

# Klotho, stem cells, and aging

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**Abstract:** Aging is an inevitable and progressive biological process involving dysfunction and eventually destruction of every tissue and organ. This process is driven by a tightly regulated and complex interplay between genetic and acquired factors. *Klotho* is an antiaging gene encoding a single-pass transmembrane protein, klotho, which serves as an aging suppressor through a wide variety of mechanisms, such as antioxidation, antisenesescence, antiautophagy, and modulation of many signaling pathways, including insulin-like growth factor and Wnt. Klotho deficiency activates Wnt expression and activity contributing to senescence and depletion of stem cells, which consequently triggers tissue atrophy and fibrosis. In contrast, the klotho protein was shown to suppress Wnt-signaling transduction, and inhibit cell senescence and preserve stem cells. A better understanding of the potential effects of klotho on stem cells could offer novel insights into the cellular and molecular mechanisms of klotho deficiency-related aging and disease. The klotho protein may be a promising therapeutic agent for aging and aging-related disorders.

**Keywords:** aging, cell senescence, klotho, stem cells, Wnt

## Introduction

*Klotho*, the gene encoding the antiaging protein called klotho, was discovered in 1997 when mice developed multiple organ failure and shortened life span resembling human premature aging after this gene was serendipitously silenced.<sup>1</sup> The klotho-deficient phenotype can be rescued by overexpressing klotho via genetic manipulation<sup>2</sup> or viral delivery.<sup>3</sup> Subsequently, *klotho* genes in humans and rats were cloned (Table 1). The chromosomal localization of mouse klotho is on 13q12, encompassing 50 kb and consisting of five exons (Figure 1).<sup>1,4</sup> Two transcripts that arise from a single *klotho* gene through alternative RNA splicing were identified, and are predicted to encode a membrane and a secreted protein. The membrane protein consists of 1,014 amino acids, with an extracellular domain of twofold internal repeats (termed KL1 and KL2 domains), each about 450 amino acids long with 20%–40% homology to  $\beta$ -glucosidases. The secreted protein derived from the alternative transcript is only 550 amino acids long without the transmembrane domain<sup>1,4</sup> (Figure 1).

The chromosomal localization of the rat klotho gene is on 12q12. The rat membrane klotho protein is also 1,014 amino acids long and is 94% and 85% homologous to those of mouse and human klotho proteins, respectively.<sup>5</sup> The human klotho gene is localized on chromosome 13q12, encompassing 50 kb and consisting of five exons. Analogous to the mouse, two transcripts also arise from a single human klotho gene through alternative RNA splicing and encode a membrane (1,012 amino acids) or secreted (549 amino acids) protein.<sup>6</sup>

Klotho is highly expressed in the kidney, brain, and to a lesser extent in other organs.<sup>1,7</sup> The extracellular domain of membrane klotho can be cleaved and shed by secretases.<sup>8–10</sup> This released extracellular domain is referred as “soluble klotho” in this manuscript to distinguish it from another short klotho protein containing only

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**Table I** Comparison of *klotho* gene and protein between humans<sup>6</sup> and rodents<sup>1,4,5</sup>

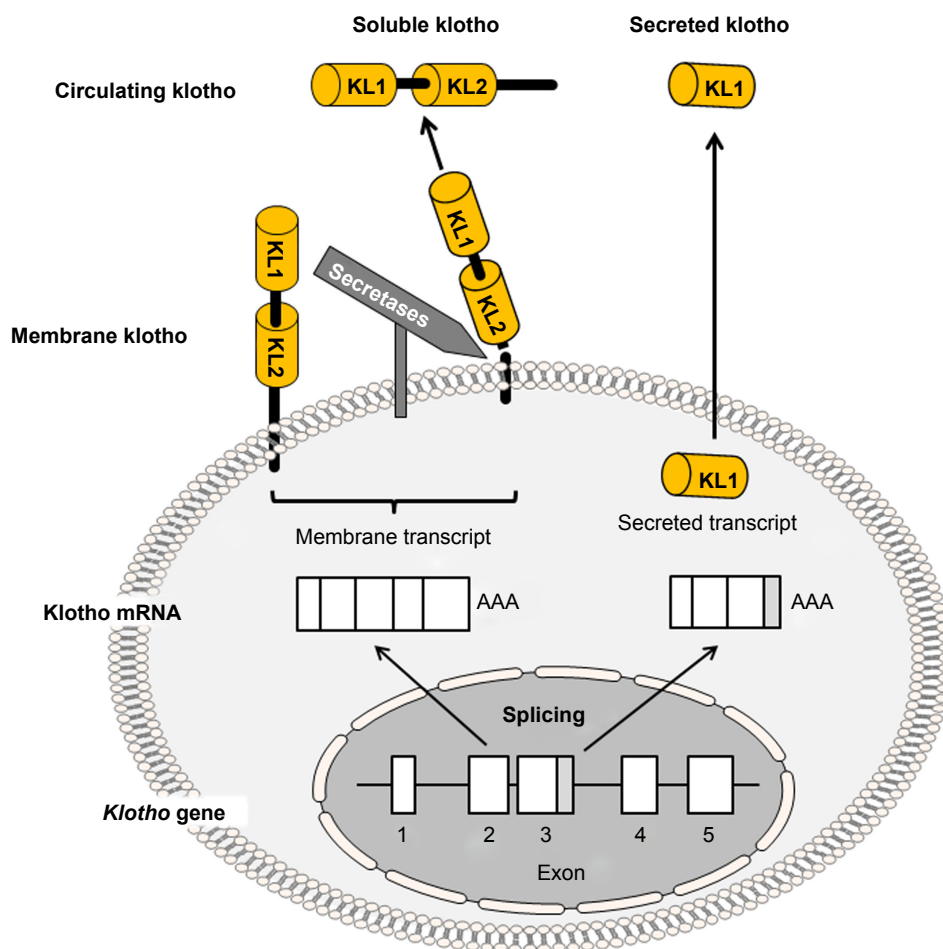
	Human	Rat	Mouse
<b>Klotho gene</b>			
Gene locus (chromosome)	13q12	12q12	13q12
Gene size (kb)	50		50
Exon number	5	5	5
cDNA size (kb)	5.2	5.2	5.2
<b>Klotho protein</b>			
Membrane protein (aa)	1,012	1,014	1,014
Secreted protein (aa)	549	NA	550
Soluble protein (Kd)	130	130	130

**Notes:** Data from Kuro-o et al,<sup>1</sup> Shiraki-lida et al,<sup>4</sup> Ohyama et al,<sup>5</sup> Matsumura et al.<sup>6</sup>  
**Abbreviation:** NA, not available.

a KL1 domain called secreted klotho, which is directly encoded by secreted klotho transcript through alternative splicing (Figure 1). Soluble klotho is the main functional form present in the circulation.<sup>2,11-13</sup> It is also present in the

cerebrospinal fluid<sup>13,14</sup> and urine of mammals.<sup>11,12,15,16</sup> As a circulating substance, soluble klotho exerts biological actions on distant organs and multiple systems.<sup>17-20</sup>

Soluble klotho can function as a  $\beta$ -glucuronidase<sup>12,21-23</sup> or sialidase<sup>24-26</sup> to regulate sodium dependent phosphate cotransporters (NaPi), organic cation transporters, renal outer medullary K<sup>+</sup> channel 1, and calcium-channel transient receptor potential vanilloid 5 in the kidney and maintain mineral homeostasis. Klotho deficiency may also decrease extracellular volume through downregulation of the Na-K-2Cl cotransporter in the loop of Henle, with consequent increases in antidiuretic hormone and aldosterone hormonal responses,<sup>27</sup> both linked to the premature aging phenotype mediated by dehydration observed in klotho-deficient mice.<sup>28,29</sup> Outside the kidney, klotho can also function as  $\beta$ -glucuronidase to enhance creatine transporter-protein



**Figure 1** Schematic diagram of membrane klotho and secreted klotho.

**Notes:** Membrane form of klotho transcript arises from the *klotho* gene. Secreted klotho arises from an alternative RNA splicing. The internal splice donor site is in exon 3 of the *klotho* gene. The resultant alternatively spliced transcript contains insertion of 50 bp after exon 3 (gray), with an in-frame translation stop codon at the end. The product is released into the blood circulation. On the other hand, membrane klotho protein encoded by the membrane form of klotho transcript is a single-pass transmembrane protein. The extracellular domain of membrane klotho containing KL1 and KL2 repeats is shed and cleaved by  $\alpha$ / $\beta$ -secretases, and released into the circulating stream. Therefore, there are two forms of klotho protein in the circulation. One is derived from cleavage of the extracellular domain of membrane klotho called soluble klotho, a well-known active form. The other is the secreted membrane protein, which is directly derived from an alternatively spliced klotho transcript, but its function is largely unknown.

**Abbreviation:** mRNA, messenger RNA.

activity and maintain neuronal function and survival,<sup>30</sup> and to increase several K<sup>+</sup> channel expression and activities: 1) KCNQ1/KCNE1, required for proper hearing and cardiac repolarization;<sup>31</sup> 2) voltage-gated K<sup>+</sup> channel (Kv1.3),<sup>32</sup> expressed in many tissues to regulate a wide variety of cellular functions, including excitability, cell proliferation, apoptosis, immune response, insulin sensitivity, and platelet function; 3) the cardiac K<sup>+</sup> channel,<sup>33</sup> a key channel for cardiac repolarization and deranged excitation following cardiac hypertrophy.<sup>34</sup> Furthermore, soluble klotho can also modulate the IGF-1<sup>2</sup> and Wnt<sup>18,35</sup> signaling pathways, playing a key role in antiaging, anti-tumor growth, and antifibrosis. Importantly, soluble klotho can suppress apoptosis and protect cells against a variety of insults, including hypoxia, hyperoxia, oxidative stress, and cytotoxic drugs.<sup>36,37</sup>

Aging is an inevitable and progressive biological process resulting in dysfunction and destruction of almost all tissues and organs. This is driven by a tightly regulated and complex interplay between genetic and acquired factors. Aging is typically characterized by an increase in senescence, a quantitative and qualitative decrease in stem cells, and abnormal structure at tissue levels. The final outcome of aging is death. In this context, klotho plays an important role in suppressing aging.

There are significant mineral-metabolism disturbances in klotho deficiency, and mineral imbalance per se can induce premature aging induced by klotho deficiency. Indeed, some of the premature aging features and early death seen in klotho-deficient mice can be rescued by reducing plasma phosphate via genetic deletion of NaPi2a in the kidney<sup>38</sup> or a low-phosphate diet.<sup>39</sup> Reduction of 1,25-(OH)<sub>2</sub>-vitamin D and its signaling pathway also rescued phenotypes in klotho-deficient mice.<sup>40,41</sup> However, there may be additional mineral-independent effects of klotho that deserve some attention. There are sparse but definite data about the potential effects of the klotho protein on stem cells, a key player in aging and tissue-regeneration processes. This manuscript summarizes current data on how klotho deficiency induces stem cell senescence and depletion, and provides novel insights into the cellular and molecular mechanisms of how the klotho protein affects stem cells and aging. We propose the klotho protein as a potential candidate therapeutic agent to halt aging and aging-associated diseases.

## Klotho deficiency induces premature aging

Klotho deficiency in hypomorphic klotho mice or silencing of the klotho gene led to similar phenotypes of premature aging and short life span.<sup>1</sup> In addition, global or renal-specific

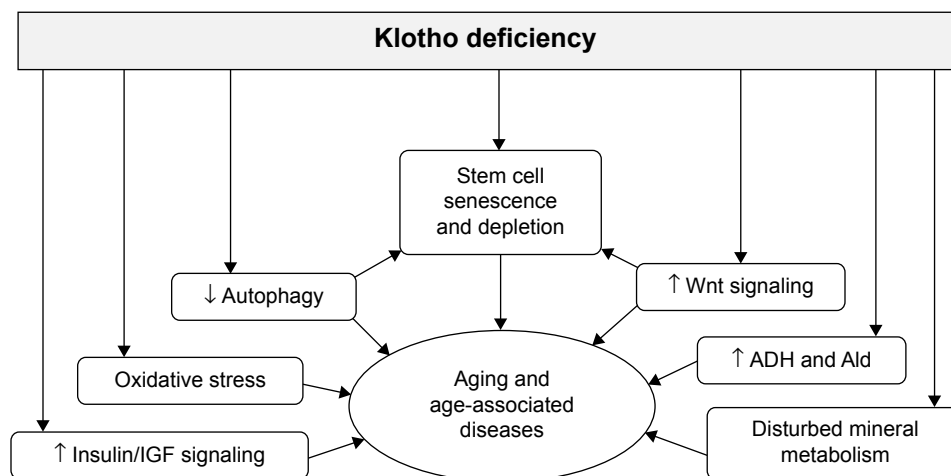
conditional knockout of klotho also led to a similar phenotype.<sup>1,42</sup> These experiments confirmed that destruction of the klotho gene or loss of klotho function leads to an accelerated aging and eventually results in death at 2–3 months, a survival of only a tenth of normal mouse life span, regardless of how klotho deficiency was induced.

In addition to shortened life span, klotho-deficient mice demonstrated growth retardation; decreased physical activity; premature thymic involution; ectopic calcification; arteriosclerosis; pulmonary emphysema; osteoporosis; atrophy of skin, intestine, spleen, and gonads; and lipodystrophy.<sup>1</sup> Early sudden death may result from cardiac arrhythmia or failure to respond properly to stress, possibly because of sinoatrial node dysfunction.<sup>43</sup> Disturbed mineral metabolism is a prominent abnormal feature, including hypercalcemia, hyperphosphatemia, and hypervitaminosis D.<sup>1,12</sup> The correction of phosphate and vitamin D levels rescues most of the premature aging phenotypes observed in klotho-deficient mice,<sup>40,41</sup> indicating that klotho may suppress aging in mammals in large part through maintenance of mineral homeostasis or that mineral dysregulation (phosphate toxicity) may also play a causal role in the premature aging process (Figure 2). Interestingly, chronic kidney disease (CKD) in both human and animals has low levels of renal and circulating klotho and shares many clinical manifestations with klotho-deficient mice,<sup>44–46</sup> suggesting that CKD may be an accelerated aging syndrome.<sup>47</sup>

## Klotho deficiency and cellular senescence

Cell senescence refers to physiological, structural, biochemical, and molecular changes that occur progressively during aging, culminating in the permanent cessation of cell division. Senescence is characterized by altered cellular morphology, increased activity of senescence-associated β-galactosidase, increased formation of senescence-associated heterochromatin foci and p16, permanent DNA damage, and chromosomal instability.<sup>48–52</sup> As a state of permanent inhibition of cell proliferation, cellular senescence initiates and promotes chronic inflammation in multiple age-related chronic diseases, such as obesity, diabetes, atherosclerosis, Alzheimer's disease, cancer, kidney disease, and degenerative disease.<sup>53–57</sup>

It is recognized that oxidative and genotoxic stress and mitochondrial dysfunction activate the senescence response by stimulating two pathways: p53/p21 and p16 pathways.<sup>51,52</sup> The p53 protein is a tumor suppressor and can be activated by the ataxia telangiectasis-mutated kinase, and in turn activates p21 that effectively arrests cell proliferation and induces irreversible cell-cycle arrest in the G<sub>1</sub>- to S-phase transition. On the other hand, p16 is a tumor suppressor and



**Figure 2** Potential effects of klotho deficiency on stem cell depletion and the aging process.

**Notes:** Klotho deficiency stimulates the Wnt signaling pathway to directly accelerate aging or indirectly induce stem cell depletion. Klotho deficiency downregulates autophagy, and low autophagy consequently accelerates aging and induces stem cell senescence and depletion. Klotho deficiency also activates the Wnt signaling pathway, and the resulting excessive Wnt signal activity also induces stem cell senescence and depletion. Both low autophagy and high Wnt signaling activity may directly accelerate aging. Klotho deficiency in turn depresses insulin/IGF signaling activity, disrupts mineral-metabolism homeostasis, and amplifies oxidative stress. Klotho deficiency is also associated with higher levels of blood antidiuretic hormone (ADH) and aldosterone (Ald). All of these effects accelerate the aging process and trigger or exacerbate age-associated diseases. Conceivably, elevation of klotho can interrupt klotho deficiency-induced pathologic processes, slow down aging processes, and attenuate age-associated diseases.

another master regulator of cellular senescence<sup>58</sup> that can be activated through p38 MAPK, enhancing senescence at later times.<sup>49,51,59,60</sup> Klotho deficiency upregulates p53/p21 expression and increases the number of senescent cells,<sup>61</sup> which can be attenuated by either knockdown of p53 or p21. Supplementation of klotho reduces H<sub>2</sub>O<sub>2</sub>-induced cell senescence and apoptosis through suppression of the p53/p21 signaling pathway.<sup>62</sup> Klotho deficiency-induced cell senescence exacerbates endothelial damage and kidney-cell apoptosis triggered by oxidative stress.<sup>62,63</sup>

## Klotho deficiency and autophagy

Autophagy is a “self-eating” process to maintain homeostasis. This process involves the sequestration of cytoplasmic components in double-membrane autophagosomes. Autophagy dysfunction is implicated in a variety of physiological and pathological processes, such as infection, cancer, metabolic and neurodegenerative disorders, and cardiovascular and pulmonary diseases, as well as physical exercise and aging.<sup>64–66</sup> Whether klotho deficiency affects autophagy activity is a very important subject to be addressed.

In klotho-deficient mice, there is severe atrophy of skeletal muscle and activation of the autophagic-lysosomal pathway. Moreover, the signaling activity of mTOR, a suppressor of autophagy, is suppressed, presumably due to deficiency of essential amino acids in the klotho-deficient mice.<sup>67</sup> Consistent with these findings in muscle, augmentation of autophagic markers, cleavage of light chain 3 (LC3),

and autophagic ultrastructural alterations were found in the brain of klotho-deficient mice, similarly to those found in aged wild-type animals.<sup>68</sup>

In contrast, some experiments have shown stimulatory effects of klotho on autophagy in tumor cells.<sup>69,70</sup> Tumor cells have low abundance of klotho expression and downregulation of autophagy. The restoration of klotho regulates IGF-1 receptor phosphorylation, activates downstream Akt-p70S6K and ERK signaling and autophagy, and subsequently suppresses tumor-cell proliferation and induces apoptosis.<sup>69</sup> We recently found that transgenic mice overexpressing klotho had more cleaved LC3 and low levels of p62 in the kidney (unpublished data). Furthermore, an *in vitro* experiment showed that klotho induced puncta patterns of LC3 in cultured kidney cells, suggesting that klotho could activate autophagy flux *in vivo* and *in vitro*. It has been shown that defective autophagy predisposes the kidney to readily develop AKI after an ischemic insult and to more severe kidney impairment in senior versus general populations. Therefore, klotho-upregulated autophagy flux may be attributable to klotho-mediated renoprotection.

## Aging of stem cells

Stem cells exist in most mammalian organs or tissues to maintain tissue homeostasis and participate in tissue repair or regeneration.<sup>71,72</sup> Stem cells exhibit functional age-related changes, which manifest the declined homeostatic and regenerative activities of aging tissues.<sup>73–75</sup> While some of the

functional changes of stem cells arise intrinsically, others are imposed by age-related changes in the microenvironment or niche. The intrinsic changes have been observed at genomic, epigenomic, and proteomic levels. Some of the changes are potentially reversible, which may result in the rejuvenation of aged stem cells.

With aging, stem cells exhibit a diminished capacity of self-renewal and proliferation, which results in increased apoptosis or senescence in the stem cell compartment and depletion of functional stem cells.<sup>76</sup> Furthermore, stem cells show changes in lineage commitment. The cell fate is determined largely by the epigenome and mediated by various signaling pathways.<sup>77–83</sup> Aging can alter the epigenome and pathways, which may lead to aberrant lineage specification of stem cell progeny, as demonstrated in such tissues as the skeletal muscle, tendon, and hematopoietic system.<sup>84–87</sup> Accumulation of these abnormal progeny contributes to the gradual deterioration of tissue structure and function associated with aging. On the other hand, accumulation of DNA mutations is a common feature of stem cell aging. Elevated levels of DNA damage have been reported in aging epidermal stem cells and hematopoietic stem cells.<sup>88,89</sup>

With aging, the number of stem cells declines significantly in some cases.<sup>90,91</sup> For example, depletion of melanocyte stem cells in the hair follicles and the appearance of mature pigmented melanocytes in the stem cell niche have been reported in both aged mice and humans,<sup>92,93</sup> leading to one of the most visible phenotypic changes during aging – hair graying. Aging or genotoxic stress induces the accumulation of DNA damage in melanocyte stem cells that results in the loss of stem cell self-renewal.<sup>92</sup> Depletion of neural stem cells, possibly also related to a specific loss of capacity for self-renewal, appears to be responsible for declining neurogenesis with age.<sup>94–96</sup> However, in other cases, stem cells do not show a significant depletion with age.<sup>97,98</sup>

As a self-renewing cell population to assure proper function and normal tissue homeostasis across the life span, stem cells are more resistant to the same factors that lead to age-related changes in their replicative or postmitotic progeny.<sup>73,99,100</sup> During DNA replication, aging-related mechanisms, such as telomere shortening, chromosome rearrangements, and single-base mutations,<sup>101</sup> can occur and ultimately lead to cellular senescence.<sup>102</sup> Stem cells possess defense and repair mechanisms that are relevant to both highly proliferative cells and to long-lived postmitotic cells.<sup>100</sup> It has been observed that adult stem cells, particularly those in continuously renewing tissues, undergo many rounds of cell division to maintain normal tissue homeostasis.<sup>77</sup> It has also

been observed that telomeres in old stem cells are still longer than those in the other somatic cells in these tissues,<sup>103,104</sup> as observed in the skin, small intestine, cornea, testis, and brain.<sup>104</sup> These observations suggest that stem cells divide at a much slower rate than their proliferative progeny or that they have evolved mechanisms to protect against telomere shortening.

A distinction among intrinsic irreversible changes (eg, genomic mutations), intrinsic reversible changes (eg, epigenomic alterations), and extrinsic influences from the microenvironment or niche in stem cells is important in studying stem cell aging. A mechanistic understanding of stem cell aging will contribute greatly to stem cell therapeutics for diseases and disorders of aging.

## **Klotho deficiency induces stem cell senescence and depletion**

As discussed earlier, adult tissue stem cells have the ability to adjust to environmental changes, affect the proliferation of neighboring cells, and consequently participate in tissue maintenance and regeneration. Aging impedes stem cell renewal and proliferation. Any factor that interferes with the capacity of stem cells to self-renew, proliferate, differentiate, and replace in adult tissues could accelerate aging (Figure 2).<sup>16,105–111</sup> Therefore, dysfunction and depletion of stem cells and progenitor cells contribute to aging. Emerging data showed that some aging-related characteristics in klotho-deficient mice may result from senescence and/or depletion of stem cells or differentiation of stem cells to promote fibrosis.<sup>18,85</sup>

In the premature aging observed in klotho-deficient mice, skin atrophy is one of the gross phenotypes.<sup>1</sup> The klotho-deficient mice had sparser hair than control mice. Histological examinations of the skin revealed a reduction in the number of hair follicles, and reduced dermal and epidermal thickness. The subcutaneous fat was barely detectable. Furthermore, there were a decreased number of stem cells, increased progenitor-cell senescence, and dramatic augmentation of Wnt protein and signaling activity in the skin,<sup>18</sup> indicating that klotho is required for maintenance of both stem cell number and function. A coimmunoprecipitation study indicated that soluble klotho binds to various Wnt family members, including Wnt1, Wnt3, Wnt4, and Wnt5a, suppresses Wnt transcription, and inhibits Wnt biological activity in the skin. An overexpression of klotho effectively antagonizes the activity of endogenous and exogenous Wnt, which induces accelerated cell senescence both *in vitro* and *in vivo*.<sup>18</sup> Therefore, overexpression of Wnt proteins may be one of



the pathogenic factors to be implicated in aging, and klotho is a secreted Wnt antagonist. In the same context of Wnt effect on skin atrophy, enhanced Wnt activity is present in degenerative skeletal muscle of aged mice, which may have affected regeneration of skeletal muscle, impaired repair, and consequently increased tissue fibrosis. All of those alterations are associated with differentiation of muscular stem cells (satellite cells) from a myogenic to a fibrogenic lineage, whose conversion results from the activation of the canonical Wnt signaling pathway in aged myogenic progenitors and can be suppressed by Wnt inhibitors, including sFRP3 and DKK1. Therefore, the Wnt signaling pathway promotes muscular stem cell aging and increased tissue fibrosis.<sup>85</sup> As a Wnt signaling antagonist, klotho conceivably rescues myogenic stem cells, improves muscle repair, and suppresses fibrosis. Therefore, klotho may be a promising therapeutic target for muscle regeneration and muscular dystrophies.

In contrast, a mouse model of X-linked hypophosphatemia with deficient Wnt coreceptor low-density lipoprotein receptor-related protein 6 and consequent reduced Wnt signaling did not alter FGF23-induced phosphaturia or reduced mineralization of the bone, suggesting a potential Wnt-independent pathway of phosphate homeostasis.<sup>112</sup>

## Klotho replacement as a possible antiaging strategy

Given that changes of functionality and a decreased number of stem cells contribute to or accelerate aging, implantation of stem cells to replenish new functional stem cells would be one means to attenuate age-associated disease by rebuilding the tissue or organ. This has been shown to be effective in preclinical and clinical trials in some diseases, including multiple sclerosis,<sup>113</sup> myocardial infarction,<sup>114</sup> ischemic stroke,<sup>115</sup> and cancer,<sup>116</sup> and even for patients undergoing plastic surgery.<sup>108</sup> However, long-term side effects of stem cell implantation are not fully recognized, and should be a concern in most cases in which stem cells are permanently injected into patients.<sup>108,115</sup> For example, recipients of genetically altered bone marrow transplants developed leukemia years after their allegedly successful transplants had cured their severe combined immunodeficiency. Despite potential side effects, recent advances in stem cell research and technology have shown promise.

On the other hand, activation or stimulation of endogenous or resident stem cells is another strategy to abate aging and age-associated disease. Current data from animal and *in vitro* cell-culture studies clearly demonstrated that klotho deficiency is associated with stem cell senescence and

depletion. Furthermore, klotho deficiency may not only be a trigger for aging but also a pathogenic intermediate for accelerated aging and development of age-associated diseases, including Alzheimer's disease, hypertension, osteoporosis, cardiovascular disease, and CKD. Conceivably, any therapy that restores or stimulates endogenous klotho or administration of exogenous klotho might provide a novel treatment strategy for aging and age-associated diseases.

## Administration of exogenous klotho as a therapeutic agent

To date, klotho gene delivery is shown to effectively rescue many phenotypes observed in klotho-deficient mice, prolonging life span,<sup>117</sup> attenuating the progression of hypertension and kidney damage in spontaneous hypertensive rats,<sup>118,119</sup> ameliorating angiotensin II-induced kidney injury,<sup>120</sup> improving endothelial function,<sup>121</sup> and protecting from uremic cardiomyopathy.<sup>122</sup> Although gene therapy is effective in animal studies, its safety is still questionable, and clinical application is not in proximity. There are few clinical trials testing gene therapy in specific diseases, usually genetic diseases, such as X-linked severe combined immunodeficiency,<sup>123</sup> cancer treatment, or delivery of vaccines.<sup>124</sup>

Compared to viral delivery of the klotho gene in animals, administration of exogenous klotho protein is a safer, easier, and more direct modality to restore endocrine klotho deficiency. Similarly to the use of erythropoietin or erythropoiesis-stimulating agents to correct anemia in CKD patients and insulin to maintain normal glucose metabolism in type I diabetes, the administration of exogenous klotho protein may be a viable and effective option in the near future to dwindle aging. Klotho protein can potentially reverse or retard stem cell depletion and abate age-associated pathological processes.

To date, no studies of klotho protein administration in humans have been reported. In contrast, animal studies have already provided convincing and encouraging data to support the proof of concept that soluble klotho protein administration is safe and effective.<sup>11</sup> We showed that soluble klotho protein attenuates kidney damage and preserves kidney function in an ischemia–reperfusion injury model causing acute kidney injury, which is a state of acute klotho deficiency.<sup>11</sup> Furthermore, klotho protein inhibited renal fibrosis in a unilateral ureteral obstruction kidney-injury model, which is also a state of low klotho expression in the kidney.<sup>125</sup> Interestingly, intraperitoneal injection every other day of soluble klotho protein effectively extended the life span of homozygous klotho-deficient mice, ameliorated premature aging-related

phenotypes, such as growth retardation, premature thymus involution, and vascular calcification, and effectively reduced cellular senescence.<sup>126</sup> Therefore, the preclinical data clearly support the therapeutic potential of soluble klotho protein for age-related disorders and klotho deficiency-associated diseases.

## Activation of endogenous klotho expression

In the context of acute kidney injury, the renal tubules, where endogenous klotho is produced, are not fully destroyed, but are suppressed in their ability to produce klotho protein. In this context, strategies to increase endogenous production will be of therapeutic benefit. In particular, while klotho gene delivery is not yet implemented and klotho protein not yet available for clinical use, upregulation of endogenous klotho expression is of high clinical relevance.

To date, several categories of drugs in the market, including a PPAR $\gamma$  agonist,<sup>127–130</sup> angiotensin II type I receptor antagonist (losartan),<sup>131–133</sup> HMG-CoA reductase inhibitors (statin),<sup>134</sup> and vitamin D active derivatives,<sup>135–138</sup> have been shown to be effective in upregulating klotho expression in vivo and in vitro. The effect of upregulating klotho is definitely not associated with those drugs well-identified original pharmacologic targets.

Antioxidants and free radical scavengers may not directly play a role in the modulation of klotho expression. However, they can ameliorate oxidative stress, which is linked to aging and suppression of klotho expression in the kidney and cultured kidney cells.<sup>139,140</sup> Klotho deficiency in turn increases oxidative stress and makes cells more susceptible to oxidative stress. Therefore, antioxidants are potentially useful in interrupting this spiral deterioration by upregulating klotho production and thus exerting antioxidant properties.<sup>141,142</sup>

## Conclusion and future directions

Since klotho was serendipitously identified in 1997, our understanding of it as an aging suppressor has been continuously growing. Klotho protein has pleiotropic actions on many organs and tissues in mammals. However, very limited and premature data about klotho effects on stem cells are available. A better understanding of the effects of klotho on stem cells not only provides novel insights into the role of stem cells in antiaging processes but could also make a significant contribution to the advancement of regenerative medicine clinical practice.

Animal models have clearly demonstrated that klotho deficiency induces shortened life span and accelerated aging.<sup>1</sup>

Epidemiological data have also shown that soluble klotho is lower in elder than young adults, and that levels of soluble klotho are inversely correlated with age,<sup>143–145</sup> indicating that aging is associated with klotho decline. Nonetheless, we do not know whether low soluble klotho is a prognostic biomarker for risk of earlier death in humans. Although we still do not fully understand why klotho is reduced with aging, maintenance of klotho levels by stimulation of endogenous klotho production or administration of exogenous klotho protein could be considered a potential therapeutic target to retard aging and attenuate age-associated diseases.

Thus far, animal experiments and in vitro cell-culture studies have shown the effects of soluble klotho protein on abating skin atrophy and skeletal muscle dystrophy during aging. It is anticipated that soluble klotho may play a pivotal role in regenerative medicine by preservation and activation of stem cells, particularly in heart tissue, where stem cells are very scarce or have low ability to replicate after injury. Therefore, if soluble klotho can activate stem cells or induce the replication of stem cells, klotho protein could be used as a promising therapeutic strategy for tissue repair and organ regeneration.

## Acknowledgments

The authors acknowledge Drs Orson W Moe and Makoto Kuro-o for long-term collaborative work and support. MCH is in part supported by the NIH (R01-DK091392, R01-DK092461) and the Charles and Jane Pak Research Foundation, AB is in part supported by a Visiting Scholar Award from the National Natural Science Foundation of China (81170660H0509, 81270408H0220) and the Provincial Natural Science Foundation of Jiangsu, People's Republic of China (BK2011849), and JAN is in part supported by the Ben J Lipps Research Fellowship Program of the American Society of Nephrology Foundation for Kidney Research and the Truelson Fellowship Fund at the Charles and Jane Pak Center of Mineral Metabolism and Clinical Research.

## Disclosure

The authors report no conflicts of interest in this work.

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