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Research article

Alterations of myelin morphology and oligodendrocyte development in early stage of Alzheimer's disease mouse model

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HIGHLIGHTS

- Myelin morphology was altered in hippocampus tissues of 2-month APP/PS1 mouse compared with age-matched WT control.
- Oligodendrocytes development was disordered in APP/PS1 mouse.
- Neuregulin 1 and active-caspase-6 might contribute to the alterations in 2-month APP/PS1 mouse.

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ABSTRACT

Alzheimer's disease (AD) is the most common cause to dementia and predicted to influence about 35 million people by the end of 2050. In this study, we discover alterations of myelin morphology in hippocampus tissues of 2-month-old APP/PS1 mouse. Myelin sheath is thicker and internodal distance is shorter in APP/PS1 mouse. Oligodendrocytes, differentiated from oligodendrocytes progenitor cells (OPCs), are responsible for formation and maintenance of myelin sheath in central nervous system (CNS). Our current results demonstrate that the oligodendrocytes development is disordered in 2-month-old APP/PS1 mouse. Neuregulin-1 type III, which is critical for both oligodendrocytes development and CNS myelination, is found up-regulated in hippocampus tissues of APP/PS1 mouse by western blots. Furthermore, we find active-caspase-6 can cleave neuregulin-1 type III at the cytoplasmic region. Given together, this study indicates the alterations of myelin morphology and oligodendrocytes development in 2-month-old APP/PS1 mouse, and the alterations might be highly associated with neuregulin-1 type III and active-caspase-6.

1. Introduction

Alzheimer's disease (AD), the most common cause to dementia in people aged over 65 years old, is predicted to influence about 35 million people by the end of 2050 [12]. Previous studies have demonstrated that compared with age-matched controls, white matter areas of AD patients are atrophied by using magnetic resonance imaging (MRI). White matters atrophy is also known as the other kind of pathological feature for AD besides amyloid plaques and neurofibrillary tangles [11,14,16].

In central nervous system (CNS), mature oligodendrocytes differentiated from oligodendrocytes progenitor cells (OPCs) are responsible for the formation of myelin sheath [17]. Increasing evidences have indicated the role of oligodendrocytes in the cellular phase of Alzheimer's disease [3,7]. Myelin, providing discontinuous insulation along axons, is a critical player in saltatory impulse conduction and very important for physiological function [13]. In human, myelin development starts from the very beginning of infant and will last to the second decade of life [2,8]. Given the critical role of myelin, disruption of myelin developments may lead to inefficient interneuronal communication [8].

Neuregulin-1 type III, encoded by *NRG1*, is well known for the necessary role in peripheral nervous system (PNS) myelination. Previous studies have shown that neuregulin-1 type III is non-essential, but do play an important role in CNS myelination [4,15]. Oligodendrocytes are responsible for CNS myelin formation and maintenance, and the developments of oligodendrocytes

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is tightly linked to neuregulin-1 from axons [1,5,10]. The former study has reported that plenty of hypermyelinated axons were found in the white matter of the mouse overexpressing cDNAs encoding neuregulin-1 type III, which suggests that neuregulin-1 type III might be associated with CNS myelination with unclear mechanisms [4].

The APP/PS1 mice is well known as the Alzheimer's disease mouse model and does not show behavior defects until at the age of 6 months. Myelin disruptions and demyelinations are found in 6-month-old and older APP/PS1 mouse, which is different with the phenotypes found in 2-month-old APP/PS1 mouse. Our present results indicate that the alterations of myelin morphology and oligodendrocytes developments in hippocampus tissues of 2-month-old APP/PS1 mouse are occured before occurrence of behavior defects and most of other pathological features. According to furthermore results, we propose the critical contributions of neuregulin-1 type III and active-caspase-6 to the alterations in APP/PS1 mouse.

2. Materials and methods

2.1. Mice

APP/PS1 (APPswe/PS1ΔE9) mice were purchased from Model Animal Research Center of Nanjing University and maintained in compliance with guidelines of Peking University Animal Care and Use. Genotyping was followed the methods described by Jackson Labs. All animal experiments were performed under the guidance of Peking University Animal Care and Use Committee.

2.2. Immunofluorescence

To fix the brain tissues, both WT and APP/PS1 mice were anaesthetized and perfused with 4% paraformaldehyde (PFA) diluted in phosphate buffered saline (PBS). 35 μm thick brain slides generated by routine methods were blocked with 5% albumin from bovine serum (BSA/PBS) and incubated with primary antibodies. Cells cultures were fixed with 4% PFA/PBS. After blocked with 5% BSA/PBS, cells cultures were incubated with primary antibodies. The following primary antibodies were used: Caspr (75-001, NeuroMab, 1:1000), MBP (40390, Abcam, 1:1000), Caspase-6 (BS7006, Bioworld, 1:100). After washed with PBS, brain slides and cells cultures were incubated with secondary antibodies for 1 h at RT. Secondary antibodies conjugated with Alex-Fluor-Dye were all purchased from Life Technology and diluted at 1:500. DAPI (Sigma) was used to stain the nuclei. We processed three pairs of APP/PS1 mice and WT controls and analyzed at least 103 myelinated axons from ten brain slices from each group.

2.3. Immunoblots

Hippocampus tissues were identified and then homogenized and extracted in lysis buffer (20 mM Tris pH 7.5, 150 mM NaCl, 1% Triton X-100) with proteinase inhibitor (Roche) and phosphatase inhibitor (Roche). After denatured at 100 °C for 5 min, proteins were separated by 10% or 12% SDS-PAGE. Following primary antibodies were used to incubate with membranes: neuregulin-1 type III (BS1173, Bioworld, 1:500), Mbp (7349, Abcam, 1:2000), NG2 (5320, Millipore, 1:2000), CNPase (BS3461, Bioworld, 1:1000), GAPDH (BE0034, EASYBIO, 1:5000). Secondary antibodies conjugated with HRP were all purchased from EASYBIO and diluted at 1:5000. HRP were then detected by Chemiluminescent.

2.4. Electron microscopy

Mice were anaesthetized and perfused with 4% PFA and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer. Hippocampus tissues were identified from both WT and APP/PS1 mice and post-fixed with 2% OsO4 for 2 h. 4% uranyl acetate was used to stain membranes. After dehydrated by a graded series of alcohol, corpus callosum was infiltrated in propylene oxide and Spurr's resin and then embedded in Spurr's resin. Ultrastructure images were obtained by transmission electron microscope (Tecnai G2 20 Twin, FEI) and analyzed by Image J. We measured at least 40 myelinated axons from each group (n = 3).

2.5. In vitro Cleavage assay

Cytoplasmic region of neuregulin-1 type III (shown as FL) was expressed and purified as described before [6]. FL neuregulin-1 type III was then diluted to 0.5 μg/μl by the same buffer used in desalting column (20 mM Tris-HCl pH 7.5 and 200 mM NaCl) and incubated with 5 ng/μl active-caspase-6 at 37 °C. Every single minute, part of protein solutions was taken out and denatured at 100 °C for 5 min until 1 h was passed. Protein samples with different incubation time were analyzed by 15% SDS-PAGE.

2.6. Statistical analysis

Data were analyzed by GraphPad Prism 6 and paired two-tailed student's *t*ests were used to declare statistical significance.

3. Results

3.1. Alterations of myelin morphology in hippocampus tissues of 2-month-old APP/PS1 mouse

Myelin basic protein (MBP), a major constituent of mature oligodendrocytes and myelin sheath, was found up-regulated in hippocampus tissues of APP/PS1 mice at the age of 2-month-old compared with age-matched WT mice (Fig. 1A). To investigate whether higher level of MBP in APP/PS1 mice contributed to abnormal myelination, we applied transmission electron microscope to study the ultrastructure of myelin sheath. We found that the ultrastructure of axon-myelin units was regular, but thickness of myelin sheath was much thicker in hippocampus tissues of APP/PS1 mice when compared with age-matched WT mice, which was caused by additional membrane wraps (Fig. 1B). Diameters of axons (d_{axon}) and fibers (axon and myelin) (d_{fiber}) were quantified and the g ratio (d_{axon}/d_{fiber}) was introduced to indicate relative thickness of myelin sheath. Different from WT (n = 41) whose average g ratio was 0.84, APP/PS1 (n = 45) were hypermyelinated with an average g ratio of 0.75 ($p < 0.001$) (Fig. 1C). Not only thickness of myelin, but also internodal length, the distance between consecutive nodes in APP/PS1 mice were quite different from WT. 35 μm brain slices of WT and APP/PS1 mice were immunostained with anti-Caspr (paranodes marker) and anti-MBP (myelin sheath marker), so that the internodal distance could be labeled and quantified (Fig. 1D). Compared with WT (n = 103), internodal lengths in APP/PS1 mice (n = 127) decreased dramatically (Fig. 1E).

3.2. Alterations of oligodendrocytes development in hippocampus tissues of 2-month-old APP/PS1 mouse

Mature oligodendrocytes, which could wrap axons to form myelin, were differentiated from oligodendrocytes progenitor cells (OPCs). Chondroitin sulfate proteoglycan (NG2) was expressed in OPCs specifically and always used as the marker of OPCs. 2′, 3′-Cyclic nucleotide-3′-phosphodiesterase (CNPase) was applied to