Experimental Gerontology 46 (2011) 533–541

Contents lists available at ScienceDirect

Experimental Gerontology

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

Age-related autophagy alterations in the brain of senescence accelerated mouse prone 8 (SAMP8) mice

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ARTICLE INFO

Article history:
Received 9 November 2010
Received in revised form 9 February 2011
Accepted 9 February 2011
Available online 6 March 2011

Section Editor: Christian Humpel

Keywords:
Autophagy
Aging
SAMP8 mouse
LC3
Beclin 1

ABSTRACT

Autophagy is responsible for the degradation of long-lived proteins and damaged organelles intracellular, even extracellular,and autophagy is proved to have relationship with Alzheimer's disease (AD) and aging. The senescence accelerated mouse prone 8 (SAMP8) was a non-genetically modified mice widely used as a rodent model of aging and senile dementia. However, little was known about the age-related changes of autophagy in the brain of SAMP8 mice. To better understand the precise relationship between aging, autophagy and neurodegeneration, we explored the time course of cognitive ability, ubiquitin-positive inclusions, ultrastructure of neurons and detected the expression of LC3 and Beclin 1 protein in different brain regions of 2, 7 and 12-month-old SAMP8 and SAMR1 mice. We found that 7 and 12-month-old SAMP8 mice presented cognitive decline and ubiquitinated proteins enhanced. In the hippocampal neurons of 12-month-old SAMP8 mice, lots of dense clumps and autophagic vacuoles were found in the cytoplasm and axons. The LC3-II expression showed an increase in hippocampus and cortex of 7 and 12-month-old SAMP8 mice. The expression of Beclin 1 displayed a significant increase in 7 months old and a decline in 12 months old mice. Based on these data, we suggest that the autophagic activity maybe increase reactively at the beginning of AD and then showed a decline with aging, and the pathological changes of 12-month-old SAMP8 mice are more similar to the late-onset AD in the perspective of autophagy.

1. Introduction

Many neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease(PD) and Huntington's disease, are characterized by the formation of intracellular protein aggregates in affected brain regions, and these protein deposits are composed of ubiquitin conjugates, suggesting a failure in the protein degradating system (McCray and Taylor, 2008). The autophagy–lysosome pathway and ubiquitin–proteasomal system (UPS) are the major systems responsible for the digestion of most cytosolic and aggregated or misfolded proteins in the cells of brain (Shintani and Klionsky, 2004). The UPS selectively degradate the short-lived misfolded proteins limited in cells (Ciechanover, 2005). In contrast, the autophagy is less selective and contributes to the degradation of long-lived proteins and damaged organelles (such as mitochondria) intracellular and even extracellular (Levine and Klionsky, 2004). Therefore, autophagy is fundamental for neuronal homeostasis through removing aged and prone aggregated potentially damaged proteins and providing macromolecules for further synthesis. The function of autophagy declines with aging and could determine cell and individual lifespan (Ettore, 2006). The lifespan of older flies could be extended by 56% when autophagy was induced in neurons, and when reducing autophagy, the lifespan could be shortened and neurodegeneration also be observed (Simonsen et al., 2008; Juhász et al., 2007).

Autophagy has several steps in the process. The formation of double membrane structures around the degradative substrates is the first step (termed autophagosomes). Then, the autophagosome fuses with lysosomes (termed autophagolysosomes) and the contents are degraded by hydrolytic enzymes inside. Finally, the degradated products are reused in the cytosol. The autophagosome and autophagolysosome are both named autophagic vacuoles (AVs), considered as the prominent morphological manifestation of autophagy (Levine and Kroemer, 2008). Microtubule-associated-protein-light-chain-3 (LC3)-II, the lipidated form of LC3, is the most widely used marker for autophagy, the only protein associating with autophagosomes specifically. LC3-II is created when autophagosome formation and is degraded as autophagosomes mature into autolysosomes. During autophagy, LC3-II on the autophagosomal membranes is delipidated to LC3-I and degraded by hydrolases in lysosome (Rubinsztein et al., 2009; Tanida et al., 2005).

Abbreviations: SAMP8, Senescence accelerated mouse prone 8; AD, Alzheimer's disease; PD, Parkinson's disease; UPS, Ubiquitin–proteasomal system; AVs, Autophagic vacuoles; LC3, Microtubule-associated-protein-light-chain-3; MCI, Mild cognitive impairment; Aβ, Amyloid-β; MWM, Morris water maze; OD, Optical density; ATG, Autophagy-related gene.

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0531-5565/$ – see front matter.
doi:10.1016/j.exger.2011.02.006

Beclin 1 is considered to be a marker of autophagic activity, because researches indicated that the levels of Beclin 1 had often been correlated with the autophagic level. Beclin 1 is reduced in the midfrontal cortex gray matter of MCI (mild cognitive impairment) and AD patients. Heterozygous deletion of Beclin 1 disrupts neuronal autophagy, which could increase Aβ deposition and promote neurodegeneration in transgenic mice (Pickford et al., 2008). Conversely, increasing Beclin 1 expression could diminish amyloid pathology in AD transgenic mice (Spencer et al., 2009).

The senescence accelerated mouse prone 8(SAMP8) is a mice strain originated from AKR/J strain of mice by Takeda et al. in the 1970s (Takeda et al., 1997), which was widely used as a rodent model of aging and senile dementia. SAMP8 mice presented not only the similar characteristics with aged human such as shorter lifespan, lordosis, reduced physical activity and hair loss (Hosokawa et al., 1984; Takeda et al., 1994), but also some neurodegenerative features, such as early onset of learning and memory deficits, altered emotions and abnormal circadian rhythm (Miyamoto, 1997), neuronal cell loss (Kawamata et al., 1997), reduction in the release of neurotransmitters in the brain (Zhao et al., 1990; Zhao et al., 1992). The SAMR1 mice do not show these senescence-related phenotypes and are used as a control strain commonly. Notably, SAMP8 mice have shown changes in protein modification, such as age-related tau hyperphosphorylation (Canudas et al., 2005) and amyloid-β (Aβ) deposition (Del valle et al., 2010), as like as pathological changes in AD patients.

By now, few reports described age-related autophagy changes in the brain of SAMP8 mice and whether these changes play a role in the aging process and cognitive decline. To better understand the precise relationship between aging, autophagy and neurodegeneration, in this study we explored learning and memory ability, ubiquitin-positive inclusion bodies, the ultrastructure of neurons and detected the expression of LC3 and Beclin 1 protein in different brain reigons of SAMP8 and SAMR1 mice in different ages.

2. Materials and methods

2.1. Animals

Male SAMP8 and SAMR1 mice in three age groups(2, 7 and 12 months, 10 mice in each age group)were used. All the mice were kept in conditions of temperature (20–22 °C) and 12-hour light-dark cycle, and they had access to the food and water *ad libitum*. All studies were performed in accordance with the guidelines established by the local animal care and use committee.

2.2. Behavioral testing

We used the Morris water maze (MWM) to assess learning ability and spatial memory. All groups were trained for swimming ability for 2 days before testing. In the first part of the test, all SAMP8 and SAMR1 mice were released in a pool to find a submerged platform (1 cm below water surface) one by one. The water in the pool contained dark ink so that the mice could not see the platform. There were geometric designs at the edge of the pool to serve as distal cues. The time they spent on finding the platform was recorded as the escape latency. About 1 min later after the mice finding the platform, they were released at another quadrant. If they did not find the platform in 120 s, they were guided gently onto it, and the escape latency was considered as 120 s. Every mouse has 4 times of swimming each day, released at the first, second, third and fourth quadrant respectively. This part of test was repeated for 4 days and the location of the platform was not changed. At the fifth day, the platform was removed and the mice were released at the first quadrant. After swimming for 120 s, they were pulled out and treated as described above. The number of times that each mouse crossed the previous platform location was considered as a measure of platform location retention. Performance in all tasks was recorded by a computer-based video tracking system (Water2020, HVS Image, UK). Data was analyzed offline by using HVS Image and processed with Microsoft Excel.

2.3. Transmission electron microscope

The mice in different age groups were anesthetized with 10% Chloral Hydrate and perfused through the left ventricle with 0.9% saline, then followed by cold perfusion fluid for electron microscope (containing 3% paraformaldehyde and 1% glutaraldehyde) for 45 min. The brains were removed from the skull, pieces of selected brain regions (cerebral cortex, hippocampus) were separated and immediately placed in 2.5% glutaraldehyde, chopped to get pieces of about 1 mm^2, and kept in 2.5% glutaraldehyde for more than 12 h at room temperature. Samples were postfixed in 1% osmium tetroxide for 1 h, dehydrated in graded increasing ethanol, and embedded in epoxy resin. Polymerization was performed at 80 °C for 24 h. Blocks were cut on a Reichert ultramicrotome into ultrathin sections (60–70 nm), which were poststained with uranyl acetate and lead citrate, and viewed under a Hitachi 7100 electron microscopy (Nikon).

2.4. Immunohistochemistry

Mice were anesthetized with 10% Chloral Hydrate and perfused with saline and then 4% paraformaldehyde through the left ventricle. The brain was removed from the skull and then postfixed for 24 h with 4% paraformaldehyde. After being progressive dehydrated, the brain was embedded in paraffins. Six-μm-thick consecutive coronal paraffin sections were collected throughout the cerebral cortex and hippocampus, according to the Mouse Brain in Stereotaxic Coordinates. Each group consisted of three mice. Briefly, sections were deparaffinized in distilled water, followed by heat-mediated antigen retrieval in 10 mM citrate buffer (pH = 6.0) and incubation with 3% H_2O_2 for 40 min at 37 °C temperature. Sections were then incubated with 10% normal goat serum for 20 min at 37 °C to block the nonspecific binding followed by incubation overnight at 4 °C with antibodies against LC3b (Abcam, USA, 1:500 dilution) and ubiquitin (Abcam, USA, 1:2000 dilution). Sections were washed in 0.1 M PBS, incubated sequentially with biotinylated goat anti-rabbit IgG and S-A/HRP-reagent, and finally rinsed with diaminobenzidine. The positive cells present a brown-yellow precipitate in the plasma. Sections were dehydrated using graded ethanol, cleared in xylene, then using Nikon 80i microscope and NIS element BR software to take photograph.

2.5. Western blot analysis

Animals were sacrificed by decapitation. The cerebral cortex and hippocampus were dissected, immediately weighed, frozen in liquid nitrogen, and stored at −80 °C. Protein extracts were prepared in the extraction buffer. The lysates were placed on ice for 30 min super-audible to shatter using ultrasound wave and centrifuged at 14,000 rpm at 4 °C for 20 min to collect the supernatants. The protein concentration was measured by using a BCA protein assay reagent according to manufacturer's instructions. Equal amount of protein (50–100 μg) from each sample was subjected to polyacrylamide gel electrophoresis, transferred to 0.22 μm PVDF membrane, and blocked in 5% skim milk for 2 h at room temperature. Samples were incubated overnight at 4 °C with primary antibodies against LC3b (Abcam, USA, 1:1000 dilution) and Beclin 1 (Cell Signaling, USA, 1:1000 dilution). Then the PVDF membranes was incubated with anti-rabbit fluorescently-labeled secondary antibody after being washed, bands were detected using an Odyssey Infrared Imaging System (LI-COR, Lincoln, NE). The usual green or red color of a band was converted to black and white colors for data presentation. The protein β-actin was used as a control. Each experiment was repeated by samples from three mice.