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Original Article

Tripartite motif-containing protein 33 (TRIM33) negatively regulates amyloid-β production by promoting proteasome-dependent degradation of BACE1

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Abstract: Recently, excessive production and accumulation of beta-amyloid (Aβ) was found to play critical roles in the physiological and pathological processes of Alzheimer's disease (AD). In the current study, we were aiming to examine the effect of Tripartite Motif-Containing Protein 33 (TRIM33) on Aβ production. We found overexpression of TRIM33 in HT22 hippocampal cells significantly decreased the intracellular and extracellular levels of $A\beta_{40}$ and $A\beta_{42}$. Moreover, we observed that the activity of BACE1 was inhibited by overexpression of TRIM33 in a dose-dependent manner, and the expression level of secreted APP-β (sAPPβ) and carboxy-terminal fragment-β (CTFβ) were also significantly decreased. Therefore, we examined the effect of TRIM33 on BACE1 expression, and we found that the mRNA level of BACE1 was not affected by TRIM33 overexpression, but the protein level of BACE1 was markedly reduced in TRIM33 overexpressed HT22 hippocampal cells in proteasome-dependent manner. Most importantly, we found that TRIM33 could interact with BACE1 and promote k48-linked poly-ubiquitination of BACE1. In conclusion, we identified TRIM33 as a negative regulator of Aβ production and suggested TRIM33as a potential therapeutic target for the treatment of Alzheimer's disease.

Keywords: Beta-amyloid, alzheimer's disease, TRIM33, BACE1

Introduction

Alzheimer's disease (AD) is an age-related chronic neurodegenerative disease and the most common cause of dementia. Currently, several hypotheses have been proposed to illustrate AD pathogenesis, and amyloid-β (Aβ) hypothesis is the most well studied and acceptable one [1]. Aβ is present in high levels in the brains of AD patients and is composed of 38-42 amino acid residues [2]. It is derived from the catalytic cleavage of a larger amyloid precursor protein (APP) by β- and γ- secretases, and Aβ in the brain exists in a dynamic equilibrium of Aβ production and clearance, however, imbalance of this equilibrium may cause dysfunction of Aβ metabolism resulting in Aβ aggregation [3]. Aβ aggregate-mediated cellular processes, including neuronal dysfunction and death, neuroinflammation, and oxidative stress, have been recognized as early events in the pathogenesis of AD [4]. Therefore, suppression of Aβ generation and deposition is expected as one of the most promising strategy to prevent AD.

To generate Aβ, APP is first cleaved by β-site APP cleaving enzyme 1 (BACE1) in the acidic compartments, and the resultant β-C-terminal fragments (CTFs) are then cleaved by γ-secretase to release Aβ [5]. Thus, because BACE1 initiates Aβ processing, inhibition of BACE1 activity may be an effective way to prevent Aβ accumulation. However, the catalytic site of BACE1 is exceptionally long, and it has been very challenging to develop small compounds that can efficiently inhibit BACE1, are able to cross the blood-brain barrier, and are reasonably stable [6]. Therefore, the strategy to control BACE1 levels by protein degradation pathway has great significance and importance.

In the current study, for the first time, we examined the effect of Tripartite Motif-Containing Protein 33 (TRIM33) on Aβ production. We

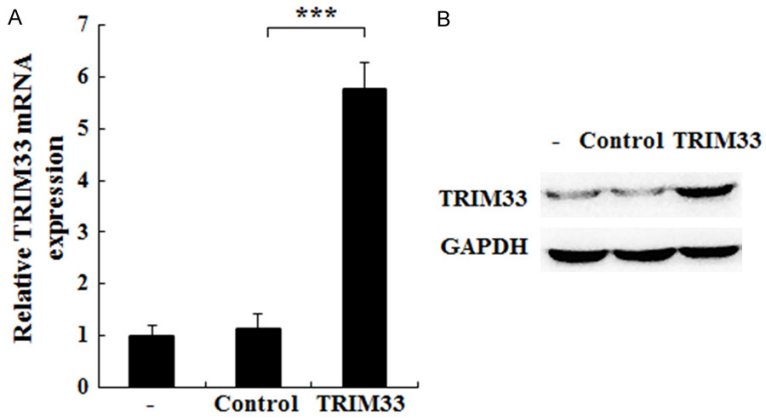

Figure 1. Overexpression of TRIM33 in HT22 cells by using lentivirus. A. HT22 cells were infected with control-lentivirus or TRIM33-lentivirus (MOI 50), the mRNA levels of TRIM33 were examined by qPCR. B. The protein levels of TRIM33 were examined by western blot. Data are representative of three independent experiments. ***P<0.001.

found intracellular and extracellular levels of $A\beta_{40}$ and $A\beta_{42}$ were all decreased in TRIM33 overexpressed HT22 hippocampal cells, as well as the inhibited BACE1 activity. Most importantly, we found TRIM33 could interact with BACE1 and promoted k48-linked poly-ubiquitination of BACE1, leading to the proteasome-dependent degradation. Overall, we suggested TRIM33 as a specific inhibitory protein of BACE1 and provided a new drug target for the treatment of AD.

Material and methods

Cells and cell culture

The HT22 hippocampal cell line was obtained from the Cell Bank at the Chinese Academy of Sciences (Shanghai, China). The cells were maintained in DMEM medium supplemented with penicillin (100 U/mL), streptomycin (100 mg/mL) and 10% FBS at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

Lentivirus preparation

Lentiviral expression plasmids for TRIM33 were constructed by MDL technology company (MDL biotech, Beijing, China). Briefly, the corresponding coding sequence was inserted into pWPXL vector (Addgene). Lentivirus particles were produced through transfection of pWPXL-TRIM33, pMD2.G and psPAX2 plasmids (Addgene) with a proportion of 20:15:7 into HEK293T cells, 3 days later the culture was harvested and enriched by PEG8000. The enriched lentivirus particle (MOI, 25 or 50) was used for HT22 cells infection in the presence of 5 μg/ml of polybrene (Sigma-Aldrich, St. Louis, USA).

Aβ detection

Intracellular and extracellular levels of $A\beta_{40}$ and $A\beta_{42}$ were detected by commercial ELISA kits (Invitrogen, #KHB3481 and #KHB3441). After 36 hours of lentivirus infection, cell supernatants were collected for detection of extracellular Aβ levels and cells were lysed for measurement of intracellular Aβ levels following the manufacture's protocol.

BACE1 activity assay

The assay was performed as previous reported [4]. The fluorescence values were measured in a 96-well black plate using an MD-M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA) with excitation at 450 nm and emission at 482 nm.

Quantitative PCR analysis

Total RNA was extracted with TRIzol reagent according to the manufacturer's instructions (Invitrogen). A LightCycler (ABI PRISM 7000; Applied Biosciences) and a SYBR RT-PCR kit (Takara Biotechnology.) were used for quantitative PCR (q-PCR) analysis. Following primer sequences were used: TRIM33: 5'-AGC ACC ATG AAT CCT TCT CC-3' and 5'-GTA CTT GGG GGT CTC ACA GG-3'; BACE1: 5'-AGACGCTCAACATCCTGGTG-3' and 5'-CCTGGGTGTAGGGCACATAC-3'; GAPDH: 5'-TGCACCACCAACTGCTTAGC-3' and 5'-GGCATGGACTGTGGTCATGAG-3'.

Immunoprecipitation and western blot analysis

HT22 cells were harvested and lysed in immunoprecipitation buffer (Beyotime, Beijing, China). Immunoprecipitation and western blot analysis were performed as described [7]. All antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz Biotechnology, CA, USA).

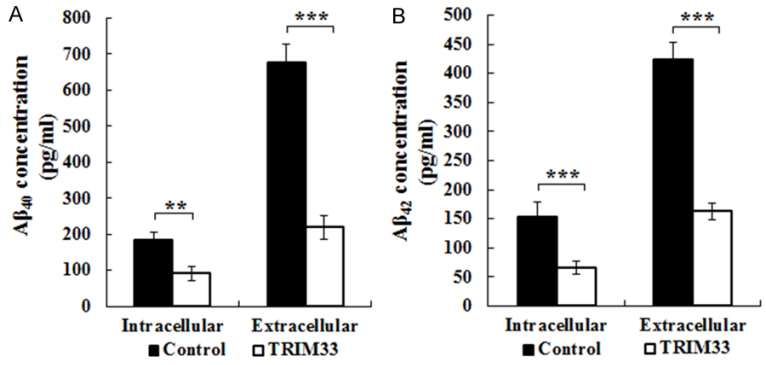

Figure 2. Overexpression of TRIM33 decreased intracellular and extracellular levels of Aβ. A and B. After 36 hours of lentivirus infection, supernatants and cells were collected for detection of intracellular and extracellular $A\beta_{40}$ and $A\beta_{42}$ levels. Data are representative of three independent experiments. **P<0.01; ***P<0.001.

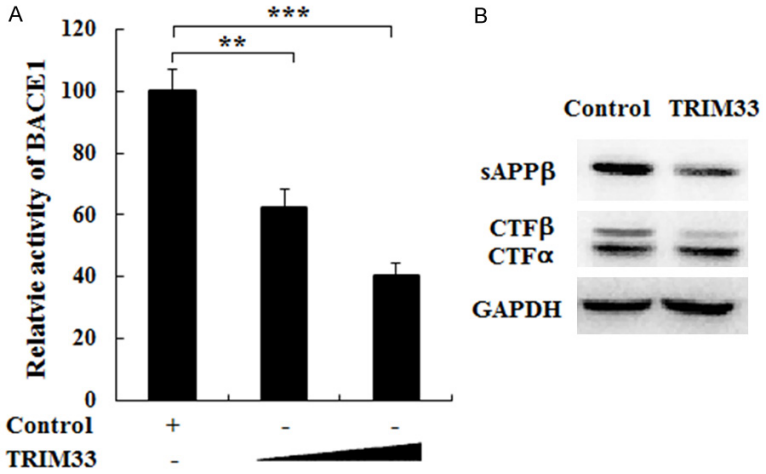

Figure 3. Overexpression of TRIM33 suppressed BACE1 activity. (A and B) HT22 cells were infected with increasing amount of TRIM33-lentivirus (MOI 25 or MOI 50), 36 hours later, the activities of BACE1 (A) or protein levels of sAPPβ and CTFβ (B) were detected. Data are representative of three independent experiments. **P<0.01; ***P<0.001.

Statistical analysis

All the data were expressed as mean ± S.D. and analyzed using analysis of variance (ANOVA) followed by Tukey test. Differences were considered statistically significant at P< 0.05.

Results

Overexpression of TRIM33 in HT22 cells by using lentivirus

In order to investigate the role of TRIM33 in Aβ production, we first constructed recombinant lentivirus containing overexpression plasmid of TRIM33 or control plasmid for the further studies, and the expression of TRIM33 in HT22 cells was detected after lentivirus infection. As shown in **Figure 1A** and **1B**, the mRNA and protein levels of TRIM33 were both significantly increased after TRIM33-lentivirus infection.

Overexpression of TRIM33 decreased intracellular and extracellular levels of Aβ

ELISA assay was used to examine the levels of Aβ in cells and culture medium. We found both intracellular and secreted levels of $A\beta_{40}$ and $A\beta_{42}$ were suppressed in TRIM33 overexpressed HT22 cells (**Figure 2A** and **2B**). These results indicated that TRIM33 could negatively regulate Aβ production in HT22 cells.

Overexpression of TRIM33 suppressed BACE1 activity

BACE1 is the most important regulator of Aβ production, therefore we examined the activity of BACE1 after TRIM33-lentivirus infection. We found the activity of BACE1 was greatly inhibited by TRIM33 overexpression in a dose-dependent manner (**Figure 3A**). Consistently, we found the levels of sAPPβ and CTFβ were decreased, however the expression of CTFα was not affected by TRIM33 overexpression (**Figure 3B**).

Overexpression of TRIM33 promoted BACE1 degradation in proteasome-dependent manner

TRIM33 was reported as an E3 ubiquitin ligase to promote nuclear β-catenin degradation [8], however the target of TRIM33 during the process of Aβ production has not been reported. We hypothesized if TRIM33 could promote BACE1 protein degradation. As shown in **Figure**

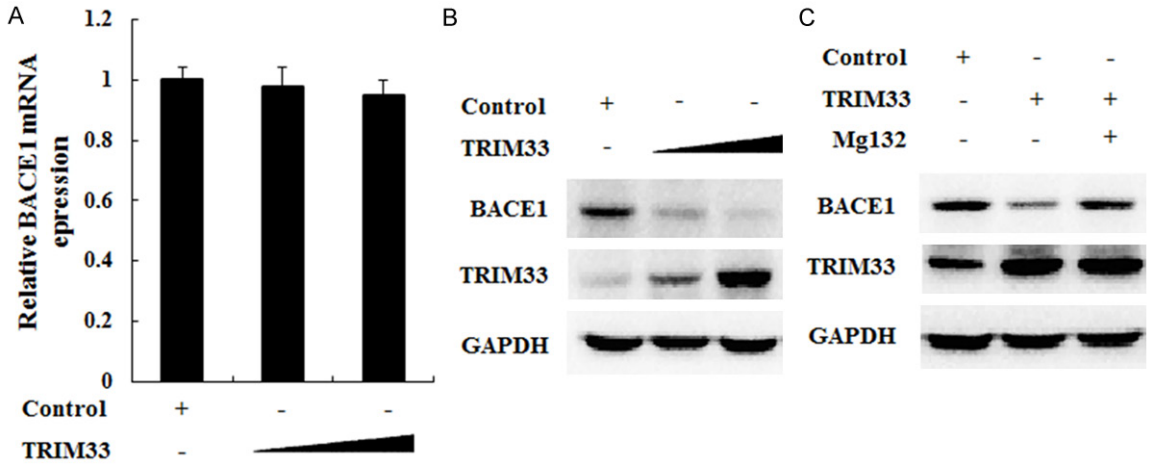

Figure 4. Overexpression of TRIM33 promoted BACE1 degradation in proteasome-dependent manner. (A and B) HT22 cells were infected with increasing amount of TRIM33-lentivirus (MOI 25 or MOI 50), 36 hours later, the mRNA (A) or protein (B) expression of BACE1 were detected (C) BACE1 protein levels were detected in the absence or presence of Mg132 in TRIM33 overexpressed HT22 cells. Data are representative of three independent experiments.

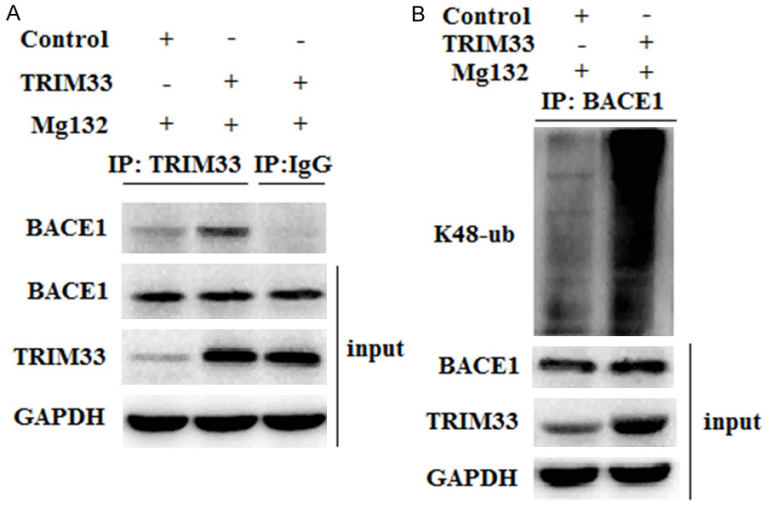

Figure 5. TRIM33 interacted with BACE1 and promoted K48-linked poly-ubiquitination of BACE1. A. Extracts of HT22 cells were subjected to immunoprecipitation with anti-TRIM33 or control anti-IgG and immunoblot analysis with individual antibodies. B. Extracts of HT22 cells were subjected to immunoprecipitation with anti-BACE1 and immunoblot analysis with K48-ub antibody. Data are representative of three independent experiments.

4A, we observed that overexpression of TRIM33 did not affect BACE1 mRNA expression. However, BACE1 protein level was obviously decreased by TRIM33 overexpression in a dose-dependent manner (**Figure 4B**). In addition, we also found that TRIM33 induced degradation of BACE1 was dependent on proteasome, because of the restored BACE1 expression in the presence of proteasome inhibitor Mg132 (**Figure 4C**).

TRIM33 interacted with BACE1 and promoted K48-linked poly-ubiquitination of BACE1

To investigate whether BACE1 is the direct target of TRIM33, we used co-immunoprecipitation experiment to detect the interaction between TRIM33 and BACE1. As shown in **Figure 5A**, BACE1 was found to directly interact with TRIM33. As a control, the interaction could not be detected with normal IgG. K48-linked protein poly-ubiquitination leads to the degradation of the corresponding protein by 26S proteasome [9], thus we examined the effect of TRIM33 on K48-linked BACE1 poly-ubiquitination level. Interestingly, we found K48-linked poly-ubiquitination of BACE1 was significantly promoted in TRIM33 overexpressed HT22 cells in the presence of Mg132 (**Figure 5B**).

Discussion

To the best of our knowledge, this study is the first to demonstrate the crucial role of TRIM33 in the process of Aβ production and Alzheimer's

disease. With the results of overexpression of TRIM33 in HT22 cells, we suggested that TRIM33 as a negative regulator of Aβ production by targeting BACE1 for proteasome-dependent degradation.

Tripartite motif-containing 33 (TRIM33) is a member of the tripartite motif superfamily that possess a RING domain, two B-boxes, and a coiled-coil domain at the N-terminus as well as a plant homeodomain (PHD) and a bromo domain at the C-terminus [10]. It has been reported that TRIM33 could interact and promote DHX33 poly-ubiquitination and be essential for cytosolic RNA-induced NLRP3 inflammasome activation [11]. Previous study also showed that TRIM33 bind an Ifnb1 regulatory region that acted as a repressor of the Ifnb1 gene in bone marrow derived macrophages (BMDMs) at the late phase of LPS stimulation [12]. Moreover, most of data from mouse models suggest a tumor suppressor role for TRIM33 [13-16]. In addition, TRIM33 has also been implicated in TGF-β signaling through its binding to phosphorylated Smad2/3 and to Smad4 to promote its ubiquitination [17]. However, the roles of TRIM33 in AD, especially in the process of Aβ production still remain unknown. In the present study, we found overexpression of TRIM33 significantly suppressed intracellular and extracellular levels of $A\beta_{40}$ and $A\beta_{42}$ in HT22 hippocampal cells, suggesting the inhibitory effect of TRIM33 in Aβ production.

BACE1 is the major neuronal β-secretase for Aβ generation. Recently, deubiquitinating enzyme USP8 was found to regulate BACE1 enzyme ubiquitination and degradation in a lysosome dependent manner [18]. Moreover, Autophagy was also found to regulate BACE1 trafficking and degradation [19]. Recent studies also revealed that miRNAs including miR-107, miR-29c and miR-135b played essential roles in the regulation of the expression of BACE1 and consequently affect the production of Aβ [20-22]. In the current study, we found TRIM33 could interact with BACE1 directly and promote K48-linked poly-ubiquitination and degradation of BACE1. Previous studies found lysosome-dependent manner is the main way for BACE1 protein degradation [18, 19, 23]. In this study, we found TRIM33 could promote BACE1 degradation in a proteasome-dependent manner, suggesting that proteasome-dependent manner may also paly crucial roles in BACE1 degradation.

In conclusion, in the current study, we investigated the role of TRIM33 in Aβ generation, and we found that TRIM33 negatively regulates Aβ production and inhibits the activity of BACE1. Furthermore, we revealed that TRIM33 interact with BACE1 and promote K48-linked poly-ubiquitination and proteasome-dependent degradation of BACE1. However, how does TRIM33 interact with BACE1 and the physiological effect of TRIM33 in AD patients still need further studies.

Disclosure of conflict of interest

None.

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