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SUMO1 modulates Aβ generation via BACE1 accumulation

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Abstract

Accumulation of disease-related proteins is a characteristic event observed in the pathogenesis of neurodegenerative diseases. β-secretase (BACE)-1, which initiates generation of β-amyloid (Aβ), is increased in the Alzheimer's diseased brain. However, the mechanisms of BACE1 accumulation in Alzheimer's disease are largely unknown. In this report, we found that small ubiquitin-like modifier (SUMO)-1 interacts with the dileucine motif of BACE1 and regulates the level of BACE1 protein. This was proved by the coimmunoprecipitation, and gain or loss of function experiments. Altering 3 SUMO isoforms affects BACE1 protein levels, and consequently results in altered amyloid precursor protein processing and Aβ generation. BACE1 levels were increased in response to Aβ or apoptosis, but not in cells lacking SUMO1. Aβ increased SUMO1 protein levels in rat cortical neurons. Moreover, SUMO1 immunoreactivity was increased in the amyloid precursor protein transgenic mice. Furthermore, the C-terminus fragments of BACE1 containing dileucine motif reduced Aβ generation by SUMO1 overexpression. Our study indicates SUMO1 is not only a novel and potent regulator of BACE1 accumulation and Aβ generation but also a potential therapeutic target for Alzheimer's disease.

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1. Introduction

One of the common observations of neurodegenerative diseases, including Alzheimer's disease (AD) and Huntington's disease, is an accumulation of proteins that is considered to involve pathological processes. Several proteins such as parkin, ubiquitin carboxy-terminal hydrolase UCH-L1 or small ubiquitin-like modifier (SUMO) have been observed in neurodegenerative diseases (Kitada et al., 1998; Leroy et al., 1998; Ueda et al., 2002).

While ubiquitination targets proteins for degradation, sumoylation modifies the interaction of target proteins with protein partners and their activity, stability, and subcellular localization (Gill, 2003; Pichler and Melchior, 2002). SUMO paralogs (SUMO1 to 3) had a wide expression in all tissue and can be covalently linked to target proteins to alter their cellular distribution, function, and metabolism (Schwartz and Hochstrasser, 2003). SUMO1 is an 11-kDa protein that is 18% identical to ubiquitin (Müller et al., 2001). SUMO2 and SUMO3 differ only in 3 N-terminal residues (Wilkinson et al., 2010). SUMO modification plays an important role in protein trafficking, nuclear bodies, the ubiquitin–proteasome system, and apoptosis. Many studies have suggested that some proteins may be preferentially sumoylated by specific SUMO types (Grégoire and Yang, 2005; Vertegaal et al., 2006).

Many SUMO target proteins have been identified in neurons that are cytosolic or membrane proteins, including glutamate receptor subunit 6 (Martin et al., 2007). Although SUMO can be covalently linked to specific proteins, it has

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been reported that SUMO even in the absence of the C-terminal diglycine motif can participate in protein–protein interaction (Yan et al., 2010).

Both extracellular β-amyloid (Aβ) deposits and the origin of β-secretase (BACE)-1 accumulation and its temporal relationship with neuritic pathology remain issues of ongoing debate in AD research. Several molecules implicated in neurodegenerative diseases including AD have been identified as SUMO targets. Tau and amyloid precursor protein (APP) have been shown to be modified by SUMO (Dorval and Fraser, 2006; Zhang and Sarge, 2008). Strong SUMO2/3 immunoreactivity has been reported in AD neurons (Martin et al., 2007), and SUMO1-positive deposits were detected in APP transgenic mice (Tg2576) (Takahashi et al., 2008). Recently, we reported that Ubc9 polymorphisms were associated with late-onset AD patients in the Korean population (Ahn et al., 2009). Two previous studies indicated that SUMO3 overexpression affects Aβ levels (Dorval et al., 2007; Li et al., 2003). However, the meaning of their results is not clear as the 2 studies observed opposite effects of SUMO overexpression on Aβ levels. Thus far, however, the role of SUMO in AD is still somewhat controversial.

BACE1 is a type I integral membrane-associated aspartyl protease (Vassar et al., 1999). An increase in BACE1 activity has been shown to be correlated with brain Aβ production in the frontal cortex (Li et al., 2004). Previously, we reported that BACE1 was degraded via the lysosomal pathway and that the dileucine motif was important to regulate BACE1 levels (Koh et al., 2005). Notably, the dileucine motif in BACE1 interacts with the Golgi-localized, γ-ear-containing adenosine diphosphate ribosylation factor-binding (GGA) family. Although depletion of GGA3 during apoptosis led to an increase in BACE1 levels (Tesco et al., 2007), previous studies showing BACE1 elevation in the presence (5XFAD mice) or absence (Tg2576 mice) of cell death imply that BACE1 accumulation was correlated with amyloid pathology (Zhao et al., 2007). Therefore, the mechanisms of BACE1 accumulation are still unknown.

In this study, we have now identified SUMO1 as a new BACE1-binding partner. All 3 SUMO isoforms (SUMO1 to 3) increased BACE1 accumulation and Aβ generation. We found that SUMO1 interacts with BACE1 and regulates BACE1 accumulation. SUMO1 depletion decreases BACE1 and Aβ generation. Moreover, we provide evidence that the dileucine motif of BACE contributes to BACE accumulation by SUMO1. The regulation of SUMO1 levels ultimately affects Aβ generation, indicating that interfering with BACE1-SUMO1 interaction may become an alternative therapeutic strategy for AD.

2. Methods

2.1. Materials

Synthetic $A\beta_{1-40}$ peptides ($A\beta_{1-40}$; Invitrogen, Camarillo, CA, USA) were dissolved in 1, 1, 1, 3, 3, 3-hexafluoro-2-propanol (Sigma, Saint Louis, MO, USA) in the vial and incubated with shaking at room temperature until dissolved completely. Staurosporine (STS; Sigma) was used for apoptosis induction. The caspase inhibitor z-Val-Ala-Asp-fmk was purchased from Calbiochem (La Jolla, CA, USA). The following primary antibodies were used: α-BACE1 (ProScience, Flint Place Poway, CA, USA), α-SUMO1 (Zymed, San Francisco, CA, USA, or Cell Signaling Technology, Danvers, MA), α-SUMO2/3 (Zymed), α-APP (6E10; Covance, Emeryville, CA, USA), α-green fluorescent protein (GFP) (Molecular Probes, Eugene, OR, USA), monoclonal α-V5 (Invitrogen), polyclonal α-V5 (Chemicon, Temecula, CA), α-Myc (Cell Signaling Technology), α-GGA3 (BD Transduction Laboratories, San Jose, CA, USA), α-eIF2α (Cell Signaling Technology), α-phospho-eIF2α (Cell Signaling Technology), α-actin (Sigma), α-nicastrin (Affinity BioReagents, Golden, CO), and α-tubulin (Sigma). SUMO1-knockout and wild type primary mouse embryonic fibroblast cells (MEFs) were a gift from Dr. Olli A. Jänne (University of Helsinki, Finland).

2.2. Animals

Eighteen-month-old APP transgenic mice were used in the present study, as previously reported (Kim et al., 2010). Eight-month-old 5XFAD transgenic (Tg) mice (B6SJL Tg [APPSwFlLon, PSEN1*M146L*L286V] 6799Vas/J) were purchased from the Jackson Laboratory and maintained by crossing hemizygous transgenic mice with B6SJL F1 mice. All studies were conducted with a protocol approved by the local Institutional Animal Care Use Committee in compliance with Korean Food and Drug Administration guidelines for the care and use of experimental animals.

2.3. Immunohistochemistry

Brains from 18-month-old APP Swedish/PS1ΔE9 Tg mice together with their wild type controls were fixed in 4% (wt/vol) paraformaldehyde. Cryostat sagittal sections were cut on a sliding microtome into 10-μm slices at −20 °C and placed on a microslide for immunostaining. The sections were immunostained with 6E10 (1:100; Covance) and SUMO1 antibodies (1:100; Cell Signaling Technology). The monoclonal antibody, 6E10, raised against 1–17 amino acids of Aβ region. Sections were then incubated for 1 hour at room temperature with secondary antibodies conjugated with Alexa Fluor 488 and 555. Axiolab-Pol polarizing (Carl Zeiss, Thornwood, NY, USA) microscopy with Axio Vision Release 4.8 software was used for analysis of 3,3'-Diaminobenzidine (DAB) photomicrographs and colocalization of immunofluorescent proteins.

2.4. Cell culture and stable cell lines

Human neuroglioma H4 cells, mouse neuroblastoma N2a cells or HEK293T cells were cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine

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