Biochemical and Biophysical Research Communications 693 (2024) 149357

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The current and emerging Klotho-enhancement strategies

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ARTICLE INFO

Keywords:
Exosomes
Epigenetic modification
Gene regulation
Klotho
Nonepigenetic modifications

ABSTRACT

Klotho is well known as a gene with antiaging properties. It has membrane and soluble forms, providing a unique system that controls various metabolic processes essential to health and disease. Klotho deficiency has been revealed to be associated with various aging-related disorders. Based on its various known and unknown protective properties, upregulating the Klotho gene may be a possible therapeutic and/or preventive approach in aging-related complications. Some agents, such as hormonal compounds, renin-angiotensin system inhibitors, antioxidants, peroxisome proliferator-activated receptor gamma (PPAR-γ) agonists, statins, vitamin D receptor agonists, antioxidants, anti-inflammatory agents, mammalian target of rapamycin (mTOR) signaling inhibitors, and receptor-interacting serine/threonine-protein kinase 1 (RIPK1) inhibitors, can possibly lead to the upregulation and elevation of Klotho levels. Demethylation and deacetylation of the Klotho gene can also be considered other possible Klotho-enhancement methods. Some emerging techniques, such as RNA modifications, gene therapy, gene editing, and exosome therapy, probably have the potential to be applied for increasing Klotho. In the present study, these current and emerging Klotho-enhancement strategies and their underlying mechanisms were comprehensively reviewed, which could highlight some potential avenues for future research.

1. Introduction

Klotho (Kl) has been well described as a gene with antiaging properties [1]. The Kl gene was initially discovered by Kuro-o et al., in 1997 in a group of mice with complex aging-like phenotypes. They found that the cause of this phenotype was a mutation in the Kl gene [2]. The Kl-deficient mice not only suffered from several age-related complications, such as renal failure, atherosclerosis, hypogonadism, infertility, growth retardation, vascular calcification, skin atrophy, osteoporosis, and hearing loss but also had short life spans. On the other hand, mice with high expression of Kl had a longer lifespan of 20–30 % [2].

The Kl family has three members, including α-Kl, β-Kl, and γ-Kl. In general, the word "Klotho" means α-Kl when no subfamily is mentioned [3,4]. The human Kl (α-Klotho) gene is located on chromosome 13q13.1 and consists of five exons. It is predominantly expressed in the kidneys and brain and to a lesser extent in the heart and parathyroid glands. The gene expresses a type 1 transmembrane protein functioning as a coreceptor for fibroblast growth factor-23 (FGF23) [5]. β-Kl is a coreceptor for FGF19 and FGF21, while γ-Kl is a half-size Kl-related protein. The high-affinity binding of FGF19, FGF21, and FGF23 to their receptors requires the presence of Kl [4,6,7].

There are also soluble forms of Kl (s-Kl) that can be produced not only by shedding the extracellular domain of Kl [through proteolytic activities of a disintegrin and metalloproteinases 10 and 17 (ADAM10/17)] but also by alternative splicing of the Kl gene. The soluble forms are mainly found in body secretions such as blood, urine, and cerebrospinal fluid (CSF) and have endocrine, paracrine, or autocrine roles independent of FGFs [6]. Collectively, membrane and soluble forms of Kl provide a unique system that not only controls a wide range of metabolic processes essential in health, such as mineral and energy metabolism and stress responses but also may play protective roles against chronic kidney disease (CKD), cardiovascular diseases (CVD), neurological diseases, cancer, and diabetes [6,8–10]. Therefore, enhancing Kl might have therapeutic benefits in various aging-related disorders. In general, the amount of a protein can be increased by taking supplements, increasing gene expression, and inhibiting protein degradation in the target tissue. Various methods have been introduced that can be considered possible approaches to increase Kl. The present study aimed

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https://doi.org/10.1016/j.bbrc.2023.149357
Received 5 September 2023; Received in revised form 24 November 2023; Accepted 4 December 2023
Available online 10 December 2023

to comprehensively review the current and emerging Kl-enhancement strategies as a possible therapeutic and/or preventive approach for various aging-related disorders.

2. Current methods

Some drugs and agents have been revealed to increase Kl levels through different nonepigenetic and epigenetic mechanisms. These agents are briefly described below.

2.1. Hormonal agents

There might be a link between Kl and the neuroendocrine system. It has been shown that some hormonal agents lead to Kl enhancement [11–14]. Hsu et al. examined the regulatory effects of testosterone on Kl gene expression in vivo and in vitro [12]. In their study, Kl mRNA and protein were upregulated in testosterone-treated NRK-52E cells and mouse kidneys. Kl was enhanced through the upregulation of the nuclear androgen receptor (AR) by testosterone because flutamide, an AR antagonist, attenuated the testosterone-induced Kl expression. This receptor directly binds to the Kl gene promoter via androgen response elements (AREs) and eventually upregulates Kl [12]. Estrogens have been shown to be potent modulators of neurotransmission, synaptic plasticity, and neurogenesis in the hippocampus. Menopause in women is associated with an increased risk of memory disorders, which can be reduced by timely estrogen therapy [11]. Sárvári et al., in a rat model of menopause, showed that the administration of 17β-estradiol (E2) induced the transcriptional activation of some hippocampal genes, including Kl, which ultimately improved hippocampus-dependent spatial memory [11]. Mizunoa et al., in an in vitro study, investigated the effect of tri-iodothyronine (T_3) on Kl gene expression in 3T3-L1 adipocytes [13]. They revealed that T_3 induced the expression of the membrane form of Kl (m-Kl), which may play a role in adipose differentiation. The expression of the secreted form of Kl was not affected by T_3; therefore, this hormone might affect the splicing of Kl mRNA [13]. Chen et al. found that insulin could enhance s-Kl production possibly by inducing the proteolytic activity of ADAM 10/17 [14]. The expression of the genes encoding these proteases was not affected by insulin. Furthermore, they showed that the effect of insulin on Kl shedding was inhibited by wortmannin, a covalent inhibitor of phosphoinositide 3-kinases (PI3Ks), suggesting that insulin might act through a PI3K-dependent pathway. It might recruit a signaling cascade and/or gene expression that induces ADAM 10/17 activity and increased shedding of Kl [14]. These results show that the expression of membrane and secretory forms of Kl can be regulated by some hormones through different mechanisms. Therefore, hormone therapy can be considered a strategy to increase Kl expression. However, more studies are necessary to confirm these impressions.

2.2. Renin-angiotensin system inhibitors

The renin-angiotensin system (RAS) is one of the main systems controlling blood pressure and body fluid homeostasis. The main biological hormone of this system is angiotensin II (Ang-II), which is produced under the influence of angiotensin-converting enzyme (ACE) on angiotensinogen. Ang-II binds to certain receptors, including the angiotensin type-1 (AT1) receptor, and affects a wide range of biological processes, from inflammation and immune responses to longevity [15]. Accumulating data suggest that there is an association between Kl and RAS [16]. Ang-II could downregulate Kl [16,17]. Mitani et al. showed that the long-term administration of Ang-II reduced mRNA and protein expression of renal Kl through the AT1 receptor [18]. It was also revealed that Ang–II–induced Kl downregulation was pressure-independent. Ang II increases the expression of transforming growth factor-β1 (TGF-β1) and p38 MAPK, leading to the activation of P53. Activated P53 forms complexes with the transcription factor SP1 in the nucleus. P53/SP1 binds to the Kl gene promoter and inhibits its transcription [18]. Angiotensin receptor blockers (ARBs), such as losartan and valsartan, and ACE inhibitors, such as enalapril and fosinopril, might enhance Kl levels by preventing the inhibitory effect of Ang-II on Kl expression [17,19,20]. In a study by Lim et al., the administration of losartan in patients with type 2 diabetes caused a significant increase in circulating s-Kl levels [19]. Therefore, inhibiting the RAS may be considered an approach to increase the amount of Kl. However, further studies are necessary to confirm the results. It is also imperative that the effects of RAS inhibitors on Kl gene expression be evaluated in tissues other than blood and kidneys.

2.3. Anti-inflammatory agents

It has been found that inflammation can decrease Kl expression [21,22]. Various proinflammatory and anti-inflammatory cytokines and related molecules may play a role in reducing Kl expression [23]. Moreno et al. showed that tumor necrosis factor-α (TNFα) and TNF-like weak inducer of apoptosis (TWEAK), as inflammatory cytokines, could downregulate Kl expression through a nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-dependent mechanism [23]. TWEAK induces RelA binding to the Kl promoter, causing its deacetylation and inhibiting Kl gene expression [23]. Teocchi et al. also revealed that TNF could downregulate Kl through NFkB in resected hippocampal tissue samples from patients with temporal lobe epilepsy [24]. Considering the role of inflammation in Kl reduction, the use of anti-inflammatory drugs may be effective in maintaining or even elevating Kl expression. Different steroidal and nonsteroidal anti-inflammatory drugs, through different mechanisms, can be effective in relieving inflammation and related complications by reducing inflammatory cytokines or blocking the relevant signals (84). However, more precise studies seem to be necessary to confirm the stimulating effect of these drugs on Kl expression.

2.4. Antioxidants

It is clear that Kl expression is repressed under sustained stress conditions and increased free radicals, indicating a close relationship between oxidative stress and Kl expression [25]. Mitobe et al. showed that hydrogen peroxide (H_2O_2)-induced oxidative stress downregulated Kl dose-dependently in the mouse inner medullary collecting duct (mIMCD3) cell line [25]. Oh et al. showed that circulating s-Kl levels were significantly associated with 8-isoprostane levels, an oxidative stress marker, in patients receiving peritoneal dialysis [21]. Antioxidant supplementation might lead to Kl enhancement [26]. Jaturakan et al. showed in an animal model study that vitamin C and vitamin E administration could upregulate Kl [27]. It has also been shown that N-acetylcysteine (a glutathione precursor) and melatonin administration could upregulate Kl by inhibiting AKT and forkhead box O (FOXO) phosphorylation, respectively, as possible mechanisms [28,29]. Based on previous studies, other agents with antioxidant effects, such as lipoic acid, and polyphenols (such as curcumin), might also induce Kl expression [26]. Therefore, antioxidant supplementation could be considered a Kl enhancement therapeutic or supportive approach for managing aging-related disorders.

2.5. Peroxisome proliferator-activated receptor gamma (PPAR-γ) agonists

Peroxisome proliferator-activated receptor gamma (PPAR-γ) is a transcription factor involved in glucose and lipid homeostasis, bone turnover, and inflammation [30]. Previous studies have found evidence of two noncanonical PPAR-γ binding sites upstream of the Kl gene, suggesting possible regulatory effects of PPAR-γ on Kl [31]. Zhang et al. showed that PPAR-γ upregulated Kl both in vitro and in vivo [32]. The expression of PPAR-γ decreases with aging [30]. Since the expression of

PPAR-γ decreases during aging, studies on the effect of PPAR-γ agonists or thiazolidinediones (TZDs) on aging-related agents have revealed that the widely used TZDs, such as Ciglitazone, Troglitazone, Pioglitazone, and Rosiglitazone, could elevate Kl levels [32–34]. Therefore, the use of PPAR-γ agonists can be considered a potential strategy to enhance Kl gene expression.

2.6. Statins

Statins are competitive inhibitors of 3-hydroxy-3 methylglutaryl coenzyme A (HMG-CoA) reductase. It is the main allosteric enzyme catalyzing the conversion of HMG-CoA to mevalonic acid, a necessary step in the biosynthesis of cholesterol and other isoprenoids. Statins such as atorvastatin, rosuvastatin, and pitavastatin are widely used in treating patients with hypercholesterolemia to reduce LDL cholesterol levels [35]. Statins have other beneficial effects, such as anti-inflammatory and antioxidant effects, by modulating nuclear factor erythroid 2–related factor 2 (Nrf2) and Nrf2/HO-1 signaling in different diseases [36]. Convincing evidence indicates that statins could also stimulate Kl gene expression by activating the FOXO signaling pathway and inhibiting the Rho/Rho-kinase pathway [37,38]. Therefore, statin administration can also be considered a possible approach to enhance Kl expression.

2.7. Vitamin D and vitamin D receptor (VDR) agonists

Vitamin D acts as a pleiotropic steroid hormone, which, in addition to its regulatory effects on calcium and bone homeostasis, exerts antiproliferative, antibacterial, immunomodulatory, and anti-inflammatory properties [39]. The biological effects of $1,25(OH)_2D_3$ (calcitriol), the biologically active form of vitamin D, are mediated by VDR, which belongs to the nuclear hormone receptor superfamily of transcription regulators [39,40] After binding to VDR, vitamin D can be involved in the regulation of the expression of various genes, including Kl [41,42]. After activation by vitamin D or its analogs, VDR forms a heterodimer with the retinoid X receptor (RXR) and translocates to the nucleus, where it binds to vitamin D response elements (VDREs) within the Kl gene promoter region, inducing its expression. Lau et al. showed that the administration of calcitriol or its analog, paricalcitol, elevated the serum levels of Kl in mice independent of parathyroid hormone and calcium level alterations [41]. Therefore, vitamin D and VDR agonists, including calcitriol, alfacalcidol, doxercalciferol, fluorocalcidol, and maxacalcitol, might have inducing effects on Kl gene expression.

2.8. Mammalian target of rapamycin (mTOR) signaling inhibitors

The mammalian (or mechanistic) target of rapamycin (mTOR) is a serine/threonine protein kinase that plays roles in regulating cellular growth, proliferation, body energy metabolism, and lifespan [43]. Some evidence has revealed that activation of mTOR signaling suppresses Kl expression, while its inhibition could upregulate it [44–46]. Sirolimus, also known as rapamycin, is an mTOR inhibitor that has immunosuppressive effects by inhibiting the activation of T and B cells. This agent has been shown to increase or retain Kl levels by inducing Kl expression [45]. Mizusaki et al. showed that renal transplantation recipients receiving Everolimus (a rapamycin derivative) had higher s-Kl levels than other patients who did not take this agent [46]. The use of mTOR inhibitors can also be considered another possible Kl-increasing approach.

2.9. Receptor-interacting serine/threonine-protein kinase 1 (RIPK1) inhibitors

Receptor-interacting serine/threonine-protein kinase 1 (RIPK1) has been identified to play roles in several cellular pathways related to cell survival, cell death (apoptosis and necroptosis), and inflammation [47]. It has been considered an encouraging therapeutic target in managing various human neurodegenerative, autoimmune, and inflammatory disorders [48]. In recent years, the therapeutic application of RIPK1 inhibitors has gained attention. The effects of the RIPK1 inhibitors GSK'772 and DNL747 have been evaluated in the management of autoimmune diseases and neurodegenerative disorders, respectively, and the results were encouraging [49,50]. Ning et al. revealed that necrostatin-1, a potent and specific small-molecule inhibitor of RIPK1, could alleviate cisplatin-induced nephrotoxicity by retaining renal Kl expression and suppressing apoptosis and oxidative stress [51]. Therefore, RIPK1 inhibition may also be a strategy to enhance Kl.

2.10. Other agents

Other factors, such as aerobic exercise [52], intermedin (a vasoactive factor) [53], and ligustilide (a natural benzoquinone derivative found in some Chinese herbal medicines) [54], may also be effective in enhancing Kl.

3. Emerging methods

Recently, some new and emerging introduced methods, such as epigenetic modifications [45,55], gene therapy [18,56], gene editing [57], and exosome therapy [58], have the potential to upregulate Kl and enhance its amount in the brain. These methods are briefly described below.

3.1. Epigenetic modifications

Epigenetics is one of the expanding fields of biology that has been applied in modern and precision medicine [59,60]. Epigenetic modifications are nuclear or molecular alterations affecting gene expression and, eventually, the final product amount of a locus or chromosome without direct interaction with the primary DNA sequence. Histone modification, DNA methylation, and microRNA (miRNA) expression alteration are the most prevalent studied epigenetic modifications [61]. Different types of histone modification, such as acetylation, methylation, phosphorylation, and ubiquitination, have been revealed to affect gene expression and protein production. Acetylation and deacetylation are mainly catalyzed by two enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs). Histone acetyltransferase adds an acetyl group to an amino acid in histones that facilitates chromatin opening, leading to gene upregulation. Histone deacetylases with contrasting functions remove acetyl groups, resulting in a more closed chromatin structure and leading to gene downregulation [61,62]. In a study by Lin et al., HDAC inhibition by trichostatin-A attenuated chronic kidney disease-associated bone injury in mice by upregulating Kl [63]. DNA methylation is a transfer process of a methyl group to the fifth carbon of a cytosine residue to form 5-methylcytosine in DNA. This process is catalyzed by a family of DNA methyltransferases and affects gene expression by applying proteins involved in gene repression or by interfering with the binding of transcription factor(s) to DNA [64]. Zhu et al. showed that methylation of the Kl gene promoter silenced its gene expression in head and neck squamous cell carcinoma [65]. Therefore, DNA methyltransferase inhibitors such as azacytidine, decitabine, and zebularine might have the potential to be used as a Kl-elevating approach. MiRNAs are a type of small, noncoding RNA that affects the expression of protein-encoding genes through the RNA interference (RNAi) pathway. They posttranscriptionally repress the target genes by attaching to the 3′UTR of the genes or by inducing mRNA degradation [55]. Liang et al. demonstrated that miR-130a promoted Kl expression, leading to protection against lipopolysaccharide-induced glomerular cell injury [55]. These encouraging results could demonstrate that epigenetic modifications have the potential to be applied to enhance Kl expression.

Table 1
The current therapies for increasing Klotho levels.

Method	Agent	Key Findings	Target	Ref.
Hormonal Agents	Testosterone	Kl mRNA and protein levels were enhanced through mediating of AR and AREs.	NRK-52E cells and mouse kidneys	[12]
	17β-estradiol (E2)	Transcriptional activation of Kl was induced. Spatial memory was improved.	Rat hippocampus	[11]
	T_3	The expression of the m-Kl was upregulated. The expression of the s-Kl was not affected.	3T3-L1 adipocytes	[13]
	Insulin	The production of s-Kl was enhanced by inducing the activity of ADAM 10/17.	COS-7 cells, rat kidneys	[14]
RAS inhibitors	Losartan	Circulating levels of s-Kl increased.	Type 2 diabetic patients	[19]
	Fosinopril Valsartan	Kl expression was upregulated.	NRK-52E cells	[20]
Anti-inflammatory and antioxidant agents	N-Acetylcysteine	Kl expression was upregulated. CsA-nephropathy was attenuated.	Rat Kidneys	[28]
	Melatonin	Kl expression was upregulated. Cisplatin-induced acute kidney injury was attenuated.	Rat Kidneys	[29]
PPAR-γ agonists	Thiazolidinediones (Troglitazone and Ciglitazone)	Kl expression was increased in a dose and time-dependent manner.	HEK293, IMCD, MCT, and MDCK cells; Mouse Kidneys	[32]
	Pioglitazone	The expression of Kl was increased and aging-related progressive renal injury was ameliorated.	Rat Kidneys	[33]
	Rosiglitazone	Cerebral Kl expression was increased and the impaired baroreflex sensitivity was restored.	Rat Brain	[34]
Statins	Pravastatin	Kl expression was upregulated in a dose-dependent manner. CsA-induced nephropathy was ameliorated.	Mouse Kidneys	[37]
	Atorvastatin Pitavastatin	The reduction of Kl expression induced by NOS inhibition was prevented.	Rat Kidneys	[38]
Vitamin D and VDR agonists	Calcitriol Paricalcitol	The serum levels of Kl were elevated independent of parathyroid hormone and calcium level alterations.	Mice	[41]
mTOR signaling inhibitors	Rapamycin	Membrane and secreted Kl were enhanced. Vascular calcification was ameliorated.	BASMCs HASMCs Rat's aorta and serum	[45]
	Everolimus	s-Kl levels were elevated.	Renal transplantation recipients	[46]
RIPK1 inhibitors	Necrostatin-1	Kl expression was upregulated and Cisplatin-induced nephrotoxicity was alleviated	Mouse Kidney	[51]
Other agents	Aerobic exercise	Kl was upregulated that induced antioxidative effects.	Rat brain and kidneys	[52]
	$Intermedin_{1-53}$	Kl was upregulated that reduced vascular calcification.	Rat aortas	[53]
	Ligustilide	Kl expression increased. Memory deficits, amyloid accumulation, tau phosphorylation, and oxidative stress were reduced.	Mouse brain and serum; 293T cell line	[54]

ADAM, a disintegrin and metalloproteinase; AR, androgen receptor; ARE, androgen receptor element; CsA, Cyclosporin A; Kl, klotho; m-Kl, membrane klotho; mTOR, mammalian target of rapamycin; PPAR-γ, peroxisome proliferator-activated receptor gamma; RAS, renin–angiotensin system; RIPK1, receptor-interacting protein kinase 1; s-Kl, soluble klotho; VDR, vitamin D receptor.

3.2. Epitranscriptomics

Biochemical modifications of macromolecules have inevitable roles in biological processes. Like DNA and histone modifications, it has been found that RNA modifications also have profound impacts on gene expression [66,67]. RNA epigenetics, also called "epitranscriptomics", is an emerging field of posttranscriptional regulation of gene expression that has recently attracted the interest of researchers [68]. It is a set of dynamic and reversible biochemical modifications in cellular RNAs (transcriptome) that can alter gene expression levels by affecting the structure and metabolism of mRNA. Some examples of known RNA modifications are N1-methyladenosine (m1A), pseudouridine (Ψ), 5-methylcytidine (m5C), and N6-methyladenosine (m6A) modifications [66,69]. They have predominantly been identified in noncoding RNAs (ncRNAs), including tRNA, rRNA, and small nuclear RNAs (snRNAs), that could affect gene expression through various mechanisms, such as affecting the structure, stability, and translation level of mRNA [67,69,70]. RNA modifications have the potential to be applied for altering the expression of a target gene such as Kl. Recently, it has been shown that m6A modification could affect Kl gene expression [71]. M6A modification can be mediated by methyltransferase-like enzymes such as METTL14, which has been revealed to downregulate Kl expression. On the other hand, removing m6A on RNA by demethylase enzymes such as FTO and ALKBH5 or inhibiting METTL14 could lead to Kl upregulation [72]. Therefore, epitranscriptomics could be considered an emerging therapeutic approach to alter the expression levels of related target genes, such as Kl, which play roles in the pathogenesis of different disorders.

3.3. Gene therapy

Gene therapy is generally a method to replace a defective gene with a normal version to work properly in the target tissue [26]. Lentivirus and adenovirus-based vectors are practical tools for transmitting genetic material. Recently, these vectors have widely been used in gene therapy due to their characteristics, such as low and acceptable immunogenicity, low toxicity, high stability, and cell-type specificity [73]. A lentivirus encoding the mouse Kl gene was applied in an animal model study by Zhou et al. to sustain Kl expression [56]. These vectors were injected into the bilateral lateral ventricles of 7-month-old mice. Gene delivery increased all forms of Kl in the mouse brain. The results also revealed that lentivirus-mediated upregulation of Kl expression alleviated aging-related memory deficits and oxidative stress in the studied mice [56].

3.4. Gene editing

Techniques for transferring exogenous genes to target sequences have improved significantly. Clustered regularly interspaced short palindromic repeats (CRISPR) are nucleic acid sequences that, in coordination with CRISPR-associated (Cas) proteins, act as the immune system that protects bacteria and archaea against the external invasion of viruses, plasmids, and phages.

Type II of this compatible bacterial immune system has been engineered in recent years and presents a new and powerful technique called CRISPR–Cas9 for precise genomic manipulation [26]. Several desired applications, such as gene editing, gene tracking, upregulation,

Table 2
The emerging therapies for increasing Klotho levels.

Method	Agent	Key Findings	Target	Ref.
Epigenetic modifications	Trichostatin-A	HDAC was inhibited leading to Kl upregulation. CKD-associated bone injury was attenuated.	Mouse kidneys	[63]
	miR-130a	LPS-induced glomerular cell injury was alleviated by upregulation of Kl expression.	HK-2 cells	[55]
Epitranscriptomic modifications	METTL14-siRNA	Kl was upregulated by METTL14 knockdown	HRGECs Mice Kidneys	[71, 72]
	FTO, ALKBH5	Kl can be upregulated by reversing m6A RNA modification.		
Gene therapy	Lentivirus encoding Kl gene	All forms of Kl increased. Aging-related memory impairments and oxidative stress were alleviated.	Mouse brain	[56]
Gene editing	CRISPR system	Two gRNA targeting the Kl promoter regions were recognized and utilized for efficient transcriptional activation of the Kl gene. Klotho gene expression was enhanced at both the gene and protein levels.	SY5Y, and HK-2 cells	[57]
Exosomes	Kl-overexpressing exosomes	Caerulein-stimulated apoptosis and inflammation were reversed.	AR42J cells	[58]

ALKBH5, alkylation B Homolog 5; CKD, chronic kidney disease; CRISPR, clustered regularly interspaced short palindromic repeats; FTO, fat mass and obesity-associated protein; HDAC, histone deacetylases; Kl, klotho; LPS, lipopolysaccharide; m6A, N6-methyladenosine; METTL14, methyltransferase-like 14.

downregulation, and gene blocking, can be provided by adding suitable and specific peptides, proteins, and enzymes to this system [26,74]. The CRISPR–Cas9 system has recently attracted inclusive attention for therapeutic applications [74]. It may also be considered a possible therapeutic approach in Kl gene editing and altering its expression. Chen et al. applied the CRISPR–Cas9 system to activate human Kl gene expression in two human cell lines, neuronal SY5Y and renal HK-2 cells [57]. Two gRNAs targeting the Kl promoter regions were recognized and utilized for efficient transcriptional activation of the Kl gene. Klotho gene expression was enhanced at both the gene and protein levels, indicating the efficacy of gene therapy for targeting Kl using the CRISPR method [57].

3.5. Exosomes

Exosomes are nanosized vesicles (50–100 nm) produced by almost all cells and have received considerable attention in recent years. Exosomes were initially thought of as tools that can be used to dispose of cellular waste. These nanovesicles are currently considered a new form of intercellular interaction [26,75]. They have roles in several pathophysiological processes. Additionally, some of their characteristics, such as tissue specificity, lack of immunogenicity, and ability to cross the cell membrane and blood–brain barrier, make them a possible tool for therapeutic approaches. They can be applied as a vehicle to transport various contents, such as DNA, miRNA, mRNA, protein, and even drugs, to target cells [26,75]. The transferred exosomal cargo can eventually alter cell function by affecting some metabolic and signaling pathways and altering the expression of target genes and proteins [75]. Thus, exosomes can be used as a possible tool for transporting soluble-Kl to several target tissues. In a study by Wang et al., exosomes were extracted from Kl-overexpressing mesenchymal stem cells and subsequently administered in cell culture (AR42J cells) and an animal model of acute pancreatitis induced by caerulein [58]. The administered Kl-overexpressing exosomes reversed apoptosis and nuclear factor-kB activation and alleviated the severity of pancreatic inflammation [58].

4. Conclusion and future research directions

Kl is well described as a gene with antiaging properties. Membrane and soluble forms of Kl provide a unique system that controls a wide range of metabolic processes essential in health and diseases. Based on its various known and unknown protective properties, upregulating the Kl gene may be a possible therapeutic and/or preventive approach in aging-related disorders. Various drugs and factors have been revealed to enhance endogenous Kl. They include hormonal agents, renin-angiotensin system inhibitors, antioxidants, PPAR-γ agonists, HMG-CoA reductase inhibitors, vitamin D receptor agonists, antioxidants, anti-inflammatory agents, mTOR signaling inhibitors, and RIPK inhibitors (Table 1). Epigenetic modifications such as demethylation and deacetylation of the Kl gene can also be deemed other possible Kl-enhancement methods. In addition, some emerging techniques, such as RNA modification, gene therapy, gene editing, and exosome therapy, have the potential to be applied to upregulate the Kl gene (Table 2).

The use of recombinant therapeutic proteins is another approach to therapeutically increase a protein in the body. Direct treatment with Kl recombinant protein has been successfully demonstrated in some recent animal model studies [76–78]. Direct administration of exogenous proteins has some advantages compared to traditional small molecule drugs, such as lower toxicity, fewer side effects, more efficacy, greater specificity, and clearer biological functions [79]. However, there are still some challenges that should be addressed, such as the inherent susceptibility of exogenous proteins to aggregation, degradation, denaturation, and concomitant loss of activity. Their untimely clearance from the body, nonspecific distribution, and immunogenicity could be considered other relevant concerns [79,80]. The relatively larger sizes of recombinant protein drugs compared to small molecule agents make it difficult to deliver most exogenous proteins to target tissues, especially in hard-to-target locations in the body, such as the brain. The stability of the recombinant protein drugs under storage conditions that could affect their therapeutic efficacy can be an added concern [80].

Overall, although Kl might be considered a therapeutic target, it is necessary that the usefulness of Kl-enhancing strategies in the prevention and treatment of aging-related disorders be investigated by various preclinical studies. In addition, the implementation possibility of Kl-increasing strategies in different tissues while considering their safety and possible side effects should be precisely evaluated by further animal model studies. These are considered potential avenues for future research.

CRediT authorship contribution statement

Haniyeh Poursistany: Writing – original draft, Investigation. **Solmaz Tabibi Azar:** Writing – original draft. **Mahsan Tabibi Azar:** Writing – original draft. **Sina Raeisi:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This work was supported by the Pediatric Health Research Center of Tabriz University of Medical Sciences, Tabriz, Iran. We would like to thank the Clinical Research Development Unit of Zahra Mardani Azari Children Educational and Treatment Center, Tabriz University of Medical Sciences, Tabriz, Iran.

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