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S-adenosylmethionine reduces the inhibitory effect of Aβ on BDNF expression through decreasing methylation level of BDNF exon IV in rats

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ABSTRACT

The structure of brain-derived neurotrophic factor (BDNF) gene is complex, which is composed of eight non-coding exons and one coding exon, each of them has its own unique promoter. Multiple BDNF transcripts have distinct functional properties and epigenetic modulation of *BDNF* gene transcription is implicated in the neurological disorders. In the present study, rat models with amyloid-β (Aβ) intrahippocampal injection and PC12 cells were used to explore the role of DNA methylation in the promoters of BDNF exon IV and exon VI in BDNF suppression caused by Aβ. We found that Aβ inhibited BDNF expression accompanying with hypermethylation in BDNF exon IV promoter, meanwhile, S-adenosylmethionine (SAM), primary methyl donor, reversed the low BDNF expression through demethylation in BDNF exon IV promoter. No methylation change was observed in BDNF exon VI promoter. The alteration of DNA methylation caused by Aβ or SAM was mediated by DNA methyltransferase 3A (DNMT3A). These data suggest that methylation change in BDNF exon IV is involved in the regulation of BDNF expression by Aβ or SAM, and further support the view of specific epigenetic modifications of a certain *BDNF* gene transcript.

1. Introduction

Alzheimer's disease (AD) is a common neurodegenerative disease of central nervous system (CNS), which is characterized by a progressive loss of memory and cognitive impairment [1]. Amyloid-β (Aβ) is a length of 38–43 amino acid residues cleaved from the amyloid precursor protein (APP) by β and γ secretase enzymes [2,3]. Pathological overproduction of Aβ leads to oxidative damage, inflammation, damage to the blood brain barrier, neurofibrillary tangles formation and impaired memory [4].

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family of growth factors [5], which plays an important role in synaptic transmission, plasticity, neuronal proliferation, differentiation and survival [6,7]. It is reported that BDNF is down-regulated in the brain of many neurodegenerative diseases including AD [8]. There is growing evidences for the role of epigenetic modifications (mainly direct methylation of DNA or posttranslational modifications of histones) in the regulation of BDNF transcription [9]. Epigenetic modulation of *BDNF* gene transcription has also been implicated in the long-term impact of learning and memory [10]. The structure of *BDNF* gene is complex. In rodents, the *BDNF* gene has eight non-coding exons and one coding exon (IX). Each exon is regulated by its own unique promoter, resulting in more than 10 different transcripts [11]. There is solid experimental evidences showing that multiple BDNF transcripts have apparently distinct functional properties [12]. It is reported that there is a significant correlation among DNA methylation and specific histone modification patterns at BDNF exon I, IV and VI, and changes in the expressions of the correspondent transcripts in fear memory, stress, enriched environment and neuropsychiatric disorders [12].

S-adenosylmethionine (SAM) is a critical metabolite that participates in multiple cellular reactions in the periphery and CNS [13]. As a global methyl group donor, SAM can affect gene expressions. The data from some clinical and animal experiments suggest that SAM can be used for the treatment of nervous system diseases including depression, drug addiction, and cognitive dysfunction

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[14]. Clinically low SAM in the cerebrospinal fluid of AD patients has been reported [15].

Our previous studies reveal that BDNF expression is inhibited by Aβ, which can be reversed by SAM administration [16]. However, the underlying epigenetic mechanisms are not clear. In the present study, rat models with Aβ intrahippocampal injection and PC12 cells were used to explore the role of DNA methylation in the promoters of BDNF exon IV and VI in these pathological processes.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley (SD) rats of 12-week-old (Beijing Vital River Experimental Animal Technology Co., Ltd, Beijing, China) were used for all the experiments. All animals were housed in Experimental Animal Center of the Capital Medical University under the standard conditions. All experimental procedures were in compliance with the Guidance for the Care and Use of Laboratory Animals formulated by the Ministry of Science and Technology of China.

2.2. Cell culture and treatment

Rat adrenal pheochromocytoma cells (PC12, well-differentiated) were maintained at 37 °C with 5% CO_2 in RPMI 1640 (Gibco, USA) containing 10% fetal bovine serum (Gibco, USA). Both $A\beta_{1\text{-}42}$ (China Peptides Co., Ltd, China) and scrambled $A\beta_{1-42}$ (peptide comprised of the same amino acid composition of $A\beta_{1\text{-}42}$ but in a randomized sequence, China Peptides Co., Ltd, China) were aggregated by incubation in distilled water at 37 °C for 72 h before use. $A\beta_{1\text{-}42}$ (20 μM), scrambled $A\beta_{1\text{-}42}$ (20 μM) and/or SAM (100 μM in water, Yuanye Bio, China) were added into the medium for 24 h.

2.3. Stereotaxic injection of Aβ and SAM treatment

After anesthesia by intraperitoneal injection of chloral hydrate (350 mg/kg), the rats were placed onto a stereotaxic frame, and microcannulae were stereotactically implanted to bilateral hippocampus (4.3 mm posterior to the bregma; 3.5 mm lateral from midline; and 3.3 mm ventral to bregma). $A\beta_{1\text{-}42}$ (20μg/3 μL) or the same volume of scrambled $A\beta_{1\text{-}42}$ was delivered using a 10 μL stepper-motorized micro-syringe at a rate of 1 μL/min. Then the rats received an intraperitoneal injection of either SAM (10 mg/kg) or saline each day for 6 weeks after surgery.

2.4. Western blot analysis

Cells or hippocampal tissues of each group were harvested and homogenized in a lysis buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% NP-40, 0.1% SDS, and protease inhibitor cocktail (PPLYGEN, China). Routine procedures were carried out as described previously [16]. Rabbit anti-BDNF polyclonal antibody (1:200, Santa Cruz, USA), rabbit anti-DNMT1 monoclonal antibody (1:1000, CST, USA), rabbit anti-DNMT3A polyclonal antibody (1:1000, CST, USA), and mouse anti-β-actin monoclonal antibody (1:500, ZSGB-BIO, China) were used. The quantification of the blots was carried out using Alpha Fluor Chem FC3 system (ProteinSimple,USA) and analyzed with Image J 16.0 (NIH, Bethesda, MD).

2.5. Quantitative real-time PCR (q-PCR)

Total RNAs were extracted from the PC12 cells using RNAsimple Total RNA kit (Tiangen, Beijing, China). Reverse transcription was performed using a FastQuant RT kit (Tiangen, Beijing, China). The q-PCR reaction was performed at 95 °C for 15 min, followed by 45 cycles of 95 °C for 10 s, 55 °C for 20 s and 72 °C for 20 s, and the melting curve was analyzed. Each q-PCR was conducted in triplicate in a CFX96 Touch system (Bio-Rad, CA). Data were analyzed using the $2^{-\Delta\Delta Ct}$ method with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as normalization control. The primer sequences are used as follows: BDNF exon IV, forward primer 5′-GCTGCCTTGATGTTTACTTTGA-3′ and reverse primer 5′-CGTGGACGTTTG- CTTCTTTC-3′; BDNF exon VI, forward primer 5′-TTTGGGGCAGACGAGAAA- GC-3′ and reverse primer 5′-GGCAGTGGAGTCACATTGTTGTC-3′; BDNF exon IX, forward primer 5′-CCATAAGGACGCGGACTTGTAC-3′ and reverse primer 5′-AGA-CATGTTTGCGGCATCCAGG-3′; GAPDH, forward primer 5′-GAC-CACC- CAGCCCAGCAAGG-3′ and reverse primer 5′-TCCCCAGGCCCCTCCTGTTG-3′.

2.6. DNA methylation analysis

Genomic DNA from the PC12 cells and the hippocampus were extracted using DNeasy tissue system (Qiagen). Genomic DNA from each sample was bisulfite-treated using the EZ-96 DNA methylation kit (Zymo Research) according to the manufacturer's instructions. Sequenom MassARRAY platform (CapitalBio, Beijing, China), which was composed of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry and combined with RNA base-specific cleavage was used to analysis DNA methylation level in BDNF exon IV (Gen-Bank Accession Number: NM_001270632.1) and exon VI (Gen-Bank Accession Number: NM_001270634.1) promoter regions. PCR primers were designed using Methprimer (http://www.urogene.org/methprimer/). For each reverse primer, an additional T7 promoter tag was added, whereas a 10-mer tag was integrated into the forward primer to adjust melting temperature. Primers used in this study were as follows: BDNF exon IV: 5′-aggaagagagTTTATTGTTTTATAGGAAGG-GGGTT-3′ and 3′-cagtaa-tacgactcactatagggagaaggctCCTTCAATAAAAAACTCCAT-TTAATCT-5′; BDNF exon VI: 5′-aggaagagagGGGAATTAGGGATATTTTAAGGGT-T-3′ and 3′-cagtaatacgactcactatagggagaaggctCTCAAAATCCACA-CAAAACTCTC-5′. Mass spectra were obtained via MassARRAY Compact MALDI-TOF (Sequenom) and the spectra methylation ratios were generated by Epityper software version 1.0 (Sequenom, San Diego, CA).

2.7. Cell transfection

Before transfection for 24 h, PC12 cells were harvested and re-suspended in a fresh medium without antibiotics to achieve a culture density of 2×10^5 cells/mL and seeded in 6-well plates. Two-milliliter transfection solution containing 12 μL riboFECT CP Reagent and 50 nM DNMT3A siRNA or 50 nM negative control siRNA (RiboBio, Guangzhou, China), were added, respectively. After transfection for 48 h, the cells were collected.

2.8. Methylated DNA immunoprecipitation (MeDIP)-qPCR

Genomic DNA from the PC12 cells was extracted using TIANamp Genomic DNA Kit (Tiangen, Beijing, China) and MeDIP assay was performed using MethylampTM Methylated DNA Capture Kit (Epigentek, USA) according to the manufacturer's instruction. The sonicated DNA was immunoprecipitated with antibody against 5-methylcytidine (Epigentek, USA), then the immunocomplex were