α-klotho and anemia in patients with chronic kidney disease patients: A new perspective (Review)

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Abstract. Normocytic normochromic anemia is a common complication of chronic kidney disease (CKD) and is associated with numerous adverse consequences. Certain symptoms previously attributed to CKD are now known to be a consequence of anemia. Anemia contributes to an increased cardiac output, and the development of left ventricular hypertrophy, angina and congestive heart failure, leading to high morbidity and mortality in patients with CKD. The multifunctional α-klotho (KL) protein, which is predominantly expressed in the kidneys, is associated with the occurrence of anemia in patients with CKD. The present review presents current evidence on the potential role of α-KL in renal anemia. Low expression of α-KL appears to improve anemia in patients with CKD, and has been hypothesized to be a compensatory mechanism to attenuate the effects of anemia in patients with CKD. Further understanding of the role of α-KL in renal anemia may offer novel insights into the treatment of patients with CKD complicated with anemia.

Contents

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1. Introduction

Normocytic normochromic anemia is one of the hallmarks of progressive chronic kidney disease (CKD). Normocytic normochromic anemia is defined by a decrease in hemoglobin (Hb) to <130 g/l in men and <120 g/l in women (1). Relatively little is known about the development and progression of anemia in patients with CKD. As kidney function declines in patients with advanced CKD, the incidence of anemia increases (2). The occurrence of anemia in patients with CKD (renal anemia) is primarily due to an absolute or relative decrease in erythropoietin (EPO) production by the failing kidney (1). However, other factors, including iron and vitamin deficiency, in addition to inflammation, contribute to the development of anemia and reduced response to treatment in patients with CKD (2).

Erythropoiesis-stimulating agents (ESAs) and adjuvant iron therapy are the primary methods for treating anemia associated with CKD. ESAs potent and can increase HGB levels significantly. However, recombinant ESAs are expensive, require cold storage and are administered by the parenteral route. In addition, with frequent subcutaneous administrations, ESA therapy is cumbersome for the long-term treatment of dialysis-independent patients with CKD. Furthermore, in patients with CKD undergoing hemodialysis, intravenous administration of ESAs increases the hospital's workload. Recent clinical trials have demonstrated that higher Hb targets (≥11.3 g/dl) and/or the application of high doses of ESAs may increase cardiovascular risk (3-5). Therefore, the identification of novel drugs to treat CKD-associated anemia is an important issue.

Klotho (*KL*) was originally identified as an anti-aging gene, which when overexpressed extended the lifespan of mice (6). The *KL* gene encodes a single-pass transmembrane protein, and is expressed in the kidney and parathyroid gland (7). Furthermore, the KL protein functions as an obligate subunit of the receptor for fibroblast growth factor 23 (FGF23) (8). FGF23 is a hormone secreted by osteocytes and osteoblasts, which acts on renal tubular cells to promote phosphate excretion into the urine and suppress the synthesis of the active form of vitamin D [1,25-dihydroxyvitamin D3; 1,25(OH)2D3] (9). Growing evidence suggests that α-KL is a significant marker for CKD (10,11) and a pathogenic factor in the progression of CKD progression (12,13). A recent study has reported that

decreasing α-KL levels are associated with the occurrence of anemia in patients with CKD (14). The present study reviews the recent advances in understanding of the role served by α-KL in the development of anemia in patients with CKD, and summarizes novel approaches for the clinical treatment of anemia in CKD.

2. Generation and function of KL

The α-KL protein is encoded by the *KL* gene, which was identified in 1997 (7). To date, three types of α-KL protein have been identified: Full-length transmembrane, soluble and secreted α-KL (15). The full-length α-KL protein is a transmembrane protein that contains two separate glycosyl hydrolase domains, KL1 and KL2. The truncated α-KL protein, also known as soluble α-KL, is released from the cell membrane and may contain KL1 or KL1 and KL2 (16). Soluble α-KL is generated from the cleavage of the membrane form of KL by α- and β-secretases (α-cut, KL1 and KL2 domains, ~130 kDa; β-cut, KL1 domain only, ~65 kDa) and by insulin stimulation (16). The cleaving process is inhibited by a phosphoinositide 3-kinase (PI3K) inhibitor, which suggests that PI3K serves a role in the cleavage process (17). Secreted α-KL (~65 kDa), generated by alternative RNA splicing, is the primary form of circulating KL and contains only the KL1 domain, in which the acid/base site is mutated and the nucleophile site is conserved (16). Total circulating α-KL may include soluble and secreted α-KL. Circulating α-KL is able to function as a hormone to regulate the functions of cells or tissues that do not express α-KL (16). Secreted α-KL and the shorter form of soluble α-KL contain the KL1 domain and have approximately the same molecular weight (~65 kDa) (16).

α-KL is a multifunctional protein that regulates essential cellular processes and is predominantly expressed in the kidneys (18). In addition to its anti-aging function, α-KL serves an essential role in the phosphatonin FGF23 signaling pathway and secreted KL functions as an endocrine hormone (19). Previous studies have identified that α-KL is associated with a range of diseases, including periodontitis (20), cardiovascular disease (21) and kidney disease (18). However, the role of α-KL in hemopathies, including anemia, remains unclear.

3. Association between α-KL and anemia in CKD

Erythropoietin (EPO) and α-KL. The release of EPO by the kidneys, a hormone that is important in the regulation of erythropoiesis, is triggered by tissue hypoxia (22). Under hypoxic conditions, EPO stimulates the differentiation of erythroid progenitor cells and normoblasts to increase the amount of red blood cell (RBCs) (22). Hypoxia-inducible factors (HIFs) are heterodimeric proteins that regulate the physiological response to hypoxia by altering the expression of downstream genes, including EPO, in interstitial cells in the renal cortex near the proximal tubule (23).

There may be a negative feedback between EPO and α-KL such that EPO promotes the expression of α-KL and increasing α-KL suppresses the production of EPO in CKD (22,24). KL deficiency is a characteristic feature of CKD with anemia (22). Hu *et al* (10) revealed that KL expression was reduced during the progression of kidney disease. In addition, the treatment

of anemia with EPO has been demonstrated to enhance renal and extrarenal production of α-KL in patients with CKD (14,24). Furthermore, EPO has been demonstrated to inhibit uremia-induced nuclear factor-κB (NF-κB) production in endothelial cells and prevented a decrease in intracellular KL (24). However, a previous study reported that KL directly regulates hematopoietic stem cell differentiation, and erythroid cell generation and maturation (22). KL deficiency in mice resulted in increased erythropoiesis through activation of the HIF signaling pathway, and subsequent upregulation of renal EPO synthesis and secretion (22). Additionally, the expression of HIF-1α and HIF-2α was significantly upregulated in KL$^{-/-}$ bone tissue, resulting in localized overexpression of EPO (22). The molecular mechanism responsible for the KL-induced inhibition of the HIF signaling pathway and EPO expression may be associated with reduced osteoblast numbers and osteopenia (22). The effects of α-KL on EPO and the HIF signaling pathway are summarized in Fig. 1.

Kempe *et al* (25) demonstrated that a lack of KL expression leads to increased cytosolic Ca^{2+} activity, leading to enhanced scrambling of cell membrane phospholipids and cell shrinkage in erythrocytes. This suggests that KL deficiency accelerates eryptosis, the suicidal death of erythrocytes. Thus, the role of KL in hemopoiesis remains unclear and further studies into this area are warranted.

Iron metabolism and α-KL. Iron-deficiency anemia (IDA) is responsible for ~50% of all anemia cases (26). It has been reported that 273,000 individuals succumb to IDA each year worldwide (26). 'Absolute' iron deficiency is common in patients with CKD and anemia (27). Different conditions, including inflammation, liver disease and pregnancy, may induce iron-restricted erythropoiesis and aggravate renal anemia (1). The distinction between these conditions is clinically relevant; however, peripheral iron indices are of little help in the differential diagnosis (28).

Iron deficiency may lead to high expression of KL in patients with CKD. A study of 70 patients with stages I-V CKD demonstrated that the FGF23 levels were elevated and KL levels were decreased, and the magnitude of these changes increased as the tumor stage increased (29). In addition, significant correlations were identified between serum KL levels and ferritin levels, and serum KL levels and transferrin saturation percentage, which suggest that KL serves a negative role in iron regulation (29). However, little is known about the underlying molecular mechanisms of these effects. A previous study demonstrated that serum iron overload decreased renal expression of KL at the mRNA and protein levels, and that iron chelation suppressed angiotensin II-induced downregulation of KL (30). These results indicate that the underlying mechanism of the angiotensin II-induced downregulation of KL is the alteration of iron metabolism in the kidney. Therefore, the renin-angiotensin system may serve a role in the regulation of iron and α-KL.

HIFs affect the majority of aspects of iron metabolism (23). HIF-2 enhances iron absorption by small bowel enterocytes and augments iron export from duodenal cells (23). Additionally, HIFs serve a role in the utilization of iron from senescent RBCs via reticuloendothelial system macrophages (31). In this manner HIFs increases iron stores through absorption
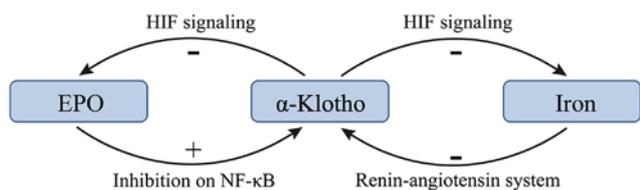
Figure 1. Low expression of α-Klotho in patients with chronic kidney disease complicated by anemia could increase serum EPO and iron levels. HIF, hypoxia-inducible factor; EPO, erythropoietin; NF-κB, nuclear factor-κB.

Figure 2. Low expression of α-Klotho in patients with chronic kidney disease complicated by anemia could increase serum vitamin D levels and inhibit inflammation.

from the duodenum and the recycling of senescent RBCs (31). The increased efflux of iron to the circulation is achieved via HIF-mediated increased expression of ferroportin in duodenal enterocytes and macrophages (31). KL deficiency in mice results in the activation of the HIF signaling pathway (22). The effects the HIF signaling pathway on iron supplies are summarized in Fig. 1.

Vitamin D and α-KL. Accumulating evidence suggests that vitamin D deficiency increased the progression of CKD (32-34). Vitamin D is important in anemia, with low vitamin D levels being associated with an increased risk of anemia (35,36). Thus, vitamin D deficiency may be a factor in the development of anemia in patients with CKD.

Vitamin D and KL exert negative feedback towards one another in patients with renal anemia. A previous study suggested that the vitamin D/parathyroid hormone (PTH) signaling pathway regulates the KL/FGF23 signaling pathway, and vice versa (37). 1,25-dihydroxyvitamin D [1,25(OH)D2], the metabolically active form of vitamin D, was demonstrated to upregulate KL expression (37). PTH may indirectly upregulate KL via upregulating 1,25(OH)D2 (38). In addition, several recent studies have demonstrated that vitamin D stimulates the expression of FGF23 and KL, and that vitamin D formation is limited by a negative feedback regulation (39-41). This negative feedback is lost in *kl⁻/kl⁻* mice and in these mice the formation of 1,25(OH)2D3 is a function of dietary vitamin D even at excessive 1,25(OH)2D3 concentrations (42). The mechanism by which KL inhibits the production of 1,25(OH)2D3 may be via inhibiting 25-hydroxyvitamin D 1α-hydroxylase (39). A summary of the current knowledge of the association between vitamin D and α-KL is presented in Fig. 2.

Inflammation and α-KL. Inflammation is an essential component to the body's defense system; however, excessive inflammation is considered to be the cause of anemia in patients with CKD (43). Inflammatory cells release large amounts of chemokines and vasoactive factors, including monocyte chemotactic protein-1 (MCP-1) and NF-κB, which induce the production of profibrotic cytokines following kidney injury (44). At the site of injury, proinflammatory factors, including interleukin (IL)-12, MCP-1 and NF-κB, stimulate the generation of myofibroblasts and the deposition of extracellular matrix, eventually leading to renal dysfunction and potentially to the indirect development of anemia (45). Notably, anemia is a common complication in patients with infections, autoimmune disorders,
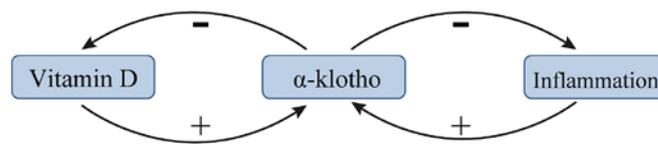
malignancies, CKD and other inflammation-associated disorders (46). Inflammatory cytokines impair erythropoiesis by inhibiting the production and function of EPO, and inhibiting erythroid progenitor cell proliferation and differentiation. Notably, inflammation induces the expression of iron regulatory hormone hepcidin and suppresses the iron exporter ferroportin, restricting the supply of iron for erythropoiesis (46).

KL is known to be an inhibitor of several inflammatory cytokines (47). The transcription factor NF-κB is an important stimulator of inflammation in CKD (48). A recent study reported that intracellular KL diminished the DNA binding ability of NF-κB and stabilized the NF-κB/NF-κB inhibitor α complex, thus preventing uremia-induced NF-κB expression (24). KL serves as an anti-inflammatory modulator through negatively regulating the production of NF-κB-associated inflammatory proteins (47). A potential mechanism for this effect is that KL inhibits the Ser[536] phosphorylation of the NF-κB p65 subunit and its subsequent recruitment to the promoter sites of multiple cytokines (49). Additionally, KL may attenuate the glucose-stimulated activation of the NF-κB by downregulating the expression of toll-like receptor 4 (50), which is associated with CKD (51). Conversely, NF-κB regulates the activity of exogenous and intracellular KL in endothelial cells (52). NF-κB also suppresses the activity of KL, potentially by promoting the production of reactive oxygen species (52). Finally, NF-κB also inhibits the proinflammatory effect of IL-12 (20). These results demonstrate the anti-inflammatory effects of KL (Fig. 2).

4. Conclusions

Although several studies have reported that there is reduction of α-KL in patients with CKD with anemia, the mechanisms are complicated. Based on previous studies, it appears that low levels of EPO in patients with CKD with anemia downregulates the expression of α-KL, which results in high serum levels of iron and vitamin D, mitigating anemia. This suggests that low expression of α-KL may be a compensatory mechanism to attenuate the effects of anemia. Meanwhile, the expression of α-KL declines when serum levels of iron increase, and the expression of α-KL increases with vitamin D. Overall, further understanding of the role of α-KL in renal anemia is needed, which may offer novel insights into the treatment of patients with CKD complicated with anemia.

Acknowledgements

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