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Brain Research

Research Report

DSCAM and DSCAML1 regulate the radial migration and callosal projection in developing cerebral cortex

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ARTICLE INFO

Article history:
Accepted 27 October 2014
Available online 4 November 2014

Keywords:
Cerebral cortex
DSCAM
Dendritic arborization
Radial migration
Callosal projection

ABSTRACT

Down syndrome cell adhesion molecule (Dscam) is essential for self-avoidance and tiling of dendritic development in sensory neurons in *Drosophila*. Recent studies also show that DSCAM together with its closely related protein DSCAML1 functions in dendritic self-avoidance of a certain types of interneuron in mammalian retina. However, the functions of these DSCAMs in developing mammalian cerebral cortex are not well understood. Here we reduced the expression of DSCAM or DSCAML1 in mouse cortical neurons by RNA interference both *in vitro* and *in vivo*. We found that knockdown of DSCAM or DSCAML1 increases the complexity of proximal dendritic branching, and impedes the axon growth in cultured neurons. *In vivo* knockdown experiments showed that both DSCAM and DSCAML1 contribute to normal radial migration and callosal projection during the postnatal development. Our results indicate an important role of DSCAM and DSCAML1 in the development of cortical neural network.

1. Introduction

Down syndrome cell adhesion molecule (DSCAM) was first isolated from the human chromosomal band 21q22.2–22.3, which is in a critical region for many of the neurological phenotypes of Down syndrome (Yamakawa et al., 1998). One of the *Drosophila* homologs (Dscam1) was found to generate thousands of isoforms from a single genomic locus through alternative splicing (Schmucker et al., 2000). These different isoforms of Dscam1 exhibit exquisite isoform-specific homophilic binding

Abbreviations: CNS, central nervous system; DIV, days *in vitro*; dpc, days post-coitum; DSCAM, down syndrome cell adhesion molecule; DSCAML1, down syndrome cell adhesion molecule like 1; RNAi, RNA interference; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; shRNA, short hairpin RNA

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http://dx.doi.org/10.1016/j.brainres.2014.10.060
0006-8993/

which mediates cell recognition and dendritic self-avoidance in the dendritic development of sensory neurons in *Drosophila* (Hattori et al., 2007; Hughes et al., 2007; Matthews et al., 2007; Soba et al., 2007), while Dscam2 mediates tiling between processes of a subset of neurons in the fly visual system (Millard et al., 2007). In addition, Dscam proteins were shown to regulate the synaptic targeting and specificity of sensory neurons (Cvetkovska et al., 2013; Kim et al., 2013; Millard et al., 2010).

In contrast to the fly Dscam1, the vertebrate DSCAM does not undergo extensive alternative splicing and only two paralogs, DSCAM and DSCAM-like-1 (DSCAML1) were identified (Agarwala et al., 2001b). These two genes are abundantly distributed in the developing and adult mouse central nervous system (CNS) including the retina, olfactory bulb, cerebral cortex, hippocampus, cerebellum and spinal cord (Agarwala et al., 2001a, 2001b; Barlow et al., 2002). It has been reported that DSCAM and DSCAML1 promote the layer-specific targeting in the chick retina (Yamagata and Sanes, 2008) and dendritic self-avoidance as well as tiling in mouse retina (Fuerst et al., 2008, 2009, 2012). In addition, DSCAM was identified as a netrin-1 receptor in commissural axon pathfinding (Liu et al., 2009; Ly et al., 2008), although this notion was recently challenged (Palmesino et al., 2012). Recent reports also showed that DSCAM and DSCAML1 affect the dendritic arborization (Alves-Sampaio et al., 2010; Cui et al., 2013; Maynard and Stein, 2012).

In order to investigate the function of DSCAM and DSCAML1 more clearly in the mammalian brain, we used the RNA interference (RNAi) to knockdown DSCAM or DSCAML1 in dissociated cortical neurons and developing mouse cerebral cortex. We found that downregulation of these two genes increases the complexity of dendritic arborization and inhibits axon growth *in vitro*. The *in utero* electroporation showed that DSCAM and DSCAML1 affect the radial migration and callosal projection of cortical neurons to different extent. Besides, expression of a shRNA-resistant DSCAM or DSCAML1 partially rescues the *in vitro* and *in vivo* phenotypes. Thus, DSCAM and DSCAML1 play similar but important roles in the development of mouse cortical neurons.

2. Results

2.1. *Expression of DSCAM and DSCAML1 in the cerebral cortex*

Previous studies showed that DSCAM and DSCAML1 are widely expressed in developing and adult mouse brain, including the cerebral cortex (Barlow et al., 2002). In order to investigate the functions of these two genes, we firstly examined their expression in the mouse cerebral cortex at postnatal day 7 (P7) using *in situ* hybridization. Similarly, our results showed both *DSCAM* and *DSCAML1* were widely expressed in all layers of cerebral cortex (Fig. 1A–D). However, two *DSCAM* paralogs exhibit differential enrichment across the cortical layers: *DSCAM* shows a relatively higher expression in deep layers (V) (Fig. 1B) and *DSCAML1* is more abundant in superficial and deep layers (II/V) (Fig. 1D). Besides, the immunohistochemical staining of DSCAM (Fig. S1) showed a similar expression pattern in cerebral cortex to that revealed by *in situ* hybridization (Fig. 1B). It is noted that the distinct expression pattern of *DSCAM* and *DSCAML1* is more evident in the CA1 region of hippocampus and the thalamus (Fig. 1A and C). Thus, the wide expression of both DSCAMs in the cerebral cortex in the end of first postnatal week suggests their potential roles in the cortical development.

2.2. *Knockdown of DSCAM or DSCAML1 increases the dendritic branching and decreases the axonal length of cortical neurons in vitro*

We started to investigate the role of DSCAMs using a loss-of-function approach. We generated two short hairpin RNA (shRNA) constructs targeting against *DSCAM* and *DSCAML1*, respectively (hereafter designated as shDSCAM and shDSCAML1). HEK293T cells were transfected with either a mixture of shDSCAM and pCAG–DSCAM or shDSCAML1 and pCAG–DSCAML1 plasmids, then DSCAM or DSCAML1 protein level was measured by western blots to test the knockdown efficiency of shRNA vectors. Comparing with the protein level in pSUPER-transfected cells (Fig. 2A and B), expressing shRNA significantly inhibited the expression of DSCAM or DSCAML1 on respective condition. We also generated two expression vectors of DSCAM or DSCAML1, which was resistant to shDSCAM or shDSCAML1, respectively (designated as pCAG–DSCAM^{R} and pCAG–DSCAML1^{R}). Using western blots, we found that shDSCAM had no knockdown effect on the expression of pCAG–DSCAM^{R} in HEK293 cells (Fig. 2A), and similarly, shDSCAML1 did not knock down the resistant form of DSCAML1 expressed by pCAG–DSCAML1^{R} (Fig. 2B).

After confirmation of the efficacy of shDSCAM and shDSCAML1 plasmids *in vitro*, we next examined their effects on cultured cortical neurons. Transfection of pSUPER or shRNA constructs was performed at DIV7 and neuronal morphology was examined at DIV9. We found that down-regulation of DSCAM or DSCAML1 caused an increase of complexity of dendritic arborization (Fig. 2C, D, and F). Sholl analysis showed that the number of intercrosses was elevated in the range of 20–40 μm distance from soma, indicating a dramatic increase of dendritic branching in the proximal dendrites of both shRNAs-transfected neurons comparing with that in control (Fig. 2H). Cotransfection of shRNA vectors and resistant form of cDNA in cortical neurons significantly decreased the dendritic branching than that in shRNA-transfected alone cells (Fig. 2D–G), as shown by the Sholl analysis (Fig. 2H). These results revealed that shDSCAM and shDSCAML1 exerted impacts on the dendritic arborization, especially the maintenance of dendritic branches. In order to test the effect of shRNAs on the initial dendritic growth, we performed a transfection by electroporation to dissociated neurons from mouse cortex before plating on the coverslips. Dendritic morphology was analyzed at DIV4. Conversely, we found an overall decrease of dendritic arborization for both shDSCAM and shDSCAML1, as compared to the control (Fig. S2). These data showed that DSCAM and DSCAML1 had different effects on the dendritic growth and arbor maintenance during development.

We then examined the functions of DSCAMs in the axonal development *in vitro*. In a similar way, dissociated neurons were