the effects of uremia on low-density lipoprotein (LDL) carbamylation and the effect of carbamylated LDL (cLDL) and oxidized LDL on the number, function, and genomic stability of endothelial progenitor cells (EPCs) obtained from healthy volunteers. EPCs were exposed to cLDL generated after incubation of native LDL (nLDL) with uremic serum from patients with CKD stages 2-4. Compared with cLDL, nLDL induced an increase in oxidative stress, depolarization and senescence in EPCs, and a decrease in EPC proliferation and angiogenesis. The authors hypothesize that cLDL triggers genomic damage in EPCs resulting in premature senescence, and that this contributes to atherosclerotic disease in uremia.

Klinkhammer *et al*[81] demonstrated that bone marrow mesenchymal stem cells (MSCs) isolated from uremic rats (both surgically induced and adenine diet) showed signs of premature senescence, and failed to accelerate healing of glomerular lesions when injected into the left renal artery of rats with acute anti-Thy1.1-nephritis when compared to MSCs obtained from control rats. The authors conclude that CKD leads to a sustained loss of *in vitro* and *in vivo* functionality in MSCs, possibly due to premature senescence. Stem cell exhaustion and the resultant decline in tissue regenerative potential has been noted as one of the hallmarks of aging[3].

In summary, aging and uremia share many important cellular characteristics such as increases in cell senescence, telomere shortening and exhaustion of stem cells. This provides further evidence that supports the contention that uremia can be considered as a form of accelerated aging[1].

Klotho

The *klotho* gene was originally identified as being involved in the suppression of aging in transgenic mouse studies[82]. Defective klotho expression resulted in mice having a premature aging phenotype, which had striking similarities to that of CKD patients, including reduced life span, arteriosclerosis, hyperphosphataemia and high concentrations of plasma fibroblast growth factor-23 {FGF23, a bone derived hormone that promotes renal phosphate excretion and reduces serum levels of 1,25-dihydroxyvitamin D3 [1,25-(OH)2VD3][83]}. This observation, coupled with the fact that, although found in multiple tissues, klotho expression is highest in the kidney (predominantly in the distal convoluted tubules[84]), suggested that CKD might be a state of klotho deficiency, and this might contribute to the accelerated aging phenotype of uremia[85].

Through alternative splicing klotho exists in membrane-anchored and soluble, secreted forms, the latter being found in mammalian cerebrospinal fluid, blood and urine[84]. These forms have distinct functions. Membrane klotho forms a complex with FGF receptors and functions as a co-receptor for FGF23. Soluble klotho functions

as an endocrine factor, and has a role in a number of processes including modulation of ion transport[86] and counteraction of the renin-angiotensin system[87]. Klotho suppresses 1α-hydroxylase in the kidney to regulate calcium metabolism[88], and participates in in the regulation of parathyroid hormone synthesis in the parathyroid gland by FGF23[84,89].

Both physiological aging and CKD are associated with reduced klotho levels. Lower renal klotho protein expression has been shown in aging rodents compared to young ones[90], and plasma klotho concentrations were found to be two-fold higher in normal children than in adults[91]. Renal klotho RNA has been shown to be reduced in CKD kidneys[92], as have urinary klotho levels[85]. Klotho concentrations in plasma, urine and kidney were found to be decreased in parallel in a rodent CKD model[85].

Klotho may influence cell death and survival pathways *via* its anti-senescence and oxidation effects. Liu *et al*[93] analysed various tissues and organs from klotho$^{-/-}$ mice and demonstrated a decrease in stem cell number and an increase in progenitor cell senescence. Tissues from klotho-deficient animals showed evidence of increased Wnt signalling. *In vivo* and *in vitro* Wnt exposure triggered by the absence of klotho accelerated cellular senescence. The authors conclude that klotho might act as a secreted Wnt antagonist and that a decrease in klotho concentration leads to an increase in Wnt signalling and this may play a role in aging.

de Oliveira *et al*[94] generated a klotho-knockdown human fibroblast, in which premature senescence was seen alongside an increase in p21 expression. p53 knockdown in klotho attenuated cells restored normal growth and replicative potential. These results suggest that klotho regulates cell senescence by suppressing the p53/p21 pathway. Ikushima *et al*[95] demonstrated that purified recombinant klotho protein could attenuate apoptosis and senescence in human umbilical vein endothelial cells. The same group went on to show that this occurred *via* mitogen-activated kinase and extracellular signal-related kinase pathways[96].

Klotho may exert an anti-aging effect by suppressing the inflammatory effect of substances secreted by senescent cells. Liu *et al*[97] have shown that cellular klotho interacts with retinoic acid-inducible gene-Ⅰ (RIG-Ⅰ) and that this interaction inhibits the RIG-Ⅰ induced expression of interleukin 6 (IL-6) and IL-8 both *in vivo* and *in vitro*.

Thus the deficiency in klotho seen in uremia and aging might underpin the enhanced cell senescence, apoptosis and stem cell depletion common to both states[81]. Given that tissue klotho expression is greatest in the kidneys a common mechanism is perhaps to be expected. Indeed recent data indicate that kidney tissue klotho expression greatly effects systemic concentrations and they concluded that the kidney is the prime mediator of klotho function[98]. Therefore klotho, a recognised anti-aging factor, is under the control of the kidney and thus lends further support to there being a molecular basis for the observed shared phenotype between uremia and aging.

Post-translational protein modification

Spontaneous post-translational protein modifications result from the non-enzymatic attachment of reactive molecules to protein functional groups. This process occurs in healthy individuals with aging, but is increased in certain disease states. Alterations to protein structure may result in functional changes, which can be pathogenetic[99]. Carbamylation is one form of post-translational protein modification specifically associated with CKD and uremia. Cyanate, a dissociation product of urea, binds to proteins and free amino acids, resulting in abnormal cellular responses that may contribute to inflammation and atherosclerosis. As carbamylation results from a direct product of uremia it may serve as a quantitative biomarker of time-averaged urea concentrations in addition to its potential use in risk assessment[99].

One of the most widely studied and publicised forms of post-translational protein modification is glycation. Advanced glycation end products (AGEs) are formed by the non-enzymatic modification of tissue proteins by physiologic sugars. AGEs accumulate in tissues as a function of increased production (*e.g.*, in diabetes mellitus), decreased renal removal of AGE precursors (*e.g.*, in advanced CKD) and time (as occurs in physiological aging)[100]. Covalent cross-linking occurs in affected proteins, leading to increased stiffness of the protein matrix, thus impeding function, and increased resistance to proteolytic removal, thus affecting tissue remodeling[101]. This contributes, for instance, to the histological and functional changes seen in diabetic glomerulosclerosis and atherosclerosis[102]. AGE accumulation also stimulates cytokine and reactive oxygen species (ROS) production through AGE-specific receptors, modifies intracellular proteins[100], and has been shown to promote senescence[103] and apoptosis[104] in the cells of affected tissues, contributing to cell death and tissue dysfunction.

Significantly elevated serum levels of AGEs are present in ESKD, with no differences between patients with and without diabetes[105], and uremic patients are known to be exposed to high levels of oxidative stress[106]. Taki *et al*[107] demonstrated that plasma levels of pentosidine, an AGE, was correlated and independently associated with coronary artery calcification score in hemodialysis patients. Pentosidine formation is accelerated by oxidative stress[108], and in this study was correlated with indoxyl sulphate. The authors thus conclude that indoxyl sulphate may enhance oxidative stress, which in turn enhances AGE generation.

Increased oxidative stress and AGE generation are known to play a role in the pathophysiology of aging[100], and both of these events are present in patients with CKD[105,106] and therefore represent two further potential crossovers between uremia and the aging process.

Table 1 Events common to aging and uremia covered by this review

Aging	Uremia
TGF-β ↑	TGF-β ↑
Autophagy ↓	Autophagy ↓
Apoptosis ↑ (muscle)	Apoptosis ↑
Senescence ↑	Senescence ↑
Telomere shortening ↑	Telomere shortening ↑
Stem cell exhaustion ↑	Stem cell exhaustion ↑
Klotho ↓	Klotho ↓
AGEs ↑	AGEs ↑
Mitochondrial dysfunction ↑	Mitochondrial dysfunction ↑

TGF-β: Transforming growth factor beta; AGEs: Advanced glycation end products.

Mitochondrial dysfunction

According to the mitochondrial free radical theory of aging, progressive, age-related mitochondrial dysfunction results in increased production of ROS, which causes further mitochondrial deterioration and cellular damage[109]. Recent data have questioned the idea that ROS have an entirely deleterious effect in aging, suggesting that they represent a stress-induced survival signal which acts to activate homeostatic responses to cellular stress and damage. As these accumulate with aging ROS eventually pass a threshold and aggravate the damage[110].

Dysfunctional mitochondria can contribute to aging independently of ROS[3]. Damaged mitochondria have an increased tendency to permeabilize in response to stress, leading to apoptotic cell death[111] and inflammation[112]. Aging associated mitochondrial dysfunction arises *via* several mechanisms[3]. For example, mitochondrial decline occurs as a consequence of telomere attrition in telomerase-deficient mice with subsequent p53-mediated repression of peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PCC1a) and PGC-1b[113], and can be partially reversed in wild-type mice by telomerase activation[114]. Sirtuins, a group of nicotinamide adenine dinucleotide-dependent protein deacetylases[115], also play a role in controlling mitochondrial function. Silent information regulator two protein 1 modulates mitochondrial biogenesis *via* the transcriptional co-activator PGC-1a[116] and the removal of damaged mitochondria by autophagy[117]. SIRT3 targets many enzymes involved in energy metabolism[118], and may directly control ROS production by deacetylating manganese superoxide dismutase, a mitochondrial antioxidant enzyme[119].

Mutations and deletions in mitochondrial DNA are known to accumulate with aging[3]. One of the most common and abundant mitochondrial DNA mutations is a 4977 base pair deletion between nucleotide positions 8470 to 13,477 (mtDNA4977)[120], which is known to accumulate in a variety of human tissues with age and has been demonstrated to be associated with several neurodegenerative diseases (including Alzheimer's) and atherosclerosis[121,122]. Defective quality control by mitophagy (organelle-specific autophagy that targets abnormal or worn out mitochondria for degradation) leads to reduced clearance and turnover of ineffective and toxic mitochondria[123]. The net result of these processes is that there is a reduction in the formation of healthy mitochondria, an increased incidence of mitochondrial damage, and a failure to clear and recycle abnormal organelles, with consequently increasing bioinefficiency, inflammation and cell death with aging.

Patients with advanced uremia are recognised to have low body temperatures, reduced stamina and low basal energy expenditure, suggesting a hypometabolic state[124]. Thompson *et al*[125] examined the forearm muscles of patients with ESKD using ^{31}P-magnetic resonance spectroscopy. They noted increased phosphocreatine depletion and increased glycolytic ATP production during exercise, suggesting mitochondrial dysfunction due to either limitation of oxygen supply, reduced mitochondrial content or an intrinsic mitochondrial defect. Exercise-related abnormalities remained despite anemia correction with erythropoietin[125].

Lim *et al*[126] demonstrated a high frequency of mtDNA4977 in the skeletal muscle of chronically uremic patients, and that this correlated with enhanced oxidative damage to DNA, lipids and proteins of mitochondria compared to healthy controls. Liu *et al*[127] found that the incidence and proportion of mtDNA4977 in hair follicles was significantly higher amongst hemodialysis patients compared to age matched controls. Therefore mitochondrial abnormalities, contributing and consequent to high levels of oxidative stress in uremia, are strongly suspected to play a role in the causation of pathological aging in CKD, acting as a nexus for several processes, including defective bioenergetics, telomere attrition, DNA mutations, autophagy, inflammation and cell death. Mitochondrial abnormalities therefore represent a further crossover point between aging and the uremia.

DISCUSSION

In this review we have sought to draw the reader's attention not just to the morphological similarities between advanced aging and uremia, but also to their shared characteristics at a cellular and molecular level (see Table 1). Experimental evidence has been provided to suggest common involvement of established cell death and survival pathways (apoptosis, necrosis, necroptosis and autophagy), and the presence of several of the recognised cellular and molecular features of the aging process in patients with ESRD and in experimental models of uremia. These include mitochondrial dysfunction, damage to genetic material, telomere shortening, impaired proteostasis, cell senescence, stem cell loss, oxidative stress, AGE accumulation, and klotho deficiency. Based on this evidence it could be posited that the physical resemblance between advanced age and uremia is underpinned by shared cellular and molecular

"abnormalities". These observations also reinforce the idea of the "uremic syndrome", in which dysfunctions in multiple body systems arise due to a pervasive defect at a cellular level.

Information gathered by research into aging pathways and "anti-aging therapies" might inform interventions to avoid, slow the progression of or even reverse some of the pathological changes seen in uremia. Given that these pathways are seen throughout most tissues and cell types it is also possible that a single intervention might treat several pathologies. However, the aging process remains incompletely understood in healthy individuals, and those pathways that are known are complex and heavily interconnected. Disentangling these in the uremic syndrome, in which multiple co-existing and interdependent metabolic abnormalities arise, will be a challenge. Additionally, many of these pathways have known (and possibly unknown) protective mechanisms (against malignant transformation, for example), thus blocking them may have unwanted and deleterious effects. What could be more immediately practicable would be employing some of the therapies known to be effective in improving the health of elderly patients, such as exercise.

The concept of accelerated aging in uremia is an intriguing and complex one that may yield important therapeutic targets and strategies to improve health outcomes in patients with CKD. Much work, however, remains to be done in understanding its cellular and molecular basis before any potential benefits can be realised.

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