SUMO negatively regulates BACE expression

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Abstract

OBJECTIVE: The effect of SUMO on the promoter activity and mRNA expression of BACE gene was investigated to find the connection between sumoylation and APP processing.
METHODS: The BACE promoter activity was measured by reporter gene analysis and BACE mRNA level was investigated using real-time RT-PCR method.
RESULTS: BACE gene promoter activity was inhibited by the over-expression of SUMO proteins and was blocked by disrupting the SP1 site. Endogenous BACE mRNA level was also negatively regulated by the induction of SUMO proteins. Using a specific inhitor of SP1, BACE promoter activity was coordinately inhibited.
CONCLUSIONS: SUMO negatively regulates the BACE expression and SP1 is involved in the process.

Abbreviations:

SUMO	- Small Ubiquitin-like Modifiers
BACE	- Beta site APP cleaving enzyme
APP	- Amyloid precursor protein
AD	- Alzheimer's Disease
Aβ	- Amyloid β
SP1	- Specificity protein 1

INTRODUCTION

AD (Alzheimer's disease) is a prevalent neurodegenerative disease affecting all brain areas including the hypothalamus (Swaab *et al.* 2011; van de Nes *et al.* 1993) with two hallmarks, deposition of extracelluar amyloid plaques and intraneuronal tangles. Amyloid plaques are mainly composed of the Aβ peptides, which are generated from the transmembrane protein APP. BACE (Beta site APP cleaving enzyme), also called β-secretase, is a pivotal enzyme in amyloid pathology, which initiates the cleavage of APP at the N-terminal end and results in the fibrillar Aβ1-40 and Aβ1-42 peptides under sequentially processing by γ-secretase(Vassar *et al.* 1999). There are numerous studies supporting the idea that abnormal BACE activity is associated with Alzheimer's disease and it also has been verified that in Alzheimer's disease subjects BACE protein level and enzymatic activity are increased(Cai *et al.* 2001; Fukumoto *et al.* 2002; Li *et al.* 2004; Luo *et al.* 2001). It may, therefore, be a promising therapeutic approach for the prevention or treatment of Alzheimer's disease to modulate β-secretase activity or regulate its expression (Potter *et al.* 2000; Roggo 2002; Vassar 2001).

SUMO (Small Ubiquitin-like modifiers) proteins share the same structural folding with ubiquitin, but are only distantly related in amino acid sequence to ubiquitin(Gill 2004). Sumoylation is a post-translational modification by which SUMO are conjugated to target proteins by a covalent isopeptide bond between the C-terminal glycine residue and ε-amino group of the lysine residue in the target protein(Johnson 2004). Thus far, it was found that from the four members of SUMO identified in humans, SUMO1, 2, 3, had a wide

expression in all tissues while the expression of SUMO4 was limited to the immune system and kidney (Guo *et al.* 2004). In the pathway of sumoylation, a heterodimer SAE1/2 is known as the activating enzyme (E1) and Ubc9 is the only conjugating enzyme (E2) identified. To date, three types of E3 ligases have been demonstrated, from which most proteins belongs to PIAS family, that are responsible for the efficient transfer of SUMO to the target protein (Johnson 2004).

Sumoylation has been related to a variety of pathological disease including Alzheimer's disease and Parkinson's disease. It has been reported that sumoylation affected the APP processing and Aβ level, but the mechanism is so far not known (Li *et al.* 2003; Zhang *et al.* 2008). The present study analyzes the effect of SUMO on BACE, the pivotal enzyme in APP processing.

MATERIALS AND METHODS

Chemical and DNA constructs

Mithramycin A (MIA) was purchased from Sigma. BACE promoter DNA construct was donated by Dr. Song and pGL3-basic and pRh-TK was purchased from Promega. Human SUMO1 and SUMO3 cDNAs were inserted into pEGFP-C1 (Clontech) between EcoRI and SalI sites.

Cell culture, Transfection and luciferase report gene analysis

293T and SH-SY5Y cells were obtained from ATCC and grown in DMEM supplemented with 10% FBS and maintained at 37 °C in an incubator containing 5% CO2. Cells were seeded into 6 or 24 well plates one day before transfection.

Transfection was performed according to the producer's manual by using Lipofectamine 2000 (Invitrogen) or Fugene HD (Roche). 24 or 48 hours, cells were collected and lysed for luciferase assay or total RNA extraction. For a luciferase assay, 20 ul cell lysates were used for a dual reporter assay and read out on Veritas microplate reader using Promega's standard protocol.

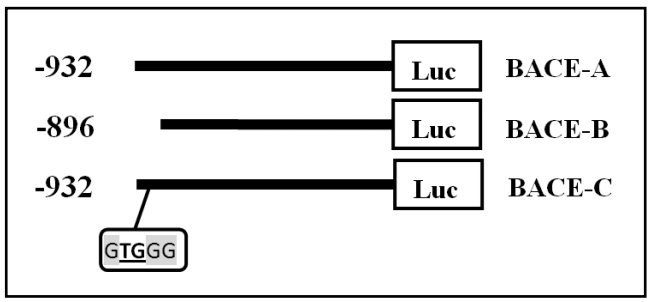

Fig. 1. Schematic map for reporter gene vectors used in this study. BACE-A contained a fragment flanking the BACE promoter region (from –932 to 292) with pGL3 backbone. In comparison with BACE-A, BACE-B lacked a short fragment including a SP1 binding site and the SP1 site was mutated in BACE-C.

RNA extraction, Reverse transcription and real time PCR

Cells were lysed with Trizol Reagent (Invitrogen) and chloform was added to precipitate DNA and protein. After centrifuge at 12,000 g for 10 minutes, RNA in the water phase was precipitated by isoprophrol alcohol and washed once with 75% ethanol and air dried, then dissolved in RNase free water. After treatment with RNase free DNaseI (Promega) for 30 minutes, RNA was quantified by UV spectrophotometry. The same quantity of RNA (1ug) was reverse transcripted into cDNA at 42 °C and real time PCR were performed using SYBR method. Primers for BACE and GAPDH were: BACE-F, GCTGGGCACCGACCTGCTTT; BACE-R, ACTCCCGCCGGATGGGTGTA; GAPDH-F, TGCTGGCGCTGAGTACGTCG; GAPDH-R, GGCATCAGCAGAGGGGGCAG.

Statistics

Statistical analysis was performed by unpaired t test or ANOVA with a post-hoc analysis using the Bonferroni's multiple comparison correction. Differences were considered significant when the *p*-value was less than 0.05.

RESULTS

BACE promoter activity was modulated by SUMO

To analyze whether SUMO proteins have an effect on BACE expression, firstly we subcloned the BACE gene promoter region into pGL3 basic vector as indicated in Fig1. Human BACE gene promoter region (–932 to +292) was subcloned into pGL3. SUMO1 and SUMO3 cDNAs were inserted into pEGFP-C1 between EcoRI and SalI sites.

We co-transfect GFP tagged SUMO or GFP with BACE promoter vectors into HEK293T cells. And pRh-TK was used as an internal control to evaluate the transfection effiency. 24 or 48 hours later, we lysed the cells and checked the luciferase activity of the cell lysates on the Veritas microplate reader. Firefly luciferase activity, which was normalized with *Renilla* luciferase activity, was markedly decreased in the SUMO groups compared with GFP group (Figure 2A).

To further determine the inhibition of SUMO on BACE promoter activity, we transiently transfect GFP tagged SUMO plasmids or GFP vector into SH-SY5Y cells, which expressed endogenously BACE mRNA or protein. 48 hours after transfection, we collected the cells and evaluated the mRNA level of BACE by normalizing with GAPDH using real time RT-PCR method. In SUMO groups BACE mRNA level was decreased as compared to the GFP group (Figure 2B).

SP1 was involved in the regulation of SUMO on BACE promoter activity

Here in the reporter gene analysis, we used a short fragment (–932 to +292) in the BACE promoter region, there was an SP1 site and it has been suggested that

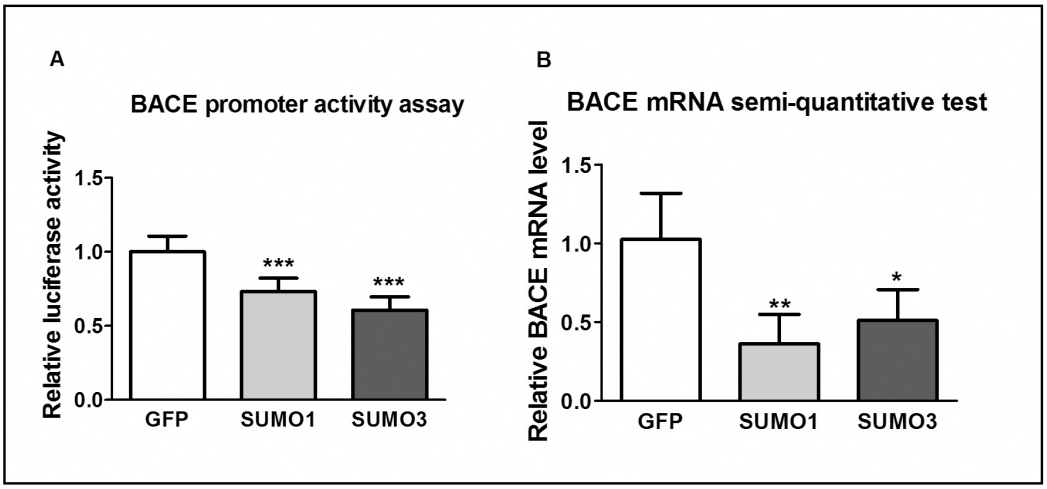

Fig. 2. BACE promoter activity is modulated by SUMO. **A:** BACE-A was co-transfected with GFP or GFP tagged SUMO protein vectors into 293T cells and subjected to report gene assay 24 or 48 hours later. In contrast to the GFP group, luciferase activity was significantly decreased in the SUMO groups (n=12). **B:** Endogenous BACE mRNA level was also down-regulated by induction of SUMO vectors into SHSY5Y cells (n=6).

SUMO would negatively regulate SP1-mediated transactivation. Thus we subcloned two vectors, BACE-B with a deletion of SP1 site, BACE-C with the SP1 site mutated. We co-transfected BACE-B or C with SUMO into 293T cells and checked luciferase activity and found that the inhibition of SUMO on BACE promoter activity was markedly blocked. This suggested that SP1 is involved in the process, and in accordance with a previous report about sumoylation, negatively regulates SP1-mediated transactivation (Figure 3).

Relation of SP1 and SUMO on BACE promoter activity

To confirm our former hypothesis, we used Mithramycin A (MIA), a selective inhibitor of SP1 in our assay. MIA acted as a dose-dependent inhibitor on SP1 mediated transactivation (as shown in Figure 4A) and we selected a moderate concentration. 24 hours after the induction of BACE-A into 293T cells, we treated the cells with MIA for 12 hours and performed a luciferase assay. In contrast to the DMSO solvent control, luciferase activity was markedly decreased in the MIA group, no matter whether SUMO1 or SUMO3 was expressed in the cells (Figure 4B). This suggested that MIA exaggerates the inhibition of SUMO proteins on BACE gene promoter activity. This observation is in conflict with a previous hypothesis stating that SP1 inhibition may block the inhibition on BACE promoter activity.

DISCUSSION

In a previous report, Li *et al.* found that over-expression of SUMO can negatively regulate APP processing and Aβ production but did not go into the possible mechanism involved (Li *et al.* 2003). Here we provided a direct connection between SUMO and BACE and thus with the pathway of APP processing into Aβ. SUMO might thus be an upstream signaling molecule of the APP pathway in Alzheimer's disease. To confirm this, and to find the relationship among these three proteins, the levels of SUMO, SP1 and BACE have to be determined human tissues. In the meanwhile, a knock down test of SP1 or SUMO is needed, to support our hypothesis.

There are a series of sites in the BACE promoter, which are recognized by different transcription factors including SP1, CREB, AP1 and HIF-1 (Christensen *et al.* 2004). As we know, all the above transcription path-

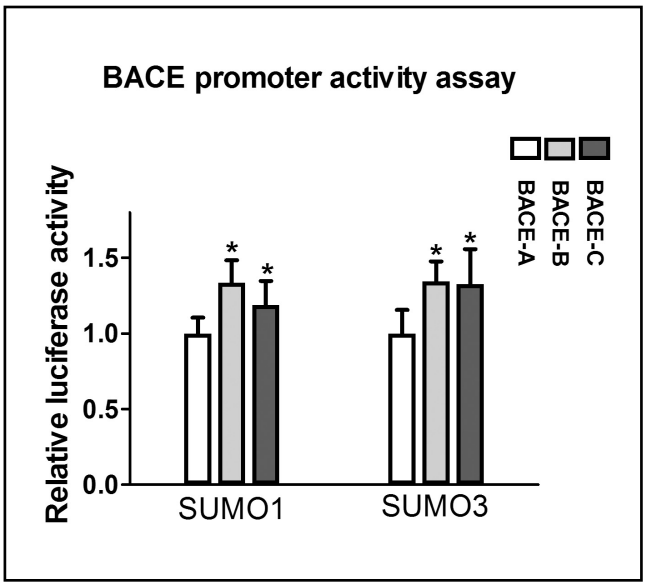

Fig. 3. SP1 is involved in the inhibition process of SUMO on BACE promoter activity. 293T cells were co-transfected with SUMO and BACE reporter vectors and the promoter activity of BACE gene was measured by dual reporter gene assay. BACE promoter activity was markedly increased by deletion (BACE-B) or mutation (BACE-C) of SP1 site (n=8).

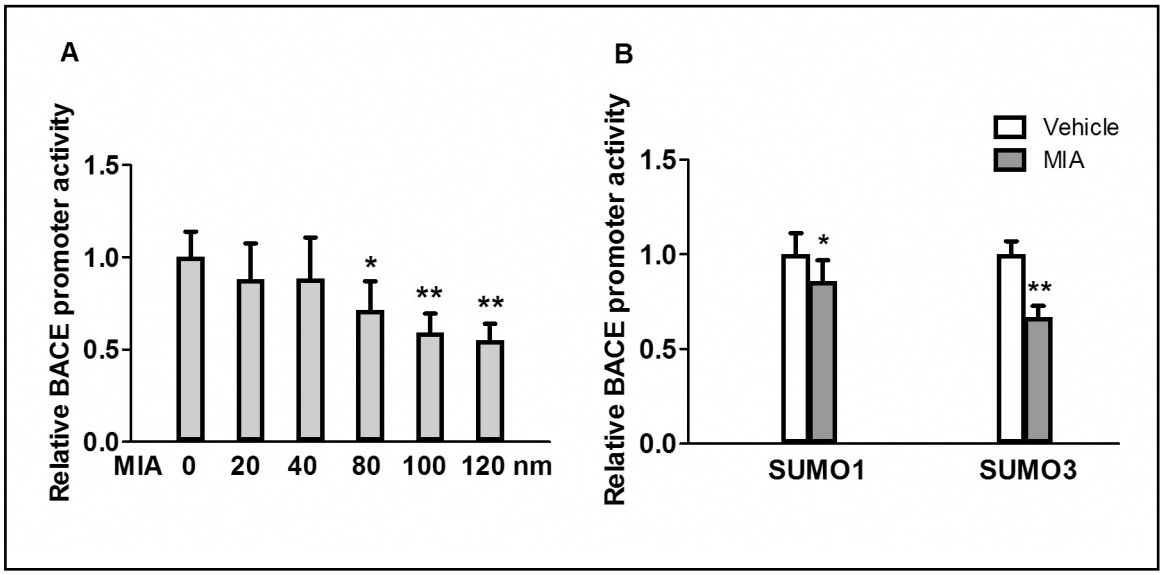

Figure 4. Relation of **SP1 with SUMO on BACE promoter activity.** A, Mithramycin A (MIA) inhibits SP1 activity in a dose-dependent manner. B, MIA (80nM) was introduced in the reporter gene assay and exhibited an inhibitory effect together with SUMO1/3 on BACE promoter activity (n=9).

ways can be regulated by sumoylation. As to BACE gene transcription, it is also a possibility that SUMO proteins regulate the other three transcription factors besides SP1, and then affect the transcription level of BACE gene.

Sumoylation, as an important post-translation modification, is attracting much more attention. Besides our finding, it has been reported that APP (Zhang *et al.* 2008) and tau protein (Dorval *et al.* 2006) can be directly modified by SUMO proteins although the functional meaning of these observations remains unclear. It is, on the basis of our results reasonable to presume that sumoylation plays a pivotal role in APP or tau metabolism and the regulation would be subtle.

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