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Oxidative stress induces DNA demethylation and histone acetylation in SH-SY5Y cells: potential epigenetic mechanisms in gene transcription in Aβ production

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ABSTRACT

Overwhelming evidence has suggested that enhanced oxidative stress is involved in the pathogenesis and/or progression of Alzheimer's disease (AD). Amyloid-β (Aβ) that composes senile plaques plays a causal role in AD, and its abnormal deposition in brains is the typical neuropathologic hallmark of AD. Recent studies have suggested that epigenetic mechanisms play an important role in the initiation and development of AD. In the present study, we investigated the epigenetic mechanisms, such as DNA methylation and histone acetylation, involved in the transcription of AD-related genes with Aβ production under oxidative stress. Human neuroblastoma SH-SY5Y cells were treated with hydrogen peroxide (H_2O_2) and used as the cell model. The intracellular Aβ level was significantly increased in H_2O_2-treated SH-SY5Y cells. The expression of amyloid-β precursor protein and β-site amyloid-β precursor protein-cleaving enzyme 1 was upregulated by demethylation in the gene promoters associated with the reduction of methyltransferases. Meanwhile, H_2O_2 induced the upregulation of histone acetyltransferases p300/cAMP-response element binding protein (p300/CBP) and down-regulation of histone deacetylases. DNA hypomethylation induced by DNA methyltransferase inhibitor could activate the DNA binding activity of transcription factor nuclear factor-κB, whereas no significant effect was observed on specific protein 1. DNA binding activities of nuclear factor-κB and specific protein 1 were activated by histone hyperacetylation induced by histone deacetylase inhibitor. These findings suggested that oxidative stress resulted in an imbalance between DNA methylation and demethylation and histone acetylation and deacetylation associated with the activation of transcription factors, leading to the AD-related gene transcription in the Aβ overproduction. This could be a potential mechanism for oxidative stress response, which might contribute to the pathogenesis and development of AD.

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1. Introduction

Alzheimer disease (AD) is defined as progressively impaired memory and cognition, and it is also characterized by the presence of extracellular neuritic plaques and intracellular neurofibrillary tangles. Altered proteolytic processing of the amyloid precursor protein (APP) plays a crucial role in the AD development, resulting in the overproduction and aggregation of neurotoxic forms of amyloid-β (Aβ) peptide. It has been postulated that different soluble or insoluble higher molecular weight forms of Aβ trigger a complex pathologic cascade that might cause synaptic dysfunction, inflammatory processes, neuronal loss, cognitive impairment, and finally the onset of the disease. An aspartyl protease (β-site APP-cleaving enzyme 1 [BACE1]) mediates the proteolytic cleavage of APP, which is a required process for Aβ generation. Most AD cases are sporadic and have no defined causes. Factors contributing to AD development are numerous and complex. In the last decade, overwhelming evidence has suggested that oxidative stress is exponentially increased with age through variations in the generation of reactive oxygen species (ROS), ROS elimination, or both (Barja, 2004). Oxidative stress is an early event in AD. It has been demonstrated that the early involvement of oxidative stress in AD includes oxidative modifications of lipids (Sayre et al., 1997; Schuessel et al., 2005), proteins (Montine et al., 1996; Smith et al., 1991), and nucleic acids (Nunomura et al., 2004, 2001) in brains from AD patients as well as in cellular and animal models of AD. Biomolecules modified by oxidative stress exist in neurons with or without neurofibrillary tangles and plaques, suggesting that oxidative stress promotes the formation of AD pathologies and is a very early contributor to the disease.

To date, AD pathophysiology remains largely unclear. Recent studies have suggested that epigenetic mechanisms play a key role

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in the initiation and development of AD (Chouliaras et al., 2010; Zawia et al., 2009). AD is among a few diseases that might display high homocysteine, and low B12 and folate in blood, suggesting that a dysregulation in the S-adenosylmethionine cycle is required for epigenetic regulation through DNA methylation (Fuso et al., 2005). It is worth noting that the expression of *APP* and *BACE1* genes is regulated via methylation of their promoters (Scarpa et al., 2006). Previous studies have demonstrated that AD might begin early in life and involve an interplay among the environment, epigenetics, and oxidative stress. Alterations in the methylation or oxidation of adjacent cytosine-guanine (CpG) dinucleotides within DNA mediate the epigenetic mechanisms that control the gene expression and promote the accumulation of oxidative DNA damage (Zawia et al., 2009). Recent studies of histone acetylation from human postmortem brain samples and transgenic animal models and cell culture demonstrated the conflicting results. Some studies reported that AD is associated with generally increased histone acetylation (Kim et al., 2007; Marambaud et al., 2003), and others showed that a decreased histone acetylation is causally linked to AD. Moreover, some other studies have demonstrated that the memory deficits can be partially recovered by histone deacetylase (HDAC) inhibitor in an AD transgenic mice model (Fischer et al., 2007; Green et al., 2008; Ricobaraza et al., 2009).

The molecular mechanism underlying the promotion of Aβ production by oxidative stress is not completely understood. It has been reported that hydrogen peroxide (H_2O_2) induces APP expression and therefore enhances Aβ production in mammalian lenses (Frederikse et al., 1996). The promoter activity of BACE1 can be potentiated by H_2O_2 at a low concentration, resulting in an enhanced Aβ expression (Tamagno et al., 2005; Tong et al., 2005) in vitro. In this study, we investigated the epigenetic mechanisms (such as DNA methylation and histone acetylation) involved in oxidative stress-induced Aβ overproduction. Because H_2O_2 is the main source of the highly reactive hydroxyl radicals in the brain, human neuroblastoma SH-SY5Y cells, as a more physiological model of neuronal Aβ generation, were treated with H_2O_2 and used as the cell model in our study. We found that H_2O_2 induced the upregulation of *APP* and *BACE1* genes through DNA hypomethylation and histone hperacetylation, correspondingly leading to Aβ overproduction. Our data suggested a novel insight to epigenetic mechanisms, by which oxidative stress might contribute to the AD pathogenesis.

2. Methods

2.1. Cell culture and treatment

Human neuroblastoma SH-SY5Y cells were maintained in Dulbecco's Modified Eagle's Medium (Gibco) supplemented with 10% fetal bovine serum (HyClone), 100 μg/mL streptomycin, and 100 U/mL penicillin (Gibco) at 37 °C in a humidified incubator with 5% CO_2.

Cells were treated with 30% H_2O_2 alone at a dose of 200 μmol/L for 1 hour, or 20 μmol/L for 24 or 72 hours, or in a combination with the following inhibitors. p38-mitogen activated protein kinase (p38MAPK) inhibitor SB203580 (20 μmol/L) and c-Jun amino terminal kinase (JNK) inhibitor SP600125 (10 μmol/L) were used to pretreat the cells for 1 hour before the H_2O_2 treatment. DNA methyltransferase (DNMT) inhibitor 5-Aza-2'-deoxycytidine (5-Aza-dC; 2 μmol/L) was used to treat cells for 72 hours alone or pretreat cells for 72 hours before the H_2O_2 treatment. HDAC inhibitor trichostatin A (TSA; 0.5 μmol/L) was used to treat cells for 24 hours alone or pretreat cells for 24 hours before the H_2O_2 treatment. Cells treated with the same volume of phosphate-buffered saline (PBS) were used as control. The viability of SH-SY5Y cells was evaluated by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay.

2.2. Quantification of Aβ level by ELISA

The Aβ level was quantified in cell lysates by enzyme-linked immunosorbent assay (ELISA) using a Biosource kit according to the manufacturer's instructions. Aβ1–40 and Aβ1–42 were evaluated according to the standard curves. The quantity of Aβ in each sample was measured in triplicate. Recombinant C99 was added to the assay to minimize the cross reaction with C99.

2.3. Quantitative real-time PCR

RNA was extracted from cells using SV Total RNA Isolation System (Promega, WI, USA) according to the manufacturer's instructions. Purified RNA was then reversely transcribed into cDNA using Reverse Transcription System (Promega) according to the manufacturer's instructions, and the synthesized cDNA was stored at −20 °C until use. To evaluate the BACE1 expression, quantitative polymerase chain reaction (PCR) was performed on a LightCycler 1.5 instrument (Roche Diagnostics GmbH, Mannheim, Germany). The following primers were used in this study: BACE1, forward primer: GTCGGAGGGAGCATGATCA and reverse primer: CCGCCG GATGGGTGTATAC; and 18S, forward primer: GTAACCCGTTGAA CCCCATT and reverse primer: CCATCCAATCGGTAGTAGCG. Quantitative PCR reactions were conducted in a final volume of 20 μL containing 1 μL of cDNA and SYBR Green PCR Master Mix (Tiangen Biotech Co, LTD, Beijing, China). Briefly, after an initial denaturation step at 95 °C for 7 minutes, the amplification was carried out with 40 cycles at a melting temperature of 95 °C for 45 seconds, an annealing temperature of 62 °C for 35 seconds, and an extension temperature of 72 °C for 60 seconds. Each experiment was performed in triplicate. A dissociation curve was analyzed to assess the amplification specificity. The relative messenger RNA (mRNA) level of the target gene was determined with the $2^{-\triangle\triangle Ct}$ method (LightCycler Software 4.1, Roche Diagnostics GmbH) using 18S as the housekeeping gene.

2.4. Western blot analysis

Cells were harvested and washed with PBS. To obtain the cell lysates, cell pellet was lysed in lysis buffer (50 mmol/L Tris-HCl, 2 mmol/L ethylene diamine tetraacetic acid (EDTA), 2 mmol/L ethylene glycol bis(2-aminoethyl) tetraacetic acid (EGTA), 1 mmol/L dithiothreitol (DTT), 0.1% sodium deoxycholate, 0.5% NP-40, 1 μmol/L sodium orthovanadate, 5 mmol/L sodium pyrophosphate, 5 μg/mL pepstatin A, 5 μg/mL leupeptin, 5 μg/mL aprotinin, 5 μg/mL chymostatin, and 50 nmol/L okadic acid, pH 7.4) and sonicated for 5 minutes. After centrifugation at 13,000 *g* for 10 minutes, the supernatant was collected and stored at −20 °C until use. Protein content was determined using BCA protein assay kit (Pierce). Samples (50 μg per lane for anti-phospho-p38MAPK, anti-phospho-JNK, anti-APP, anti-tumor necrosis factor-alpha convertase [TACE] and anti-methyl-CpG binding domain (MBD) 2; 60 μg per lane for anti-HDAC3 and anti-p300/CBP; 80 μg per lane for anti-DNMT1, anti-DNMT3a, anti-a disintegrin and metalloproteases [ADAM] 10, and anti-BACE1) were loaded in 8%, 10%, or 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis, respectively. After the electrophoresis, proteins were electro-transferred onto nitrocellulose membrane. Blots were blocked with 5% milk in Tris-buffered saline containing 0.1% Tween 20 (TBS-T, pH 7.5) at room temperature for 1 hour and then incubated with primary antibodies in TBS-T at 4 °C overnight. The following primary antibodies were used in this study: rabbit anti-phospho-p38MAPK monoclonal antibody (1:1000, Cell Signaling), rabbit anti-phospho-JNK monoclonal antibody (1:1000, Cell Signaling),

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