Int J Clin Exp Med 2017;10(3):4342-4349
www.ijcem.com /ISSN:1940-5901/IJCEM0044613

Review Article

Klotho in diabetes and diabetic nephropathy: a brief update review

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Received November 19, 2016; Accepted January 6, 2017; Epub March 15, 2017; Published March 30, 2017

Abstract: The growing data demonstrate that Klotho (KL) is deeply implicated in the diabetic nephropathy. The circulating form of α-Klotho (α-KL) named as soluble KL functions as an endocrine substance that exerts heterogeneous actions including the modulation of renal function upon hyperglycemia, regulation of cell compensation, downgrade inflammation and anti-oxidation. There is a positive correlation between progression of renal disease/other complications and systemic KL deficiency in diabetes mellitus patients. Restoration by exogenous supplementation or stimulation of endogenous KL may prevent and/or ameliorate kidney injury and mitigate development of diabetes mellitus. KL signaling is intertwined with mTOR, NF-κB, Wnt and PPAR-γ. KL can possibly emerge on the horizon as a candidate for an unprecedented sole biomarker and intervention in patients with diabetes mellitus or the complication like diabetic nephropathy.

Keywords: Klotho, diabetes mellitus, diabetic nephropathy, review

Introduction

Klotho (KL) is originally identified as an anti-aging protein, but is subsequently discovered to have a multitude of biological effects [1]. KL is expressed in multiple tissues and organs, but by far, its highest expression is in the distal convoluted tubule (DCT) of the kidney [2]. Recently, the accumulated evidences show that α-Klotho (α-KL) has extreme pleiotropic functions. It can regulate the parathyroid hormone (PTH) release in the parathyroid gland [3], production of 1,25 $(OH)_2$ vitamin D3 [4], anti-oxidation [5], anti- apoptosis [6], anti-senescence [6], promotion of angiogenesis and vascularization [7], inhibition of fibrogenesis [8] and preservation of stem cells [9]. All of the above properties of α-KL can potentially mediate its renoprotective effects demonstrated in animal models. In recent years, the roles of α-KL in diabetes mellitus (DM) and diabetic nephropathy (DN) have attracted more attention [10], but little is known about circulating α-KL levels in DM/DN. Meanwhile, thus far, recent studies in patients with DM report conflicting data. Some studies showed that renal α-KL expression is markedly decreased in DN in humans and mice [11-14]. In contrast, some other researches find that the serum α-KL level is not significantly different between patients with diabetes without nephropathy and non-diabetic controls [15, 16]. Several recent reviews have comprehensively addressed the physiology of α-KL in aging [17], renal calcium, phosphate and potassium transport [18], and its pathophysiologic role in acute kidney injury, development and chronic kidney disease progression and its complications [19]. This study primarily devoted to discussing the potential effects of insulin on α-KL, and the diagnostic, prognostic and therapeutic roles of α-KL in DN.

Distribution, conversion and major functions of KL

α-KL was firstly discovered by Kuro-o *et al.* in 1997 [2]. It was named after KL, one of the Moirae (the fates) in Greek mythology who

spun the thread of life from her distaff onto her spindle [2]. α-KL is predominately expressed in both the apical and basolateral membrane of kidney distal convoluted tubules and brain choroid plexus [20-22]. Mice lacking KL exhibit many changes that occur during aging, including osteoporosis, infertility, and cognitive decline. They also have a short life span [2]. In contrast, mice overexpressing KL live 30% longer than wild-type mice and are more resistant to oxidative stress [22].

Human KL gene is on the chromosome 13q12, which contains 5 exons and 4 introns [23]. Human KL protein CDNA transcribes a single-pass transmembrane protein with 1014 amino acids [24]. Most amino acids in the KL peptide reside in the amino-terminal extracellular domain, which is followed by a 21-amino-acid transmembrane domain, and an 11-amino-acid short intracellular carboxy terminus [22].

α-Kl can be cleaved on the cell surface by membrane-anchored proteases, including by a desintegrin and metalloproteinase (ADAM)-10, and by ADAM-17. Freathy *et al.* [25] demonstrated that TAPI-1 and insulin exhibit the same effects on KL secretion *ex vivo* in rat kidney slices and overexpression of either ADAM10 or ADAM17 leading to an increase in both KL1 (molecular mass of 65-70 kDa) and KL2 (molecular mass 135 of kDa) fragments, whereas silencing of either ADAM10 or ADAM17 with siRNA leading to a decrease of both fragments. So, α-KL protein exists in two forms, membrane KL (TM-KL) is bound to the cell membrane and functions as a co-receptor for fibroblast growth factor 23 (FGF23), which is required for FGF23 regulation of both renal handling of phosphate and renal synthesis of calcitriol induced phosphate excretion in kidney [26], and circulating form of KL, detectable in plasma and urine, which is also named soluble or secreted Klotho (s-KL). S-KL is derived from the proteolytic cleavage of the extracellular portion of the TM-KL and consists of two internal repeats, known as short-form Klotho (KL1) and full- length Klotho (KL2), respectively. KL1 may be produced through alternative mRNA splicing [22, 27]. S-KL level may be mainly determined by two possible mechanisms as follows: i) cleavage of α-KL protein by proteases such as ADAM 10 or 17 [28], and ii) secretion of splice variant form of α-K into blood or urine.

Evidence shown that TM-KL mainly regulates the PTH release in the parathyroid gland [3], the production of 1,25 $(OH)_2$ vitamin D3 by negatively regulating the expression of 1a-hydroxylase [29] and transepithelial calcium transport in the DCTs via activation of the transient receptor potential vanilloid 5 (TRPV5) channel [30]. In contrast, soluble KL (major product KL2) has been shown to function as an endocrine substance and to inhibit four signaling pathways simultaneously, offering a major advantage over numerous individual inhibitors in clinical and preclinical development, including IGF-1 receptor antibodies, tyrosine kinase [31] and Wnt signaling inhibitors [32], TGF-β1 neutralizing antibodies, soluble TGF-βR2, TGF-β receptor kinase inhibitors [33, 34] and ROCK signaling inhibitors.

Interactions of insulin and KL

It has been tested that α-KL is mainly cleaved on the cell surface by membrane-anchored proteases by ADAM-10 and ADAM-17. Some studies have found that insulin also has the similar function. But the precise mechanism of insulin-induced shedding of α-KL is unknown.

One possible mechanism is that insulin can activate the ADAM17 by the down-regulation of Timp-3, an ADAM17 inhibitor. Findings from the insulin receptor heterozygous mice ($Insr^{+/-}$) that develop diabetes with more than five times increased insulin level in the serum. These mice have reduced Timp-3 and increased ADAM17 activity [35, 36].

Another possible mechanism is that insulin can enhance the activity of ADAM10 and ADAM17. Shiraki-Iida *et al.* [27] find that insulin can enhance the activity of sheddase, which suggests the involvement of the insulin signaling pathway in the release of KL from cell membranes. So the authors propose a possible negative feedback loop of insulin regulation by KL, in which insulin initiates a signaling cascade and/or gene expression that results in the trafficking and/or activation of ADAM10 and/or ADAM17. This result, in turn, increases the release of the KL proteins (including KL1 and KL2 fragments) and other ADAM10 and ADAM17 substrates into the medium. KL has been shown to block insulin and insulin-like growth factor 1 receptor phosphorylation of the insulin receptor substrate (IRS) and also subse-

quent downstream activation of PI3K and Akt-1 [37, 38]. The KL fragments can then feedback through an as-yet-unknown process to turn off insulin signaling. TNF-α also has been shown to contribute to the inhibition of the insulin signaling pathway [39]. It has been reported that, in CHO cells, insulin stimulates a 2- to 3-fold increase in the endocytic recycling pathway, implicating that the vesicle- associated proteins have an increased chance to be at the cell surface [40]. In further support for the role of insulin in vesicle trafficking is the recent report that in adipocytes insulin causes the fodrin/spectrin remodeling, leading to the translocation of GLUT4 to the membrane [41].

KL in diabetes and DN

Experimental research: There are numerous experimental studies showing that KL orchestrate various pivotal functions though heterogeneous mechanisms in diabetes. Growing evidences showed that KL exerts antioxidant effects and can provide effective protection against the oxidative stress through KL expression in DN. It has been established that high glucose caused an excessive production of ROS [42]. Improvements in diabetes- induced renal dysfunction and DN by antioxidants are evidence for an important role of ROS in kidney damage [43]. KL plays a major role in the protection of kidney due to anti-oxidation in diabetes rats [44-46]. It has been demonstrated that KL-overexpressed mice showed increased superoxidative dismutase (SOD2) expression in muscles and low levels of phosphorylated Forkhead box O proteins (FOXOs), in addition to the reduced oxidative stress as evidenced by lower levels of urinary 8-OHdG, a marker of oxidative damages to DNA [47]. KL could activate FOXOs, induce SOD2 expression, and confer resistance to oxidative damages and apoptosis induced by paraquat or hydrogen peroxide [48].

The RhoA/Rho-associated coiled-coil kinase (ROCK) signaling pathway has been implicated in DN. Regulation of KL expression can be achieved through inhibition of RhoA/ROCK signaling pathway [49]. Another study [50] also demonstrated that exogenous recombinant adeno-associated virus (rAAV) carrying mouse KL full-length cDNA (rAAV. mKL) transfection inhibited the expression of fibronectin (FN), decreased the protein expression of vimentin (VIM), which may contribute to the inhibition of the mRNA expression and protein activity of ROCK.

There are growing evidences demonstrated that TGFβ1 and mTOR signaling may contribute to the exacerbation of early DN in $KL^{+/-}$ mice. TGFβ1 has been shown to be linked to renal fibrosis in DN in animals and humans [52-54]. Suppression of TGFβ1 inhibited hyperglycemia-induced collagen synthesis and prevented glomerular fibrosis and renal insufficiency in db/db mice [53, 55]. One study [56] reveals that deficiency of renal KL in $KL^{+/-}$ mutant mice increased phosphorylation of Smad2, a key downstream signaling of TGFβ1, in diabetic kidney. This result supports a notion that endogenous KL in kidney may be an important negative regulator of the TGFβ1 signaling in diabetic mice. Several findings have shown that activation of mTOR increases the synthesis of matrix proteins that contributes to basement membrane thickening and glomerular mesangial matrix expansion [57]. A number of studies have shown that activation of mTOR plays a crucial role in renal hypertrophy and podocyte injury, which may contribute to the progressive loss of renal function in DN [56, 58]. $KL^{+/-}$ mutant mice showed exacerbated kidney damage that is likely attributed to KL deficiency-induced enhancement of mTOR signaling.

According to literatures [59], there is a close link between PPAR-γ and KL. PPAR-γ is a key transcription factor controlling adipogenesis and insulin sensitivity. PPAR-γ dimerizes with retinoid X receptor and activates the gene expression by binding to the cognate PPRE within the regulatory region of the target genes. A study showed that troglitazone, an agonist for PPAR-γ, augmented the renal KL mRNA expression in OLETF (Otsuka Long- Evans Tokushima Fatty) rats [60]. It has also been recently described that KL promotes adipocyte differentiation in cultured preadipocytes [61] and that overexpression of KL slows the aging process through induction of insulin resistance. Recently, Zhang *et al.* [59] established a novel transcriptional mechanism that controls the expression of KL by showing that KL is a target gene of PPAR-γ in the cultured kidney cells as well as in mouse kidneys *in vivo*.

NF-κB pathway maybe is one of the mechanisms related to the function of KL in DN [62]. It's reported that both exogenous soluble α-KL

administration and overexpression of membranous α-KL in kidney cell culture suppress NF-kB activation and subsequent inflammatory cytokine production in the response to TNF-α stimulation suggest that α-KL serves as an anti-inflammatory modulator [14].

KL may preserve beta cells against development of diabetes. *Lin et al.* [51] found that β-cell-specific expression of KL attenuated the development of diabetes in db/db mice, decreased intracellular superoxide levels, oxidative damage, apoptosis, and endoplasmic reticulum stress in pancreatic islets. Furthermore, β-cell-specific expression of KL increased expression levels of Pdx-1 (insulin transcription factor), PCNA (a marker of cell proliferation), and LC3 (a marker of autophagy) in pancreatic islets in db/db mice.

Although this review is focusing on KL's role on diabetic nephropathy, it is noted that mTOR, Wnt, NF-κB and PPAR-γ signaling are intertwined each other. Therefore, *in vivo* expression of KL may offer a new and effective therapeutic strategy not only in β-cell dysfunction but also in systemic pathology in DM [63-66].

Clinical research: In recent years, studies on soluble KL levels in diabetic patients are scarce and inconclusive [67-69]. Several researches [70, 71] showed that the KL gene expression and protein levels were decreased in patients with even early DN [12-14, 72-75]. Of interest, studies further demonstrated that restoration of α-KL abundance in the kidney by gene transfer could ameliorate angiotensin II-induced proteinuria [76]. Other studies also found that the replacement or endogenous upregulation of α-KL protects the kidneys from renal insults, preserves kidney function, and suppresses renal fibrosis [77].

In contrast, several papers didn't find the increase of KL in patients with DN [15, 16, 78]. The controversial findings may be multifactorial. Firstly, different testing methods can affect the results. A reliable ELISA-based assay to measure s-KL levels has only recently become available [79], these conflicting data have been obtained using various commercially available assays [80, 81]. Secondly, many factors can affect the soluble KL level. Besides insulin, AMAD10 and AMAD17, some research showed that renal α-KL expression levels were inversely correlated with urinary calcium augmentation [82, 83], the use of ACE-inhibitors and angiotensin II receptor may have negative effect on soluble KL production in type2 diabetes with nephropathy [39, 84]. Therefore, clinically we need to interpret these data with caution in a certain patient.

On the other hand, more solid evidence shown that the increase of soluble KL in DN, but little is known about the regulatory mechanism of KL in DN. A study found that miR-199b-5p targeted KL at two binding sites using the MicroRNA.org data bank and that the activation of miR-199b-5p inhibited the 3'UTR activity of KL and down-regulated its expression level in HK-2 cells. Some other authors also observed the similar research findings [85-87]. Therefore, at present, it can be hypothesized that the miR-199 family may be at least one of the regulatory mechanisms of KL in DN. This warranted the further studies.

Conclusions

In conclusion, KL may be an early biomarker and a potential therapeutic target in patients with DN. Numerous efforts have been made to identify the mechanisms of DN, and indeed, significant progress has been made. Growing studies strongly pose the potential utility of endogenous KL restoration or exogenous KL replacement as therapeutic options in DN. Recombinant KL administration is efficacious in animal studies, but prior to launching clinical trials, many further studies still needed.

Disclosure of conflict of interest

None.

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