Hippocampal overexpression of Down syndrome cell adhesion molecule in amyloid precursor protein transgenic mice

Y.L. Jia¹,2, Z.X. Fu2, B.H. Zhang2 and Y.J. Jia¹

¹Department of Neurology, the First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan Province, China
²Department of Neurology, The Central Hospital of Kaifeng, Kaifeng, Henan Province, China

Abstract

Down syndrome cell adhesion molecule (DSCAM) is located within the Down syndrome critical region of chromosome 21. DSCAM is a broadly expressed neurodevelopmental protein involved in synaptogenesis, neurite outgrowth, and axon guidance. We previously demonstrated DSCAM overexpression in the cortex of amyloid precursor protein (APP) transgenic mice, suggesting possible regulatory interactions between APP and DSCAM. APP mice exhibit deficits in hippocampus-dependent learning and memory. In this preliminary study, we examined age-related changes in DSCAM expression within the hippocampus in 16 APP transgenic mice (1, 3, 6 and 12 months old). Hippocampus-dependent spatial memory was assessed in APP mice and age-matched wild type littersmates (WTs) using the Morris water maze (MWM). The cellular distribution of hippocampal DSCAM and total expression at both mRNA and protein levels were measured by immunohistochemistry, qRT-PCR, and western blotting, respectively. APP mice exhibited spatial memory deficits in the MWM. Intense DSCAM immunoreactivity was observed in the dentate gyrus granule cell layer and hippocampal stratum pyramidale. Total hippocampal DSCAM mRNA and protein expression levels were substantially higher in APP mice than WTs at 1 and 3 months of age. Expression decreased with age in both groups but remained higher in APP mice. DSCAM is overexpressed in the hippocampus over the first 12 months of life in APP mice, but especially during maturation to adulthood. In conclusion, these results suggest an association between DSCAM and APP mice, which is characterized by neuropathology and behavioral deficits. These results provide some clues for future studies on the role of DSCAM overexpression in the precocious cognitive decline observed in APP transgenic mice.

Key words: Down syndrome cell adhesion molecule; Amyloid precursor protein; Transgenic mouse; Hippocampus; Dementia

Introduction

Down syndrome (DS) phenotypes are determined by trisomy of a particular region of chromosome 21 known as the Down syndrome critical region (DSCR). Increased copy number of the genes in the DSCR are believed to cause the clinical manifestations of DS through overexpression of their protein products (1). Neuropathological research has identified congenital and developmental abnormalities associated with precocious dementia in DS. The former include a decrease in granular cell number in the cerebral cortex, most likely the aspinous stellate cell type (2). The DS brain exhibits decreased synaptic spine number and abnormal spine morphology from infancy through adulthood (3,4). Subjects with DS also develop senile plaques and neurofibrillary tangles in the prefrontal and hippocampal cortex with concomitant age-related dementia resembling that observed in Alzheimer’s disease (AD).

Correspondence: Y.J. Jia: <yanjie5113@163.com>

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protein (APP) transgenic mouse model of AD. The finding that APP overexpression is associated with DSCAM overexpression suggests regulatory interactions between APP and DSCAM, or possibly reactive DSCAM overexpression due to the neuropathological sequelae of APP overexpression, such as deposition of AD-like plaques and neurofibrillary tangles. APP mice also exhibit age-related deficits in hippocampal learning and memory, even prior to substantial Aβ deposition (13). Thus, we examined age-related changes in DSCAM expression in the hippocampus of the APP mouse.

**Material and Methods**

**Animals**

APP transgenic mice (C57BL/6J-APP mice; n=16/group, 1–12 months) were obtained from the Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College (CAMS&PUMC). The mice were generated using a full-length mutant human APP cDNA [695 V717I]. The exogenous Aβ was approved by the Ethics Committee of the First Affiliated Hospital, Zhengzhou University.

**Immunohistochemistry**

Paraffin-embedded sections were deparaffinized and incubated for 9 min at 90°C in 0.01 mol/L sodium citrate buffer solution, pH 6.0, for antigen retrieval. The sections were immersed in 3% hydrogen peroxide in deionized water for 10 min to quench endogenous peroxidase activity. Non-specific binding was blocked by incubation for 30 min in normal goat serum at room temperature (RT). Sections were incubated overnight at 4°C in phosphate buffered saline (PBS) containing anti-DSCAM antibody (1:50; N-16, sc-79437; Santa Cruz Biotechnology, USA) (2), followed by a 30-min incubation in biotinylated goat anti-rabbit IgG antibody (Zymed Laboratories, USA) at RT. Sections were incubated with horseradish peroxidase (HRP)-conjugated streptavidin (Zymed) for 30 min at RT. Between staining steps, the sections were thoroughly washed in PBS for 2 min. The specimens were counterstained with hematoxylin and mounted with appropriate mounting medium.

**Protein extraction and western blotting**

For protein extraction, hippocampus tissue samples were thawed and homogenized in lysis buffer containing 40 mg/mL CHAPS, 50 mM Tris-HCl, pH 7.6, 0.4808 mg/mL urea, 1 mM phenylmethylsulfonyl fluoride, 5 μg/mL apro tinin (Amresco, USA), and 5 μg/mL leupeptin (Amresco). Lysates were centrifuged at 14,000 g for 5 min at 4°C, and the supernatants were collected. Supernatant proteins (30 or 60 μg/lane) were separated on 10% SDS-polyacrylamide gels and then electrophoretically transferred to nitrocellulose membranes (Invitrogen, USA). Membranes were blocked in 8% skim milk for 1 h at RT, then incubated in anti-DSCAM (1:1,000; N-16, sc-79437; Santa Cruz) overnight at 4°C and anti-β-actin as an internal reference (1:1,000; sc-81178; Santa Cruz). Immunolabeled membranes were then incubated in HRP-labeled goat anti-rabbit
IgG (1:4,000; Zymed) for 45 min at RT. Bands were visualized using enhanced chemiluminescence and autoradiography (Hyperfilm-ECL; Amersham, Sweden). We analyzed band optical density using an image analysis system (Gel-Pro Analyzer Version 3.0, Media Cybernetics Inc., USA) and calculated the ratio of DSCAM to β-actin optical density for estimation of DSCAM expression.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was extracted from tissue blocks using the RecoverAll Total Nucleic Acid Isolation kit for FFPE (Thermo Fisher Scientific, USA) according to the manufacturer’s instructions. For determination of RNA concentration and purity, the solution (2 μL) was tested with a NanoDrop 2000 (Thermo Fisher Scientific) at absorbance 260/280. A value of 1.8–2.0 was considered acceptable. The RNA samples were stored at –80°C.

RNA was reversely transcribed using the RevertAid First Strand cDNA synthesis kit (#K1622; Thermo Fisher Scientific), according to the manufacturer’s instructions, using 2 μL of RNA sample, 1 μL of oligo(dT)18 primer, 4 μL of 5 x reaction buffer, 1 μL of Riboblock RNase inhibitor, 2 μL of 10 mM dNTP mix, 1 μL of RevertAid M-MuLV reverse transcriptase, and 9 μL of nuclease-free water. Reaction conditions were: 1) 25°C for 5 min; 2) 42°C for 60 min, and 3) 70°C for 5 min. The cDNA concentration was measured with the NanoDrop 2000 (Thermo Fisher Scientific). The cDNA was stored at –20°C.

The primers for qRT-PCR are shown in Table 1 and were synthesized by Shanghai Shenggong Biology Engineering Co., Ltd. (China). The reaction mixture contained 10 μL of DreamTaq Green PCR Master Mix, 1 μL of each primer, 1 μL of template cDNA, and 7 μL of nuclease-free water. Amplification was performed in a LightCycler 1.5 qRT-PCR system (Roche Diagnostics, Switzerland). The reaction conditions were: 1) 95°C for 3 min; 2) 32 cycles of 95°C for 30 s, 57°C for 30 s, and 72°C for 35 s, and 3) 72°C for 10 min. β-actin was used as the internal reference. Each sample was tested six times and the average value was used for analysis. The 2^-ΔΔCT method was used to represent the relative expression of the target gene.

Statistical analysis

SPSS 10.0 (IBM, USA) was used for all analyses. Results are reported as means ± SD. One-way analysis of variance was used to compare group means followed by least significant difference (LSD) t-tests for pair-wise comparisons. A P < 0.05 was considered to be statistically significant.

Results

Learning and memory functions

We first confirmed that the APP transgenic mice examined in this study exhibited age-dependent deficits in hippocampus-dependent spatial reference memory, as shown previously (13). Spatial memory was assessed in
the Morris water maze by a single probe trial measuring the mean time to find the quadrant formerly containing the escape platform (target quadrant). APP mice demonstrated significantly longer search times compared with age-matched WTs for all age groups tested (Figure 1).

**DSCAM expression in mice**

Immunohistochemistry of brain sections revealed that DSCAM is highly expressed in the cerebral cortex, granule layer of the dentate gyrus, pyramidal cell layer of Ammon’s horn of the hippocampus (stratum pyramidale), thalamus, and cerebellar Purkinje cells of both WT (Figure 2A,C,E) and APP transgenic mice (Figure 2B,D,F).

**DSCAM expression in the hippocampus by age and genotype**

RT-PCR (Figure 3B) and western blotting (Figure 3A) demonstrated substantial overexpression of DSCAM mRNA and protein in the hippocampus of APP mice compared with WTs during maturation to adulthood (1 and 3 months of age; P<0.05). The results of qRT-PCR showed that DSCAM mRNA expression in transgenic mice was higher than that in

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**Figure 2.** Immunohistochemistry of mouse Down syndrome cell adhesion molecule (DSCAM) in sagittal brain sections from 3-month-old mice. DSCAM is expressed in the hippocampus of (A) wild-type (WT) and (B) amyloid precursor protein (APP) transgenic mice. ca1, ca2, ca3: CA1, CA2 and CA3 regions of Ammon’s horn; dg: dentate gyrus. DSCAM expression in cerebellar Purkinje cell layers of (C) WT and (D) APP transgenic mice (indicated by arrows). DSCAM expression in middle-layer pyramidal neurons (arrows) of cerebral cortex of (E) WT and (F) APP transgenic mice. Brown staining is DSCAM; blue staining is hematoxylin.
wild-type mice of the same age ($P < 0.05$). Intragroup comparison showed that DSCAM mRNA expression was higher at 1 and 3 months compared with that observed at 6 and 12 months ($P < 0.05$), while the difference was not significant between 1 and 3 months ($P > 0.05$; Figure 3).

Discussion

In this study, it was found that DSCAM was markedly overexpressed in the hippocampus of APP transgenic mice, suggesting that dysregulation of DSCAM may contribute to the neuropathological and behavioral phenotype of APP mice. Indeed, these mice demonstrate deficits in learning and memory even before detectable plaque formation (13), during which hippocampal DSCAM expression is elevated by $\sim 50\%$ relative to levels in WT mice. Moreover, DSCAM expression was enriched in highly plastic hippocampal neurons critical for learning and memory (14). In *Aplysia californica*, postsynaptic overexpression of a truncated DSCAM containing the intracellular domain blocked the clustering of $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, which are necessary for expression of long-lasting forms of synaptic plasticity that underlie learning (15). Thus, overexpression of DSCAM may contribute to certain APP phenotypes, although additional studies in mice selectively overexpressing DSCAM are needed to test this hypothesis.

DSCAM immunoreactivity has been found in the core of senile plaques and in surrounding synapses, suggesting some role for DSCAM in plaque formation (16). In addition, DSCAM overexpression may contribute to DS pathogenesis through inhibitory action on synaptogenesis and neurite outgrowth (4,8–12,17–22). In fact, DSCAM trisomy was found in a human DS patient with no signs of AD-like neuropathology (23), suggesting that DSCAM overexpression alone may lead to cognitive deficits, but additional studies are necessary since this patient was also trisomic for other genes on chromosome 21.

In the present study, DSCAM was highly expressed in the cerebral cortex, hippocampus, thalamus, cerebellar Purkinje cells, and brainstem neurons of APP transgenic mice (2), which are regions involved in a variety of higher-order functions such as somatosensory information processing, voluntary movement, motor learning, explicit learning, and memory. Hippocampal DSCAM expression was significantly higher in APP transgenic mice compared with age-matched WT controls, particularly during young adulthood. Explicit memory depends on intact fibers linking dentate granule cells with hippocampal pyramidal cells as well as pathways linking the hippocampal formation with other regions such as the frontal cerebral cortex. Both over- and under-expression of the *Drosophila* DSCAM homolog resulted in defective axon guidance and abnormal neural network formation (24). That neurons may be particularly sensitive to DSCAM dosage is further suggested by the finding that 10–13% of embryos heterozygous for DSCAM show defects in Bolwig’s nerve axon guidance (24). In addition, *A. californica* and *Drosophila melanogaster* indicate that the

![Figure 3](image-url)

**Figure 3.** Expression of Down syndrome cell adhesion molecule (DSCAM) mRNA and protein in hippocampus of mice in different groups. DSCAM expression was measured by western blot (A) and qRT-PCR (B) in hippocampal lysates from amyloid precursor protein (APP) transgenic (Tg) and wild-type (WT) mice (1, 3, 6, and 12 months [mo] old). *P < 0.05 vs the age-matched WT group; #P < 0.05 vs 3- and 12-mo mice of the same genome type (n=10) (ANOVA followed by least significant difference t-tests for pair-wise comparisons).
increased production of neural proteins of the immunoglobulin superfamily inhibits synapse growth or migration under some conditions (25). It is thus possible that elevated DSCAM may contribute to the abnormal gross architecture and cortical lamination defects seen in DS patients (26).

We propose that hippocampal DSCAM overexpression may be involved in learning and memory defects in APP transgenic mice, possibly by altering the adhesive properties of neural cells, inhibition of synaptogenesis and/or neurite outgrowth, disturbed axon guidance, and the proper formation of neuronal connections. In addition, DSCAM may facilitate Aβ plaque formation or enhance the neurodegenerative potential of these inclusions.

This study is not without limitations. Even if an association were observed between DSCAM expression and APP mice, this study was not designed to assess the cause-effect relationship leading to this association. Studies are warranted to investigate whether DSCAM is overexpressed in the hippocampus of DS and AD patients, how APP and DSCAM interact at both the expression and functional levels, and if and how DSCAM alone alters hippocampal synaptoplasmic processes underlying learning and memory. These results suggest an association between DSCAM and APP mice, which is characterized by neuropathology and behavioral deficits. These results provide some clues for future studies on the role of DSCAM overexpression in the precocious cognitive decline observed in APP transgenic mice.

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