

Salidroside Could Delays Aging by Regulating the Expression of TERT through Wnt/ β -Catenin Pathway

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Abstract This paper attempted to investigate whether salidroside could delay brain hippocampal neuron senescence in mice. **Methods** The C57/BL6N mice were normally fed to natural aging and then treated with salidroside. The Morris water maze (MWM) test was used to evaluate the behavioral function of mice. HE staining were used to observe neuron damage in the hippocampal tissues of mice. The expression levels of telomerase reverse transcriptase (TERT) were detected by Western blotting. **Results** The results showed that salidroside could improved the cognition of aging mice. Additionally, salidroside improved the pathological changes of hippocampal neurons of aging mice. Western blotting manifested that salidroside could promote the protein expressions of TERT by activating Wnt/ β -catenin pathway. **Conclusion** It is suggested that salidroside could delay aging of the hippocampal neurons by up-regulating TERT expression through activating Wnt/ β -catenin pathway.

Keywords: salidroside, aging, telomerase reverse transcriptase, Wnt/ β -catenin pathway

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1. Introduction

Aging is a natural biological process leading to a progressive decline of physiological functions and a series of aging-related diseases [1]. The central nervous system is significantly affected by aging, and brain aging is the primary risk factor for developing neurodegenerative diseases which trends to raise year by year [2]. Previous study has shown that telomere attrition is an important factor in accelerating aging [3]. When telomere is shortened to a limit length, DNA damage response trigger, cell cycle arrest and cell senescence or apoptosis [4]. Telomere length is mostly maintained by telomerase which is composed of telomerase reverse transcriptase (TERT) and telomerase RNA component (TERC) [5]. As a reverse transcriptase catalytic subunit, TERT is regarded as the key structure and main regulatory subunit of telomerase and only consist in telomerase-positive cells. However, recent studies have shown that telomerase activity can be activated and up-regulated by internal and external environmental factors [6]. Telomerase-deficient mice showed neuronal loss in the hippocampal region and frontal cortex associated with short-term memory deficits [7] and anxiety behaviors [8]. Telomerase reactivation in adult mice can delay age-related decline in cognitive performance [9], indicating that telomerase is required for normal brain function.

Telomerase expression is principally regulated by

TERT at the transcriptional and translational level. Up-regulation or phosphorylation of TERT can activated telomerase activity. Wnt protein is a secreted glycoprotein, β -catenin is a key mediator in the Wnt signaling pathway and participates in various cellular processes consisting of cell adhesion, growth, differentiation, and transcription including Wnt responsive genes [10]. TERT plays a crucial role in the Wnt/ β -catenin signaling pathway as an auxiliary factor in the β -catenin transcription complex to regulate directly Wnt/ β -catenin signaling transduction. This research indicated that the protein component of telomerase, TERT, could interact to Brahma-related gene 1 (BRG1) which is an chromatin remodeling agent. As an auxiliary factor, TERT promote the transcription of β -catenin target genes and participate in the Wnt signaling pathway through the recruitment of BRG1 [11]. In addition, overexpression of TERT in aging mice resulted in activation of the Wnt/ β -catenin pathway in several organs including the kidneys [12].

Natural bioactive compounds hold great potential for delaying senescence. Some natural molecules of herbals can play a role in delaying senescence by influencing telomerase activity [13,14,15]. Salidroside is widely used in traditional folk medicine in Asian countries. Modern pharmacological studies have revealed that salidroside has various effects such as anti-fatigue, anti-depression, anti-inflammatory, anti-oxidant, anti-tumor, neuroprotective

and cardioprotective effects [16,17,18,19,20]. Salidroside is generally recognized as a safe and effective substance, and has no obvious adverse effects in pre-clinical and clinical trials, which makes it a promising clinical drug [21]. Recent researches have shown that salidroside plays a role in the treatment of age-related diseases, especially in neurodegenerative diseases [22]. However, And the effects of salidroside on telomerase activity have not been yet reported and the specific mechanism and target of action of salidroside are not very clear. Therefore, this experiment attempted to investigate whether salidroside may act on telomerase through the Wnt/ β -catenin pathway to delay aging.

2. Materials and Methods

2.1. Animals and Treatments

Total of fifty male C57/BL6N mice (8-week-old) were purchased from Weitong Lihua Experimental Animal Technology (Beijing, China, SCXK: 2016-0011). All animals were maintained in a controlled environment at standard temperature and relative humidity with free access to food and water. All animal experiments were approved by the Ethics Committee of Qingdao University Medical College (QDDX-20241130) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals was used as a guide for the design of all animal-related studies.

The mice were randomly divided into five groups ($n = 10$), including young group, old group, salidroside-low dose (Sal-L) group (25mg/kg), salidroside-high dose (Sal-H) group (50mg/kg), cycloastragenol (CAG) group (20mg/kg). Mice were raised until 12 months of age as naturally aged mice. The mice in old group were gavaged with normal saline at an equal volume once a day for 8 weeks. Sal group was administered Sal (purity $\geq 98\%$, Aladdin, Shanghai, China) at 25 or 50 mg/kg via gavage once daily for 8 weeks. CAG group received 20mg/kg CAG by gavage daily for 8 weeks. 8-week-old mice were used as the young group and gavaged with normal saline at an equal volume for 8 weeks.

2.2. Behavioral Test

The behavioral tests were performed after the treatment. The Morris water maze (MWM) test was performed to examine the learning and memory of the mice that consisted of a circular pool (150 cm in diameter, 50 cm in height). The pool was filled with water (22 ± 1 °C at a depth of 30cm and divided into four quadrants. A hidden platform (10 cm in diameter) submerged 2cm below the surface of water was placed at the midpoint of one quadrant. all mice were given a training test for 5 consecutive days and a probe trial on day 6. For the training test, mice were given 60 s to reach the hidden platform and allowed to remain on the platform for 30 s, repeating once for each quadrant per day. The escape latency time was recorded. The platform was removed on day 6 for the probe trial, and mice were placed into the pool from the diagonal quadrant of the target quadrant,

with their heads facing the wall. Each mouse was allowed to explore for 60 s. Swimming trajectory, the time spent in target quadrant and the number of crossing the original platform were recorded and analyzed by the video tracking analysis system.

2.3. He Staining

After designated treatment and behavioral test, five mice in each group were deeply anaesthetised by 10% chloride (300 mg/kg) and reperfed with 20 mL of 4% formaldehyde from the heart into the aorta. Then, the brains were removed and dehydrated by the conventional alcohol gradient, embedded in paraffin, and cut into 7 μ m thick coronal slices. The hippocampal sections were dewaxed and rehydrated, and stain by HE staining kit (Solarbio, China) according to the manufacturer's instructions. Normal neurons have relatively big cell bodies with round nuclei, while damaged neurons exhibited shrunken cell bodies with many empty vesicles and condensed nuclei. Images from hippocampal CA1 region (at 400 magnification) were captured by an inverted microscope to obtain the images.

2.4. Western Blot Analysis

After the behavioral tests, the mice were sacrificed and the hippocampus was isolated for further studies. After designated treatment and behavioral test, five mice in each group were deeply anaesthetised and the hippocampus tissue 50 mg was isolated into EP tube, and then lysed by RIPA lysis buffer (Beyotime, China) and centrifuged at $12,000 \times g$ for 5 min to collect the supernatant. Subsequently, BCA protein assay kit (Beyotime, China) was performed to determine total protein concentrations following the manufacturer's instructions. The sample loading buffer was then added and heated to 95°C for 5 min. Samples containing 30-40 μ g total protein were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electro-transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Middlesex County, MA, USA). The membranes were blocked with 5% skim milk in Tris-buffered saline Tween-20 (TBST) at room temperature for 2 h, and then washed in TBST for three times (5 min each). After incubating at 4°C overnight with the primary antibodies (Wnt 1:1,000, β -Catenin 1:1,000, Cell Signaling Technology, USA), (TERT 1:2,000, NB100-317, Novus, UK), (β -actin 1:10,000 AF7018, Affinity, USA), the membranes were washed and incubated with the HRP-goat anti-rabbit IgG 1:15,000, S0001, Affinity, USA) at room temperature for 1.5 h. Protein signals were detected using enhanced chemiluminescence reagents (Millipore, Middlesex County, MA, USA). Images were visualized by BioSpectrum 810 imaging system (UVP, USA) and analyzed by Image J software. β -actin levels served as internal loading controls.

2.5. Statistical Analysis

All statistical analyses were analyzed by GraphPad Prism 8. Values were expressed as mean $\pm$ standard

deviation. One-way analysis of variance was used to assess multiple groups of data and Student's t-test was used to compare between two groups. $P \leq 0.05$ was considered statistically significant. All experiments were performed at least three times for quantitative comparison.

3. Results

3.1. Salidroside Improved Learning and Memory Impairments of Aging Mice

Morris water maze experiment was used to evaluate the effect of salidroside on spatial learning and memory in mice (Figure 1A). The mean escape latency was decreased after five training days among all the groups (Figure 1B). The aging mice showed significantly higher escape latency than the young mice ($P < 0.05$), whereas the treatment with Sal and CAG effectively reduced this latency ($P < 0.05$). The time spent in the target quadrant after training revealed the degree of memory consolidation. Mice in Sal groups and CAG groups spent more time on the target quadrant compared with the aging group ($P < 0.05$). In summary, aging mice had significant impairment in spatial learning and memory ability comparison with the aging mice, whereas treatment with Sal can improve the impairment.

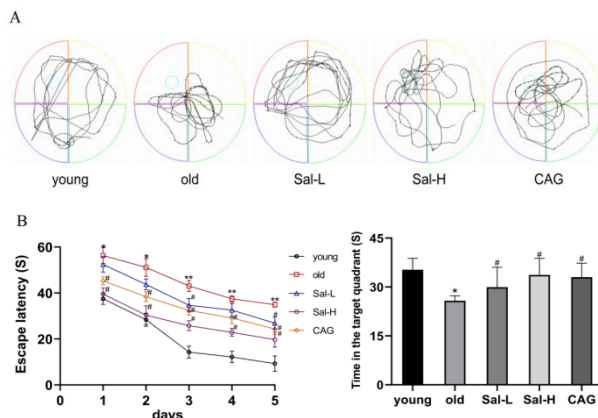


Figure 1. Salidroside improved learning and memory impairments of aging mice. (A) Representative searching strategy of mice on day 6 for the probe trial. (B) Left: escape latency to find the hidden platform in the test during the five consecutive days training. Right: the time of the mice spent in the quadrant where the platform was placed. * $P < 0.05$, ** $P < 0.01$ vs young group; # $P < 0.05$ vs old group

3.2. Salidroside Improved the Pathological Changes of Neurons in the Hippocampal CA1 Region of Aging Mice

The results of HE staining showed that neurons in the hippocampal CA1 area of young mice had a clear morphology and structure with normal morphology, round nucleus and clear nucleolus (Figure 2). In the old group, the number of neurons decreased, the cell bodies shrank with pyknotic nuclei. After the treatment of Sal and CAG, the volume of parts of neurons increased, the nuclear shape was regular, and the distribution of chromatin was uniform. These indicates that Sal can improve the morphology of aging brain tissue in mice.

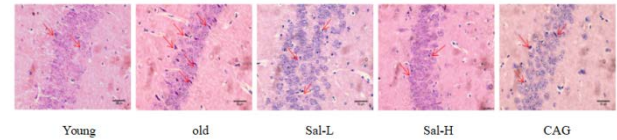


Figure 2. Effects of Salidroside on morphology in hippocampal CA1 in naturally aging mice ($\times 400$). Salidroside can improve the morphology of aging brain tissue in mice

3.3. Salidroside Increased Protein Expression of TERT Via Wnt/ β -catenin Pathway

We then investigated the effects of Sal on the expression of TERT protein in hippocampus of aging mice. Compared with the young mice, the expression of TERT protein in the aging mice were significantly decreased ($P < 0.05$), while that were significantly up-regulated after the treatment with Sal and CAG (Figure 3A) ($P < 0.05$). The results showed that compared with the aging group, Wnt expression levels were markedly increased ($P < 0.05$) in Sal groups. β -Catenin, a major downstream target of Wnt, was also upregulated ($P < 0.05$) (Figure 3B). These results suggested that salidroside can up-regulate the expression of TERT via the Wnt/ β -catenin pathway.

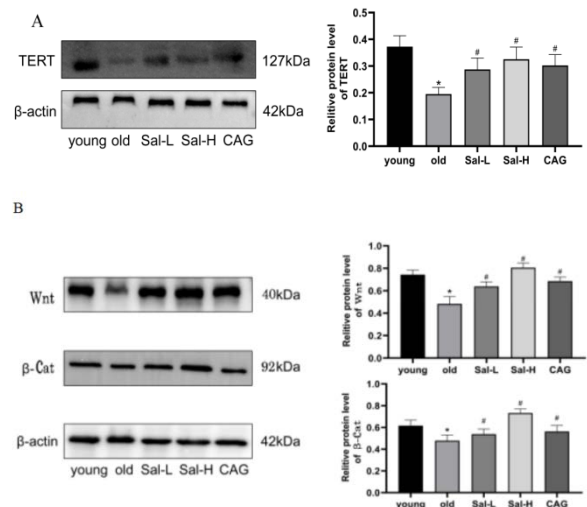


Figure 3. Salidroside increased protein expression of TERT in hippocampus of aging mice via Wnt/ β -catenin pathway. (A) The effect of salidroside on TERT protein expressions in hippocampus of aging mice. β -actin was considered as loading controls. (B) The original bands of Wnt, β -catenin and β -actin. The ratio of different proteins to β -actin was calculated by the band density. * $P < 0.05$ vs young group; # $P < 0.05$ vs old group

4. Discussion

Aging is accompanied by cognitive decline in the brain, and the hippocampus seems to be particularly vulnerable to age in humans and animals [23]. Age-related memory damage results from changes in hippocampal connectivity and plasticity that are expressed in different ways of hippocampal areas known as cornu ammonis area 1 (CA1), cornu ammonis area 3 (CA3) and the dentate gyrus (DG),

such as the CA1 area is particularly susceptible to Alzheimer's disease. In this study, we focused on the effect of salidroside on the aging of brain hippocampal cells. We found that salidroside can improve the learning and cognitive ability of the aging mice. Moreover, salidroside alleviated neuronal damage in the hippocampus CA1 region of aging mice to a certain extent.

Telomerase is a specialized reverse transcriptase, which can add TTAGGG repeats onto the chromosome ends to compensate telomere attrition and prevent cellular replication senescence, that makes the activation of telomerase an effective way to delay senescence. Telomerase is composed of TERC and TERT which is regarded as the key structure and main regulatory subunit of telomerase and only consist in telomerase-positive cells [24]. TERT expression is regulated by a variety of transcription factors. Therefore, we first investigated whether the anti-aging ability of salidroside was related to increase in TERT expression. Western blot analysis also showed that salidroside could up-regulate the expression of TERT.

Wnt/ β -catenin pathway could directly activate TERT transcription and telomerase activity in normal stem cells. Recent research indicated that β -catenin could directly bind to the promoter region of TERT and recruit the lysine methyltransferase Setd1a into the promoter region, therefore, Setd1a catalyzes the trimethylation of histone H3K4 at the promoter site so that to mediate TERT transcription and regulate telomerase activity [25,26]. Therefore, TERT is a direct transcriptional target of the Wnt/ β -catenin signaling pathway. β -catenin plays a key role in TERT expression and telomerase regulation. Compared to wild-type mice, the embryonic stem cells of β -catenin deficient mice exhibit lower telomerase activity, while higher levels of telomerase were detected in the embryonic stem cells of expressing activated β -catenin [27]. In addition, the activated TERT Wnt/ β -catenin axis could stimulate epithelial mesenchymal transition (EMT) in stem cells [28]. The above data revealed the link between stem cell proliferation, TERT, and Wnt pathways, and activated TERT-Wnt/ β -catenin axis may increase the proliferation of normal stem cells.

In conclusion, the results showed that salidroside delayed the aging of hippocampal cells and could also active the Wnt/ β -catenin signaling pathway. It is at least assumed that salidroside is associated with increased telomerase activity with up-regulation of TERT expressions.

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Ethics Approval and Consent to Participate

All related experiments were approved by the Ethics Committee of Qingdao University Medical College (QDDX-20241130)

Conflicts of Interest Statement

The authors declare that there are no conflict of interest.

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