Life Sciences 200 (2018) 56–62

Contents lists available at ScienceDirect

Life Sciences

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

Lentivirus-mediated klotho up-regulation improves aging-related memory deficits and oxidative stress in senescence-accelerated mouse prone-8 mice

Hong-Jing Zhou, Chen-Ye Zeng, Ting-Ting Yang, Fang-Yi Long, Xi Kuang, Jun-Rong Du*

Department of Pharmacology, Key Laboratory of Drug Targeting and Drug Delivery Systems, West China School of Pharmacy, Sichuan University, Chengdu 610041, China

ARTICLE INFO

Keywords:
Klotho
Aging
Lentivirus
Memory deficits
Oxidative stress
FoxO1 signaling

ABSTRACT

Aims: Oxidative stress caused by aging aggravates neuropathological changes and cognitive deficits. Klotho, an anti-aging protein, shows an anti-oxidative effect. The aims of the present study were to determine the potential therapeutic effect of klotho in aging-related neuropathological changes and memory impairments in senescence-accelerated mouse prone-8 (SAMP8) mice, and identify the potential mechanism of these neuroprotective effects.
Materials and methods: A lentivirus was used to deliver and sustain the expression of klotho. The lentiviral vectors were injected into the bilateral lateral ventricles of 7-month-old SAMP8 mice or age-matched SAMR1 mice. Three months later, the Y-maze alternation task and passive avoidance task were used to assess the memory deficits of the mice. In situ hybridization, immunohistochemistry, immunofluorescence, Nissl staining, quantitative real-time PCR and Western blot assays were applied in the following research.
Key findings: Our results showed that 3 months after injection of the lentiviral vectors encoding the full-length klotho gene, the expression of klotho in the brain was significantly increased in 10-month-old SAMP8 mice. This treatment reduced memory deficits, neuronal loss, synaptic damage and 4-HNE levels but increased mitochondrial manganese-superoxide dismutase (Mn-SOD) and catalase (CAT) expression. Moreover, the up-regulation of klotho expression decreased Akt and Forkhead box class O1 (FoxO1) phosphorylation.
Significance: The present study provides a novel approach for klotho gene therapy and demonstrates that direct up-regulation of klotho in the brain might improve aging-related memory impairments and decrease oxidative stress. The underlying mechanism of this effect likely involves the inhibition of the Akt/FoxO1 pathway.

1. Introduction

Aging is a major risk factor for most chronic diseases such as vascular disease [1], heart disease [2], diabetes [3], cancer [4], pulmonary obstructive diseases [5] and neurodegenerative diseases [6]. Oxidative stress is defined as an imbalance between oxidants and antioxidants, which can cause tissue damage, and the brain is one of the most sensitive organs to oxidative stress. Cognitive impairment is closely related to high levels of oxidative stress [7]. Studies have consistently revealed that Forkhead box class O (FoxO) transcription factors, which might regulate the expression of the key antioxidant enzymes manganese-superoxide dismutase (Mn-SOD) and catalase (CAT) [8], are important determinants in aging and longevity [8].

In 1997, Kuro-o and colleagues discovered a new gene named klotho, which showed an excellent effect in regulating aging [9]. Since the discovery of the klotho gene, researchers have identified many types of klotho proteins, including α-klotho, β-klotho and klotho-related protein (Klrp) [10], among them, α-klotho and β-klotho are the most intensively studied. Although these two forms of klotho proteins have high homology, their distribution and function are very different. β-Klotho is mainly distributed in the cell membrane of liver cells and adipocytes and might participate in metabolic regulation, glucose uptake, bile acid synthesis and fatty acid metabolism which are independent of α-Klotho [11]. However, α-Klotho is mainly expressed in the choroid plexus in the brain and distal convoluted tubules in the kidney. The absence of α-klotho in mice leads to a tremendously shortened lifespan and causes a series of pathologic changes related to aging [9], whereas the overexpression of this gene markedly extends the lifespan of mice [12,13]. There is an increasing evidence of the possible neuroprotective effect of α-klotho and its potent role in improving cognitive function [14,15]. Therefore, the present study focused on the α-klotho.

Markedly decreased expression of klotho is observed in the brains of old rhesus monkeys, mice and rats compared with the levels in young animals [16]. Additionally, the klotho levels in the cerebrospinal fluid (CSF) are also decreased in older normal adults [17]. The sequence of

* Corresponding author.
E-mail address: dujunrong@scu.edu.cn (J.-R. Du).

https://doi.org/10.1016/j.lfs.2018.03.027
Received 11 December 2017; Received in revised form 2 March 2018; Accepted 12 March 2018
Available online 13 March 2018
0024-3205/ © 2018 Elsevier Inc. All rights reserved.

the klotho gene includes five exons and four introns. Due to alternative splicing of the third exon, the klotho gene has two transcripts, one encoding a transmembrane form (m-KL) and the other encoding a secreted form (s-KL, detected in mice and humans CSF) [15]. The m-KL which is mainly expressed in the choroid plexus in the brain and distal convoluted tubules in the kidney [13,18], may be cleaved into an extracellular form (c-KL) and intracellular form by α-secretases (ADAM10 and ADAM17) [19]. For a long time, most of the studies have focused on the function of the m-KL, but in recent years, increasing numbers of researchers have paid attention to the s-KL and c-KL. Many studies have provided evidence that c-KL exerts a neuroprotective effect in vitro [20]. Furthermore, researchers have confirmed that s-KL, which is present at a much higher level in the brain than m-KL, may protect against age-dependent memory deficits in mice [21].

Lentivirus-based vectors, which are derived from human immunodeficiency virus type 1, are effective tools for delivering genetic materials. These types of vectors are safe and have low toxicity, high stability and cell-type specificity [22], so they are widely used in gene therapy. In the present study, we used a lentivirus to increase the expression of klotho in SAMP8 mice brains. Our aims were to determine whether the lentivirus-mediated up-regulation of klotho expression in SAMP8 mice would improves aging related memory deficits and oxidative stress and to identify the potential mechanism of these neuroprotective effects.

2. Materials and methods

2.1. Materials

HEK293 cells were obtained from the American Type Culture Collection (Manassas, VA, USA). The Lenti ORF clone of KL (mGFP-tagged) - Mouse KLotho (encoding the transmembrane full-length form of klotho) was obtained from OriGene Technologies (Rockville, MD, USA) and the vector was obtained from BGI (Shenzhen, Guangdong, China). A Lipofectamine® 3000 Transfection Kit was purchased from Invitrogen (Carlsbad, CA, USA). GM easy™ lentiviral mix and lentiviral enrichment reagents were purchased from Genomeditech (Shanghai, China). High-glucose Dulbecco's Modified Eagle's medium (DMEM) and foetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY, USA). A mouse klotho mRNA in situ hybridization (ISH) kit was purchased from Boster Biological Technology (Wuhan, Hubei, China). All the primer pairs (Table 1) were obtained from BGI (Shenzhen, Guangdong, China). The primary antibodies used in this study, including klotho, NeuN, synaptophysin, 4-HNE, Akt, p-Akt (Ser473), FoxO1 and p-FoxO1 (Ser256), are summarized in Table 2.

2.2. Animals

Male 7-month-old SAMP8 and SAMR1 mice were purchased from the First Teaching Hospital of Tianjin University of Traditional Chinese Medicine (Tianjing, China). The animals were housed under fixed conditions of temperature (24 ± 1 °C) and humidity with a 12-hour light/dark schedule. Animals were given free access to food and water. The procedures were approved by the Animal Research Committee of the West China School of Pharmacy. All the animal studies were conducted in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China. SAMP8 mice were randomly allocated to 2 groups, and each group contained 10 mice. An age-matched SAMR1 mouse group that also contained 10 mice was used as the normal controls.

Table 1
Nucleotide sequences of primers used for the real-time qRT-PCR experiments.

Gene	Primer sequence forward (5′-3′)	Primer sequence reverse (5′-3′)
GAPDH	AGCGAGACCCCACTAACATC	GGTTCACACCCATCACAAAC
CAT	AGCGACCAGATGAAGCAGTG	TCCGCTCTCTGTCAAAGTGTG
Mn-SOD	CAGACCTGCCTTACGACTATGG	CTCGGTGGCGTTGAGATTGTT
Klotho	GGCTTTCCTCCTTTACCTGAAAA	CACATCCCACAGATAGACATTCG

Table 2
Primary antibodies used in this study.

Antibody	Application	dilution rate	Source
Klotho	IHC	1:200	TransGenic Inc, Japan
NeuN	IF	1:200	Abcam, USA
4-HNE	IF	1:10	Abcam, USA
Synaptophysin	WB	1:2000	Santa Cruz Biotechnology, USA
Akt	WB	1:1000	Cell Signaling Technology, USA
pAkt (Ser473)	WB	1:2000	Cell Signaling Technology, USA
FoxO1	WB	1:1000	Cell Signaling Technology, USA
pFoxO1 (Ser256)	WB	1:1000	Cell Signaling Technology, USA

IHC: immunohistochemistry. WB: Western blot. IF: immunofluorescence.

2.3. Lentivirus production

HEK293 cells were used to produce the lentivirus. In brief, when cells reached 90–100% confluence, the medium was replaced with fresh culture medium. Lipofectamine 3000, GM easy™ lentiviral mix and the Lenti ORF clone of KL (mGFP-tagged) - Mouse KLotho or vectors were mixed and added to the culture dishes. After 72 h, the culture medium was collected and filtered through a 0.45-mm-pore-size filter, and then lentiviral enrichment reagents were added to the medium. After overnight storage at 4 °C, the mixture was centrifuged at 4000 × *g* for 25 min at 4 °C. The lentivirus was then resuspended with sterile phosphate-buffered saline (PBS) and stored at −80 °C. The lentiviral titers were determined by transduction into HEK293 cells [22].

2.4. Lentivirus injection

At the age of 7 months, mice were anaesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally), and then lentivirus encoding the mouse klotho gene (LV-KL) or GFP (LV-GFP) (3 μl per side, 2.15×10^7 TU/ml, at a rate of 0.5 μl/min) was injected into the bilateral lateral ventricles (stereotaxic coordinates: AP, −0.2 mm; ML, ± 1.0 mm; DV, −2.5 mm). The skull was closed with bone wax, and the scalp was sutured and disinfected. The lentivirus injection was performed only once for each mouse during the experiment.

2.5. Behavioural experiments

2.5.1. Y-maze alternation task

Three months later, the Y-maze alternation task was performed. In brief, each mouse was placed at the end of an arm and allowed to move freely during a 5-minute session, and the sequence of arm entries was recorded. The number of overlapping entries into the three individual arms (e.g. ABC, BCA, and CAB) was counted as the number of alternations. The alternation measure was calculated as a percentage: percent alternation = (number of alternations) / (total number of arm entries − 2) × 100%.

2.5.2. Passive avoidance task

The passive avoidance task was performed 24 h after the Y-maze alternation task. In brief, each mouse was placed on a rubber cylindrical platform for the training test. During the test, when the mouse stepped down from the platform and placed all of its paws on the grid floor, a continuous electric shock was delivered. The time for the mouse to jump down from the platform for the first time was recorded as the “latency time”, and the number of times that the mouse stepped down from the platform in 5 min was recorded as the “error number”. A

Download English Version:

https://daneshyari.com/en/article/8535136

Download Persian Version:

https://daneshyari.com/article/8535136

Daneshyari.com