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Research report

mTOR and autophagy in normal brain aging and caloric restriction ameliorating age-related cognition deficits

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HIGHLIGHTS

- Hippocampal autophagic deficits contribute to age-related cognitive disorder in mice.
- CR activated hippocampal autophagy and ameliorated age-related cognition deficits.
- The activity of mTOR and its upstream signaling in hippocampus declines with aging.

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ABSTRACT

Defect of autophagy is common to many neurodegenerative disorders because it serves as a major degradation pathway for the clearance of various aggregate-prone proteins. Mammalian target of rapamycin (mTOR) signaling, which is recognized as the most important negative regulator of autophagy, is also involved in neurodegenerative diseases. However, the role of mTOR and its dependent autophagy in normal brain during aging remains unknown. Furthermore, caloric restriction (CR) is frequently used as a tool to study mechanisms behind aging and age-associated diseases because CR can prevent age-related diseases and prolong lifespan in several model organisms. Inhibiting mTOR and promoting autophagy activity play roles in aging delayed by CR. However, whether CR can ameliorate age-related cognition deficits by inhibiting mTOR and activate autophagy in hippocampus needs to be further investigated. Here we showed a decline of autophagic degradation in mice hippocampus in correlation with age-dependent cognitive dysfunction, whereas the activity of mTOR and its upstream brain-derived neurotrophic factor (BDNF)/phosphatidylinositide 3-kinase (PI3K)/protein kinase B (Akt) signaling was decreased with aging. In addition, facilitating the mTOR pathway successfully declines and sustains autophagic degradation with aging in hippocampus by CR treatment and is involved in CR by ameliorating age-related cognitive deficits.

1. Introduction

Treating cognitive problems common in the elderly requires a deeper understanding of how a healthy brain ages [1]. Even in healthy individuals, brain aging is associated with structural and functional changes that invariably lead to cognitive function deficits, as well as changes that increase the brain's susceptibility to neurodegenerative disorders. Therefore, understanding the mechanisms of age-related cognition deficits and thus improving health is critical to increasing the quality of life in the elderly population, especially given the dramatic increase of global aging.

Aging represents one of the main risk factors for neurodegenerative diseases, including Alzheimer's disease (AD), Huntington's

Abbreviations: Akt, protein kinase B; AL, ad labitum; BDNF, brain-derived neurotrophic factor; CR, caloric restriction; LC3, microtubule-associated protein 1 light chain 3; mTOR, mammalian target of rapamycin; MWM, Morris water maze; OFT, open field test; PI3K, phosphatidylinositide 3-kinase; S6K1, ribosomal protein S6 kinase 1; 4E-BP1, initiation factor 4E-binding protein 1.

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disease (HD), and Parkinson's disease (PD). A key pathological hallmark shared by many of them is the accumulation of toxic protein aggregates [2]. As post mitotic cells, neurons have highly dynamic cellular processes for their proper functions, such as cell growth, synaptic formation, or synaptic plasticity by regulating protein synthesis and degradation. Long-lived or misfolded proteins and damaged organelles need to be periodically degraded because they can incur damage (e.g., oxidative changes) that renders them nonfunctional or toxic to the cell. The inability of neurons to clear mutant and/or misfolded proteins leads to their aggregation and to the cellular damage that ultimately causes cell death [3].

Macroautophagy (hereafter referred to as *autophagy*), literally means "self-eating." It is a highly regulated and evolutionarily conserved catabolic process that serves to degrade these damaged cytosolic components upon the fusion with lysosome and recycle their biochemical components for use in energy production and other biosynthetic reactions, which contribute to protein quality control and cell homeostasis [4]. Autophagy has recently been highlighted because it can be stimulated by multiple types of cellular stressors, including starvation, hypoxia, or endoplasmic reticulum stress [5]. Many lines of evidence now suggest that defects in the activation of autophagy are common to many neurodegenerative disorders [6–8].

Over the last decade, mTOR pathway, which is regulated by BDNF via the PI3K/Akt pathway, has been recognized as the major negative regulator of autophagy. Its implications for neurodegenerative diseases have been intensively investigated [9,10]. mTOR, a serine/threonine kinase, signals in at least two distinct multiprotein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), distinguished by their partner proteins and differing sensitivities to rapamycin. mTORC1 is rapamycin-sensitive and thought to mediate protein homeostasis by facilitating protein translation and inhibiting autophagy through ribosomal protein S6 kinase 1 (S6K1) and initiation factor 4E-binding protein 1 (4E-BP1) [11]. In many neurodegenerative diseases, such as AD and PD, overactivated mTOR is observed [9,10]. In mice, reduced mTOR signaling by chronic rapamycin treatment extends life span and can effectively treat age-related neurodegenerative diseases [12]. In this context, we may assume that it is aging that produces the overactivation of mTOR and its dependent inhibition of autophagy; consequently, it is responsible for the onset of age-related neurodegenerative diseases. However, other data show that mTOR activation is indispensable for dendritic morphogenesis, synaptic plasticity, and the consolidation of long-term memories [13,14]. Outwardly, these are contradictory results. Therefore, the role of mTOR and its dependent autophagy in the healthy brain during aging do need to be researched.

Furthermore, overwhelming evidence shows that CR prevents age-related diseases and increases lifespan in several model organisms ranging from yeast to mice and even primates. Consequently, CR is frequently used as a tool to understand the mechanisms behind aging and age-associated diseases [5,15,16]. Numerous studies indicate that deactivating mTOR signaling and activating autophagy play an important role in CR increasing lifespan and preventing age-related diseases [17–19]. Some studies have indicated that CR can prevent age-related cognitive dysfunction [20,21], but whether CR plays a role in ameliorating age-related cognition deficit by regulating mTOR and autophagy remains unknown.

Here we found that inhibiting mTOR signaling and activating autophagy by CR responded to CR ameliorating age-related cognition deficits. Furthermore, autophagy activity declined in hippocampus of mice with aging, and this was not induced by the negative regulation of mTOR pathway because its activity also decreased with aging. Our results provide the first evidence that the inhibitory role of mTOR signaling is not indispensable for autophagy regulation during normal brain aging.

2. Materials and methods

2.1. Animals and caloric restriction

A total of 75 C57BL/6J male mice were acquired from the Beijing HFK Bioscience Co., Ltd. (Beijing, China). Five groups of mice were included in the present study: AL mice at 3 months and AL and CR mice at 12 (middle-aged) and 20 months (old), respectively ($n = 15$/group). All animals were arranged to behavioral testing before they were sacrificed at the assigned age. Mice were sacrificed between 9:00 a.m. and 11:00 a.m. Fifty-five ($n = 11$/group) mice were used for Western blot analyses of different target proteins and 20 ($n = 4$/group) mice were used for immunohistochemistry (IHC) staining. All mice were housed individually on a 12-h light-dark cycle in the animal facility of Tianjin medical university. AL mice were AL fed (AIN-93G diet) lifelong.

Three-month-old CR mice were initiated by incremental reduction of 10% food intake per week over a 3-week period until CR mice received 70% of food intake compared to the age-matched AL mice. A vitamin and mineral fortified version of the AIN-93G diet was given to CR mice. Food consumption by AL mice was monitored weekly and throughout the study. The CR mice were fed daily, at approximately 70% of the average amount of daily food consumed by age-matched AL animals during the preceding week.

2.2. Behavioral testing

A standardized Morris water maze (MWM) procedure was used to evaluate hippocampus-dependent spatial learning and memory of the mice [22]. The equipment of the MWM consisted of a 90 cm-diameter circular tank that was filled with water to the depth of 45 cm and maintained at $23 \pm 1\,^{\circ}C$ by an automatic heater. Milk was added to make the water opaque. The tank was divided into four equal quadrants (1, 2, 3, and 4) by two imaginary perpendicular lines crossing in the center of the tank. A movable white circular platform (5 cm in diameter) was located in the center of quadrant 3 (the target quadrant) and submerged 1–2 cm below the water surface. A camera located above the center of the maze and a computerized animal tracking system was used to monitor and relay images. The environment was kept dark and noiseless, maintaining visual extra-maze clue and furnishment immobile and minimizing noise disturbance. Mice were given 4 training trials per day for 5 days and were trained to locate the hidden platform. They were randomly placed into water facing the pool wall individually from the preset starting points. The starting location varied among four equidistant points around the perimeter of the apparatus. Subsequent starting points proceeded in a clockwise manner for the ensuing trials. Each trial was terminated after 60 s or when a mouse found the hidden platform. Mice that failed to locate the platform within 60 s were guided manually to the platform and remained for at least 5 s. Sixty-second probe trails were conducted 24 h after the lasting trail, during which the platform was moved away. Mice were released into water individually from the starting point that was opposite the target quadrant. Performance parameters in MWM determined included latency to the platform, target quadrant dwell time, and times of crossing.

Open field test (OFT) was used to assess anxiety-like behavior [23]. The apparatus consisted of a square arena (100 cm long × 100 cm wide × 50 cm high) with a specified inner area. The floor was divided into 25 equal squares by black-colored grids. In brief, each mouse was placed in one center of the inner area and allowed to explore for 5 min. Locomotor activity was recorded and measured by the number of line crossings and rearings over a 5-min period. A well-trained observer who was blind to the treatments