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Caspr interaction with Amyloid Precursor Protein reduces amyloid-β generation in vitro

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HIGHLIGHTS

- Caspr is abnormally expressed in the cerebral cortex of APP/PS1 mice.
- Caspr overexpression reduces level of APP, while it does not alter APP mRNA.
- Caspr interacts with APP.
- Caspr decreases Aβ40 and Aβ42 generation.

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ABSTRACT

Contactin associated protein (Caspr), an adhesion molecule, plays roles in formation of paranodal junctions in myelinated axons, neurite outgrowth, synaptic plasticity in nervous system. Here we have shown a novel function of Caspr in pathogenesis of Alzheimer's disease (AD). Caspr distributes around amyloid plaques in APP/PS1 mice. Levels of Caspr increase in the cerebral cortex of 7-month-old APP/PS1 mice comparing to wild-type littermates. Caspr decreased protein levels of APP in both HEK-293 cells stably transfected with Indiana mutant APP (V717F; HEK-APP) and CHO cells which express endogenous APP, while it did not alter mRNA levels of APP. Furthermore, Caspr co-localizes and interacts with APP. Amyloid-β (Aβ) 40 and Aβ42 generation were also reduced in HEK-APP cells by Caspr overexpression.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that results in loss of memory and cognitive function. Amyloid plaques and neurofibrillar tangles are two pathological makers of AD. Amyloid plaques are formed by aggregation of amyloid-β (Aβ) peptides [12]. Aβ induces neurotoxicity through induction of apoptosis and inflammation, disruption of calcium homeostasis, oxidative stress and activation of complement [12]. Recent reports indicate that oligomeric Aβ reduces density of spines and suppresses Long-Term Potentiation (LTP) [21]. Therefore, reduction of Aβ generation or Aβ-induced cell toxicity is considered as one of the prime strategies of AD therapy [12]. Aβ is generated from the proteolytic cleavage of Amyloid Precursor Protein (APP). APP is cleaved by β-secretase at its extracellular domain and generates a soluble extracellular fragment called sAPPβ and a transmembrane fragment CTFβ (C-terminal fragment). CTFβ is further cleaved by γ-secretase and releases Aβ [12]. The cleavage of APP by β- and γ-secretase is termed the amyloidgenic processing pathway. Proteolytic cleavage by α- and γ-secretase precludes Aβ generation and so is known as the non-amyloidogenic processing pathway [3]. Therefore, identification of proteins which modulate cleavage or expression of APP may provide potential drug targets for AD therapy.

Contactin associated protein (Caspr) is a transmembrane protein with a large extracellular domain that contains a series of laminin-G-like domains and EGF repeats and a short intracellular domain [19]. Caspr is well known for its function in the formation of axoglial paranodal junctions surrounding the nodes of Ranvier in myelinated axons through interaction with F3/Contactin and neurofascin 155 [20]. Caspr interacts with Nogo-A at the paranodes in myelinated axons [17]. Caspr also promotes neurite outgrowth by binding to Prion protein (PrP) [4]. Caspr interacts with

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AMPA receptors and regulates the trafficking of AMPA receptors to synapses [22]. Caspr was recently identified in a γ-secretase associated complex, suggesting its possible function in AD pathology [11]. This report demonstrates that Caspr distributes surrounding amyloid plaques in the cortex of APPswe/PSEN-1 (Δ9) double transgenic mice (APP/PS1). Levels of Caspr in the cerebral cortex of 7-month-old APP/PS1 mice increase when comparing compared to wild-type littermates. Co-immunoprecipitation shows that Caspr interacts with APP. Furthermore, over expression of Caspr in HEK-293 cells stably transfected with Indiana mutant APP (V717F; HEK-APP) results in a significant reduction of APP levels compared to the control cells. However, over expression of Caspr in HEK-293 cells does not affect levels of APP mRNA. Moreover, the amounts of Aβ40 and Aβ42 in HEK-APP are reduced significantly by Caspr over expression. Thus, Caspr may be a novel regulator of Aβ generation.

2. Materials and methods

2.1. Plasmids and mice

PCMV-Caspr-myc plasmid was purchased from Origene. pcDNA-Caspr4 and PCMV-Tead2 plasmids were purchased from Shanghai NiuEn Biotech., Ltd. APP/PS1 mice were obtained from the Jackson Laboratory (stock number 004462).

2.2. Antibodies

Rabbit anti-Caspr antibody was generously provided by Prof. Melitta Schachner. MAB348 (Millipore), A8717 (Sigma), 6E10 (Covance), anti-γ-tubulin (Sigma), anti-β-actin (Sigma).

2.3. Immunofluorescence staining

Immunofluorescence staining was performed as described previously [15].

2.4. Immunoprecipitation

Mouse brains were lysed with RIPA buffer (50 mM Tris–HCl, PH 7.4, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) containing protease inhibitors and phosphorylation inhibitors. Brain lysates was incubated with antibodies together with protein A-coupled sepharose. The immunocomplexes were washed with PBS containing 0.5% NP-40, and re-suspended and boiled in 2× Laemmli sample buffer. The supernatant was subjected to immunoblotting analysis.

2.5. Aβ ELISA analysis

Aβ ELISA assays were performed using human Aβ40/Aβ42 ELISA kits according to the instructions of the manufacturer (Invitrogen).

2.6. Cell viability determination

Cell viability was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described previously [25].

2.7. Quantitative real-time PCR

Quantitative RT-PCR (qPCR) was performed using FastStart Universal SYBR Green Master (Roche) in combination with target-specific primers for human Caspr (5′-CCCTGAAGCCATTTGTAGTGT-3′, 5′-GGAGCAGAGGTCCTGAAGTA-3′), human APP (5′-GAGGAGGATGACTCGGATGTCT-3′, 5′-AGCCACTTCTTCCTCCTCTGCT-3′), and human GAPDH (5′-CAAGGTCATCCATGACAACTTTG-3′, 5′-GTCCACCACCCTGTTGCTGTAG-3′). APP mRNA level was calculated relative to GAPDH using the delta-delta computed tomography method.

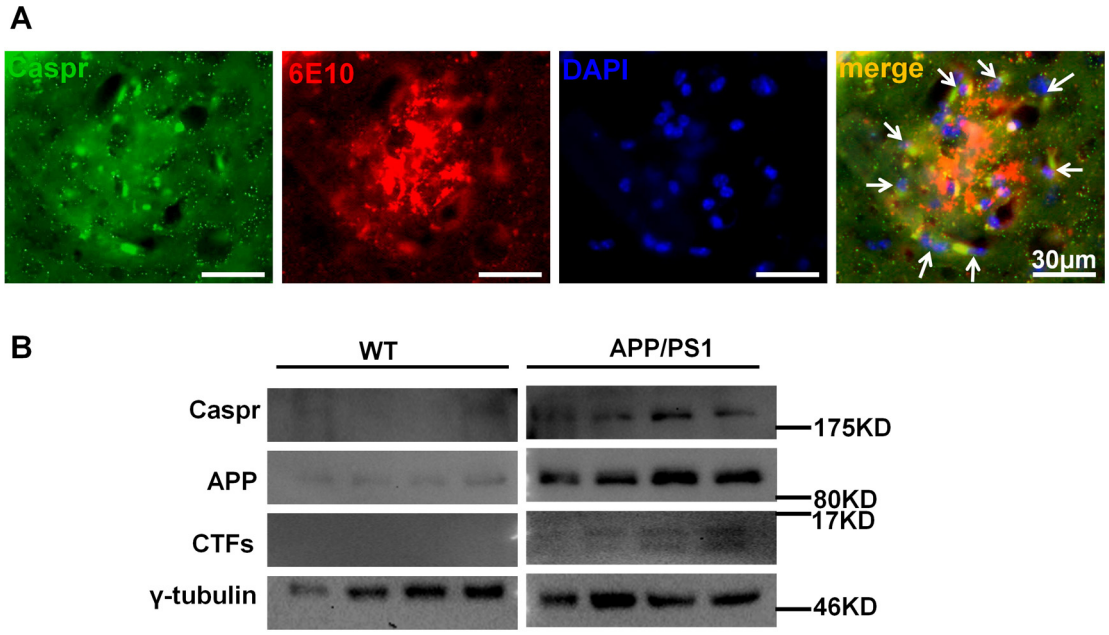

Fig. 1. Abnormal expression of Caspr in the cerebral cortex of APP/PS1 mice. (A) Immunofluorescence staining with antibodies against Caspr (Green) and Aβ (6E10, red) in the coronal cerebral cortex of 7-month-old APP/PS1 mice. Scale bar: 30 μm. (B) Immunoblotting analysis of Caspr expression in the cerebral cortex of 7-month-old APP/PS1 and wild-type littermates. γ-Tubulin was probed as the loading control. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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