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REVIEW ARTICLE

The metabolic, neuroprotective cardioprotective and antitumor effects of the Klotho protein

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Abstract

Klotho is a transmembrane protein with a wide spectrum of activity. The human Klotho gene shows 86% amino acid identity with the mouse protein.

Many important pleiotropic functions of the Klotho protein have been revealed. Amongst them, there is a regulation of nitric oxide production, suppression of oxidative stress and inflammation, influence on the insulin-like growth factors and fibroblast growth factors signaling, modulation of calcium and phosphate metabolism, synthesis of vitamin D and other. Two forms of the Klotho protein are known. The secreted form strongly inhibits the oxidative stress, and, in humans, is more dominant than the membrane form.

Studies on a mouse model resulted in the finding of the anti-aging effect of the Klotho protein. This activity is mainly associated with the suppression of oxidative stress, as well as it could be related to neuroprotective, cardioprotective, and metabolic functions.It might be speculated that Klotho, regarded as a neuroprotective factor, may have therapeutical applications in the future in the treatment of demyelinating and neurodegenerative disorders, especially multiple sclerosis (MS) and Alzheimer's disease (AD).

The Klotho through inhibition of oxidative stress possesses cardioprotective properties. Of note, one functional variant of Klotho is a risk factor for coronary disease as well as some nucleotide polymorphisms are associated with carotid arteriosclerosis. Moreover, the Klotho protein can inhibit Angiotensin II-induced cardiomyocyte hypertrophy. All those effects indicate that the Klotho protein may be useful in the therapy of heart failure and hypertension.

Undoubtedly, metabolic disturbances play an important role in the pathogenesis of many neurodegenerative and cardiovascular diseases. The metabolic effects of the Klotho protein are strongly connected with its neuroprotective and cardioprotective activity. This protein affects adipogenesis, metabolism of glucose and lipids as well as calcium-phosphate system by influence on the activity of fibroblast growth factors (FGF19, FGF23, FGF21).

Finally, it has been revealed that the Klotho protein has antitumor activity. Besides, the FGF-Klotho system may have a role in longevity and aging-related disorders.

THE KLOTHO PROTEIN – STRUCTURE, MECHANISM OF ACTION

Klotho is a transmembrane protein with a wide spectrum of activity. On one hand, the results of studies on a mouse model with Klotho deficiency indicated that these animals present systemic aging, infertility, arteriosclerosis, neural degeneration, gonadal atrophy, pulmonary emphysema and calcification of soft tissue (Kuro-o *et al.* 1997). On the other hand, the overexpression of Klotho leads to suppression of aging in mice (Kurosu *et al.* 2005).

The human Klotho gene is located on chromosome 13q12 (Matsumura *et al.* 1998), and shows 86% amino acid identity with the mouse protein. The expression of Klotho was discovered in some organs such the kidney, brain (especially in the choroid plexus), epithelium, parathyroid gland, placenta, ovary, prostate, and small intestine (Matsumura *et al.* 1998; Shiraki-lida *et al.* 1998; Chihara *et al.* 2006., Lorenzi *et al.* 2010). The Klotho protein is found in both human sera and cerebrospinal fluid (CSF) (Imura *et al.* 2009).

The analysis of Klotho cDNA revealed two spliced transcripts, which encode two proteins: membrane Klotho and secreted Klotho, which are mainly produced in the brain and kidney (Matsumura *et al.* 1998). In detail, the secreted form in CSF is derived from the choroid plexus of the brain, however, the secreted form in serum is derived mainly from the kidney (Wang *et al.* 2009).

In humans, the secreted form is more dominant than the membrane form and strongly modulates the oxidative stress as it is involved in the regulation of nitric oxide production in the endothelium (Saito *et al.* 1998; Saito *et al.* 2000). Besides, this dominant form influences calcium homeostasis (Imura *et al.* 2007) as well as inhibits insulin-like growth factors signaling (Kurosu *et al.* 1997).

The membrane form is known to be a co-receptor for the fibroblast growth factor (FGF)-23, and FGF activates specific receptors with α or β isoforms of the Klotho co-factors (Salminen *et al.* 2017). It has been found that the membrane form of Klotho plays a role in the mechanism of phosphate excretion in urine (Urakawa *et al.* 2006), and calcium homeostasis (Negri *et al.* 2005; Imura *et al.* 2007).

Furthermore, Klotho is involved in the regulation of calcium and phosphorus homeostasis and synthesis of vitamin D (Imura *et al.* 2007; Nabeshima, 2008; Nabeshima *et al.* 2008; Zeng *et al.* 2019).

Interestingly, an anti-aging effect of Klotho is related to resistance to oxidative stress and inhibition of the insulin-like growth factor (IGF) signaling (Yamamoto *et al.* 2005).

Referring to the above-mentioned data, it could be stated that the Klotho protein possesses some important pleiotropic functions including the regulation of fibroblast growth factors (including FGF19, FGF21 and FGF23) signaling, suppression of insulin-like growth factor signaling, regulation of nitric oxide production suppression of oxidative stress, and regulation of calcium homeostasis (German *et al.* 2012; Jeinsen *et al.* 2019).

THE METABOLIC EFFECTS OF THE KLOTHO PROTEIN

According to data from the literature, the Klotho protein is involved in the regulation of metabolic processes (Long *et al.* 2011; Razzaque, 2012; Berezin and Berezin,2019; Silva *et al.* 2019).

Briefly, Klotho may influence adipogenesis, glucose, and lipid metabolism (Razzaque, 2012) by mediating FGF19 and FGF21 (Long *et al.* 2011). Furthermore, the involvement of Klotho in the mechanism of adipocyte maturation, adipocyte differentiation of preadipocytes to adipocyte cells and glucose metabolism has been also reported (Arking *et al.* 2003; Chihara *et al.* 2006; Zhang *et al.* 2008; Razzaque, 2012).

Besides, the Klotho protein plays a role in the regulation of calcium and phosphate metabolism by influence on the activity of FGF19, FGF23, FGF21 (Nabeshima, 2008; Nabeshima *et al.* 2008; Wu *et al.* 2009; Kurosu *et al.* 2009; Long *et al.* 2011). Interestingly, it has been revealed that the regulation of phosphate metabolism by Klotho, which is co-receptor of FGF23, and FGF23 may affect aging processes (Kuro-o, 2010). Notably, growing data indicate that FGF19, FGF21 and FGF23 and Klotho, which influence on fibroblast growth factors, may participate in many metabolic processes, including the metabolism of glucose, lipids, phosphate, calcium, and vitamin D. All these functions suggest a potential Klotho's role in the pathophysiology of metabolic diseases such as diabetes, obesity, kidney disorders and bone diseases (Kurosu *et al.* 2009; Kuro-o, 2010). In details, the following metabolic activities of the FGF-Klotho system have been reported:

- FGF19, secreted from the intestine, plays a role in the synthesis and metabolism of bile acids.
- FGF21 affects energy metabolism by activation of the AMPK – protein kinase through the FGF R1/β klotho proteins signaling or indirectly by stimulation of expression and secretion of adiponectin and corticosteroids by activating the hypothalamo-pituitary-adrenal axis (Salminen *et al.* 2017). Moreover, FGF21 stimulates glucose uptake and lipolysis in adipose tissue and activates thermogenesis (Kharitonenkov, 2009, Fisher *et al.* 2012). Besides, FGF21 may regulate insulin-independent glucose transport in adipocytes (Kharitonenkov, 2009). Interestingly, an impairment of FGF21 signaling in adipose tissue may be connected with the downregulation of FGF21 co-receptor or βKlotho during the onset of diet-induced obesity. The group of Markan (Markan *et al.* 2017) showed that βKlotho levels are decreased in white adipose tissue but not in liver or brown adipose

tissue. The authors concluded that FGF21 resistance is not mediated by the downregulation of β Klotho expression in white adipose tissue (Markan *et al.* 2017).
- FGF23, secreted from bones, regulates phosphate metabolism in kidneys.

Animal studies showed that in the Klotho mutant mouse with low Klotho protein concentration a decreased insulin production and increased insulin sensitivity were present (Utsugi *et al.* 2000). Noticeably, a decrease of plasma the Klotho protein was described in patients with anorexia nervosa as well as in obese individuals (Amitani *et al.* 2013, Wolf *et al.* 2016).

Taking into account that the metabolic disturbances play a pathogenetic role in many neurodegenerative and cardiovascular diseases, it could be presumed that the beneficial metabolic effects of the Klotho protein are involved in the mechanism of neuro- and cardioprotection.

THE ANTI-AGING AND NEUROPROTECTIVE EFFECTS OF THE KLOTHO PROTEIN

Data from the literature suggest that the anti-aging properties of Klotho are related to increased resistance to two unfavorable factors, oxidative stress (Kurosu *et al.* 2005), and inflammation (Liu *et al.* 2011). It is worth to notice that the Klotho gene polymorphisms and the FGF-Klotho system were associated with longevity and aging-related disorders (Zeng *et al.* 2018, Zhu *et al.* 2019, Kuro-o M 2019). Besides, the Klotho activity is also among factors that impact on the length of life and disease related to age (Arking *et al.* 2002; Arking *et al.* 2003; Arking *et al.* 2005; Invidia *et al.* 2010; Laina *et al.* 2018; Zhu *et al.* 2019).

Animal studies indicated that the secretory form of Klotho possesses neuroprotective properties (Chen *et al.* 2015). The Klotho secreted by the choroid plexus may prevent myelin degeneration in the aging brain in the mechanism of enhancement of the maturation of oligodendrocyte progenitors cells (OPCS) and myelination in the central nervous system (CNS) (Chen *et al.* 2013; Chen *et al.* 2015).

The neuroprotective effects of Klotho are also mediated via the regulation of members of the redox system (Zeldich *et al.* 2014). These findings indicate that the Klotho protein may participate in the pathophysiological mechanisms of neurodegenerative disorders (Prokhorova *et al.* 2019).

Furthermore, Kuroda *et al.* showed that FGF21 drives the proliferation of oligodendrocyte precursor cells (OPCs) through interaction with βKlotho, a co-receptor of FGF21 (Kuroda *et al.* 2017). In detail, these authors demonstrated in the in vitro experiment that human OPCs expressed βKlotho and proliferated in the response to FGF21.

It has been commonly accepted that the demyelination and axonal injury observed in multiple sclerosis (MS) result, at least partially, from autoimmunological processes. Without any doubt, the following features characterize multiple sclerosis: demyelination, axonal injury, and inflammation (Kamm et la., 2014).

It needs to be highlighted that Klotho can suppress TNFα-induced expression of adhesion molecules in the endothelium as seen in a model of endothelial inflammation (Maekawa *et al.* 2009). Besides, in a study by Abraham and co-workers the Klotho protein caused a decrease of NF-Kβ activation, IkB phosphorylation, and inhibition of eNOS phosphorylation induced by TNFα (Abraham *et al.* 2012).

The experiments on Klotho knockout demonstrated that the number of mature oligodendrocytes was lower in (Chen *et al.* 2015). Of note, the experimental results in the mouse model also revealed that the Klotho protein enhanced oligodendrocyte maturation and myelination and inhibited the oxidative stress (Chen *et al.* 2013). Data from human studies showed a decreased concentration of Klotho in CSF of patients with relapsing-remitting multiple sclerosis (RRMS) (Emami *et al.* 2015). In a group of individuals with active RRMS with prolonged duration, the levels of Klotho tended to increase after therapy with immunomodulatory drugs, (Ahmadi *et al.* 2016).

Interestingly, increasing Klotho levels may protect myelin integrity and prevent myelin degeneration in the aged brain. Moreover, Zeldich and colleagues suggested that the Klotho protein may have a beneficial role in remyelination in the white matter and it may be a potential therapeutical target for myelin repair in multiple sclerosis (MS) (Zeldich *et al.* 2015).

Indeed, it is believed that the Klotho protein may be a new therapeutic target in selected diseases, including MS and, interestingly, schizophrenia, in which myelin dysfunction play a pathogenic role (Taveggia *et al.* 2010). However, Yazici and colleagues investigated the relationship between neurodegeneration and Klotho protein, vitamin D and homocysteine levels in patients with schizophrenia and these authors demonstrated that in schizophrenia Klotho levels were non-significantly elevated as compared with the control group. Additionally, homocysteine, vitamin B12, and folic acid were significantly higher (Yazici *et al.* 2019).

It could be speculated that the Klotho protein regarded as a neuroprotective factor being downregulated with age may have future therapeutical applications in demyelinating and neurodegenerative diseases including not only multiple sclerosis but also Alzheimer's disease (AD) (Abraham *et al.* 2012).

There is a piece of growing evidence that Klotho may impact on the pathogenesis of Alzheimer's disease. It has been shown that loss of Klotho expression leads to cognitive deficits (Chen *et al.* 2013). Interestingly, the clinical studies indicated that Klotho concentration in cerebrospinal fluid of patients with Alzheimer's dis-

ease was significantly lower as compared with controls (Semba *et al.* 2014).

The Klotho protein is a substrate for α, β and γ-secretase and inhibitors of β secretase reduces amyloid-beta in Alzheimer's disease (Bloch *et al.* 2009). Recently published results of experiments with the involvement of Alzheimer's disease mouse model showed that intracerebroventricular injection of a lentiviral that encoded Klotho may promote the autophagic clearance of amyloid-beta and protect against the cognitive deficit in AD mouse (Zeng *et al.* 2019). These results suggest a potential therapeutical application of Klotho usage in AD.

However, it should be noted that the results of Porter and colleagues did not support the previous hypothesis that Klotho allele status affects Aβ (amyloid β), APOE E4 (apolipoprotein E) and cognitive decline in preclinical Alzheimer's disease (Porter *et al.* 2019).

Furthermore, the Klotho protein by inhibiting the insulin/IGF 1 signaling and decreasing oxidative stress in the brain may prevent the development of Alzheimer's disease. Data concerning ligustilide, which inhibits IGF1 with simultaneous Klotho upregulation, suggests that Klotho may contribute to the neuroprotective effect of ligustilide in AD (Kuang *et al.* 2014).

Finally, regarding other brain diseases and brain damage, the group of Hu found that decreased plasma Klotho concentration was related to the poor prognosis in patients with intracerebral hemorrhage (Hu *et al.* 2019).

THE CARDIOVASCULAR EFFECTS OF KLOTHO

The animal and clinical studies showed strongly suggested that the Klotho protein may play an important role in the pathophysiology of not only neurogenerative but also in cardiovascular complications (Shiozaki *et al.* 2008). The results from experiments on animal model demonstrated a decrease in nitric oxide production, endothelial dysfunction, and arteriosclerosis in Klotho deficient mice (Kuro-o *et al.* 1997; Nagai *et al.* 2000; Shiozaki *et al.* 2008). The group of Maekawa found that Klotho reduces the expression of adhesion molecules and recovers suppression of endothelial nitric oxide synthase (eNOS) induced by immunomodulation of TNF (Maekawa *et al.* 2009). The Klotho protein acting through inhibition of oxidative stress reveals cardioprotective, and this property might implicate forthcoming therapeutical application of Klotho in patients with heart failure (Song *et al.* 2015).

The studies on animal models also showed that Klotho inhibits Angiotensin II-induced cardiomyocyte hypertrophy (Yu *et al.* 2016). Therefore, these results suggest that Klotho may act as an endogenous antihypertrophic factor and, consequently, in the future, it may be a curative agent in heart failure and hypertension (Yu *et al.* 2016).

It has been reported that polymorphism in the human Klotho gene may be connected with age-related diseases such as stroke (Majumdar *et al.* 2010) coronary disease (Arking *et al.* 2003; Yamada *et al.* 2005; Semba *et al.* 2011) hypertension (Oguro *et al.* 2010) osteoporosis (Yamada *et al.* 2005).

The polymorphism in the Klotho gene may be associated with the risk of ischemic stroke (Kim *et al.* 2006). These authors demonstrated that the G-395 A in the Klotho gene might be a risk factor for cardioembolic stroke. Low plasma Klotho in patients with cardiovascular disease is a predictor of increased mortality (Semba *et al.* 2011). The functional KL-VS variant of the Klotho gene was associated with early-onset ischemic stroke (Majumdar *et al.* 2010). Moreover, this particular variant of Klotho was related to longevity and coronary disease (Arking *et al.* 2003; Arking *et al.* 2005; Invidia *et al.* 2010). Additionally, a relationship between the functional variant of the Klotho gene and HDL cholesterol, blood pressure, and stroke was documented (Arking *et al.* 2005).

The single nucleotide polymorphisms (SNP) of Klotho have been found to associated with carotid atherosclerosis measured with mean carotid artery intima-media thickness) (IMI) in patients with hypertension (Oguro *et al.* 2010).Interestingly, the Klotho gene polymorphisms are associated with longevity and cardiovascular disease (Laina *et al.* 2018). It has been observed that the functional variant of Klotho (KL-VS) is an independent risk factor for coronary artery disease (Arking *et al.* 2003). Furthermore, plasma Klotho levels negatively correlate with the presence of coronary disease and lower Klotho mRNA levels in the thoracic aorta (Corsetti *et al.* 2016). Besides, a relationship between the Klotho protein, FGF23, and mortality in cardiac diseases was confirmed. Silva and co-workers reported that Klotho and FGF 23 were associated with cardiovascular risk in the early stage of cardiovascular diseases (Solva *et al.* 2019). Higher plasma Klotho concentrations were connected with lower cardiometabolic risk (Amaro-Gahete *et al.* 2019). On the contrary, the reduction of soluble Klotho concentrations was strongly associated with a proinflammatory status with lower IL10 concentrations and higher TNFα/IL10 and CRP levels (Martin-Nunez *et al.* 2019).

In the literature, data are indicating that FGF23 levels are increased in patients with congestive heart failure (CHF) and FGF23 is independently associated with unfavorable outcomes in CHF (Jeinsen *et al.* 2019). Moreover, the relationship between soluble Klotho and the cardiometabolic risk was also documented in healthy, sedentary middle-aged adults (Amaro-Gahete *et al.* 2019). Interestingly, it has been revealed that metabolic disturbances in prediabetes and diabetes mellitus with accompanying impairment of the FGF23-Klotho axis may lead to cardiovascular dysfunction (Berezin and Berezin, 2019) resulting in enhanced cardiovascular risk in patients with diabetes (Silva *et al.* 2019).

It is accepted that FGF23 is expressed in the bone and the myocardium. Although FGF23 plays an important role in phosphate and vitamin D homeostasis, there is also a documented association between FGF 23 and outcome in patients with congestive heart failure (von Jeinsen *et al.* 2019). These authors suggested that cardiovascular dysfunction may lead to increased bone FGF 23 expression. Furthermore, influencing the heart – bone axis may have therapeutic applications in the future (Jeinsen *et al.* 2019).

It should be noticed that FGF21 by binding to two kinds of receptors, FGFR 1C and FGFR 2C, in the presence of co-receptor βKlotho acts as a metabolic regulator of glucose and lipids, improves insulin sensitivity and glucose uptake, and suppress lipogenesis, lipid oxidation (Cheng *et al.* 2016). Besides, serum FGF21 concentration changes are positively associated with atherosclerosis, coronary heart disease, myocardial ischemic cardiac hypertrophy, diabetic cardiomyopathy. Therefore, taking into account the favorable influence of FGF21 on metabolic and cardioprotective activity, it could be speculated that FGF21 may be used as pharmacotherapeutic agent in the future. There are speculations that supplementation of FGF21 might induce beneficial effects in patients with cardiovascular diseases (Cheng *et al.* 2016).

THE ANTITUMOR ACTIVITY OF KLOTHO

The results of many studies indicated that Klotho has antitumor activity in humans (Zhou *et al.* 2015; Rubinek and Wolf, 2016; Zhu *et al.* 2019). The mechanism of this ability is based on the inhibition of insulin growth factor 1 (IGF1) and fibroblast growth factor (Abramowitz *et al.* 2011). Anticancer effects of Klotho were reported in tumors of many organs including breast (Wolf *et al.* 2008), stomach (Wang *et al.* 2011), pancreas (Jiang *et al.* 2014), endometrium (Wójcik-Krowiranda *et al.* 2018), cervix (Lee *et al.* 2010), thyroid (Motylewska *et al.* 2018), and brain especially glioma (Peshes-Yeloz *et al.* 2019). The group of Peshes-Yeloz found that Klotho messenger ribonucleic acid expression predicted survival in patients with high-grade glioma (Peshes-Yeloz *et al.* 2019). Noticeably, immunohistochemical studies of Pawlikowski and colleagues. demonstrated that a decreased expression of α-Klotho is involved in thyroid carcinogenesis (Pawlikowski *et al.* 2019).

Finally, it has been shown that α Klotho is increased in active acromegaly and normalizes after treatment. (Jawiarczyk-Przybyłowska *et al.* 2016). The authors suggested that Klotho protein may be a new marker of acromegaly activity.

CONCLUSIONS

1. The Klotho protein is involved in the metabolic processes of glucose, lipids, calcium-phosphate, and vitamin D, as well as in the pathophysiology of metabolic diseases like diabetes, obesity, kidney diseases, and bone diseases.
2. The Klotho protein may participate in the pathophysiological mechanisms of neurodegenerative and cardiological diseases.
3. Metabolic, neuroprotective, cardioprotective, and antitumor activity of Klotho may indicate that Klotho and FGF system plays an important role in longevity and some aging-related disorders and may have therapeutical applications in the future.
4. The antitumor activity of Klotho protein was documented in patients with cancers of many organs.

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DISCLOSURE

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