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Research Report

Synaptic adhesion molecule OBCAM; synaptogenesis and dynamic internalization

Mayumi Yamada[a], *Takashi Hashimoto*[a], *Noriko Hayashi*[a], *Maiko Higuchi*[b], *Akira Murakami*[b], *Toshihiro Nakashima*[a], *Shohei Maekawa*[c], *Seiji Miyata*[a,*]

[a]*Department of Applied Biology, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan*
[b]*Department of Biomolecular Engineering, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan*
[c]*Division of Bioscience, Graduate School of Science and Technology, Kobe University, Nada-ku, Kobe 657-8501, Japan*

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ABSTRACT

Opioid-binding cell adhesion molecule (OBCAM) is the member of the IgLON family, a subgroup of the immunoglobulin superfamily. In the present study, the functions and dynamics of OBCAM were investigated in hippocampal neurons *in vitro*. Western blotting revealed that OBCAM expression was low at early stages of culture but it was increased as culture development. Double labeling immunofluorescence microscopy showed that OBCAM immunoreactivity was localized mainly at postsynaptic spines labeled with phalloidin and anti-PSD-95. The inhibition of OBCAM function with the specific antibody resulted in a significant decrease in the number of synapses on dendrites compared with control mouse IgG. The suppression of OBCAM expression using the antisense oligodeoxynucleotide also impaired the formation of synapses compared with control universal ones. The overexpression of OBCAM mRNA using a plasmid vector augmented the formation of synapses. Moreover, the internalization of OBCAM was promoted with increased neuronal activity by 4-aminopyridine. This internalization was reduced with the treatment of filipin, a sterol agent, indicating that this process is a raft-dependent pathway. These results indicate that OBCAM is a synaptic cell adhesion molecule concerning synaptogenesis and its surface localization is dynamically regulated in response to neuronal activity.

1. Introduction

At a synapse, presynaptic and postsynaptic specialization is considered to be connected by cell adhesion molecules (CAMs) that are concerned with synaptogenesis during neuronal development and synaptic plasticity in learning and memory (Yuste and Denk, 1995; Matus, 2000;Goda and Davis, 2003; Rougon and Hobert, 2003). N-cadherin moves in and out of the pre- and postsynaptic membranes in response to synaptic activity (Tanaka et al., 2000) and the blockage of N-cadherin in

* *Corresponding author*. Fax: +81 75 724 7760.
E-mail address: smiyata@kit.ac.jp (S. Miyata).

Abbreviations: 4-AP, 4-aminopyridine; CAMs, cell adhesion molecules; DIV, days *in vitro*; DRG, dorsal root ganglia; GPI, glycosylphosphatidylinositol; IgSF, immunoglobulin superfamily; LAMP, limbic system-associated membrane protein; Ntm, neurotrimin; ODN, oligodeoxynucleotides; OBCAM, opioid-binding cell adhesion molecule; PBS, phosphate-buffered saline; PBST, PBS containing 0.3% Triton X-100; PFA, paraformaldehyde; TBST, Tris-buffered saline containing 0.5% Tween-20

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hippocampal neurons during synaptogenesis results in alterations of dendritic spine morphology (Togashi et al., 2002). SynCAM mediates homophilic adhesion and initiates synapse formation with the neuroligin–neurexin system (Biederer et al., 2002). Inhibition of nectin-based adhesion in hippocampal neurons results in a decrease in synaptic size and a concomitant increase in synaptic number (Mizoguchi et al., 2002). Much more synaptic CAMs and complicated mechanisms should be participated in these processes, although a few CAMs have been identified as synaptic CAMs concerning synaptogenesis and plasticity.

Notable CAMs are the limbic system-associated membrane protein (LAMP) (Levitt, 1984), opioid-binding cell adhesion molecule (OBCAM) (Schofield et al., 1989), neurotrimin (Ntm) (Struyk et al., 1995), and Kilon (Funatsu et al., 1999), which comprise the IgLON family, a subgroup of the immunoglobulin superfamily (IgSF). Each of the IgLON members is highly glycosylated and attached to the lipid membrane by the glycosylphosphatidylinositol (GPI)-anchor, and has three C2-type Ig-like domains. The first identified IgLON protein is LAMP, which is expressed in the limbic cortex and the medial nucleus of the thalamus, parts of the mammalian brain involved in behavior and memory (Levitt, 1984; Horton and Levitt, 1988; Pimenta et al., 1996; Reinoso et al., 1996). Subsets of fetal neurons are LAMP-immunoreactive on their soma, dendrites, and axons, however, the LAMP immunoreactivity eventually lost postnatally as neuronal maturation to maintain at the postsynaptic elements (Horton and Levitt, 1988; Zacco et al., 1990). Ntm is shown to have a largely complementary distribution pattern to that of LAMP (Struyk et al., 1995); LAMP expression is principally confined to the limbic cortex, whereas Ntm is expressed mostly in the sensorimotor cortex. Several *in vitro* experiments have revealed that LAMP stimulates neurite outgrowth of limbic axons (Pimenta et al., 1995), and modulates branching and layer specificity of thalamic axon systems (Mann et al., 1998; Zhukarera and Levitt, 1995), but blocks neurite outgrowth of dorsal root ganglia (DRG) neurons. On the other hand, Ntm supports DRG neurite outgrowth but inhibits neurite extension of sympathetic neurons (Gil et al., 1998, 2002). These studies indicate that LAMP and Ntm promote neurite outgrowth via homophilic adhesion mechanism, while Ntm inhibits the outgrowth via a heterophilic mechanism.

OBCAM was originally purified rat brains as an opioid-binding protein (Cho et al., 1986), however, later it is recognized as one of CAMs belonging to the IgLON subgroup on the basis of cDNA sequence (Schofield et al., 1989). Two isoforms, 46 and 51 kDa are derived from different addition of N-linked carbohydrate chains, since N-glycanase treatment is shown to change two isoforms to a single band (Hachisuka et al., 1996). The OBCAM is shown to have a much more restricted distributional pattern, with the higher expression levels in the cerebral cortex and hippocampus, compared with that of LAMP and Ntm (Struyk et al., 1995; Miyata et al., 2003a).

IgLON proteins have been supposed to be concerned with synaptic plasticity in learning and memory, since the expression of IgLON proteins is high in the limbic system and cerebral cortex (Levitt, 1984; Pimenta et al., 1996; Struyk et al., 1995; Funatsu et al., 1999). However, no studies have

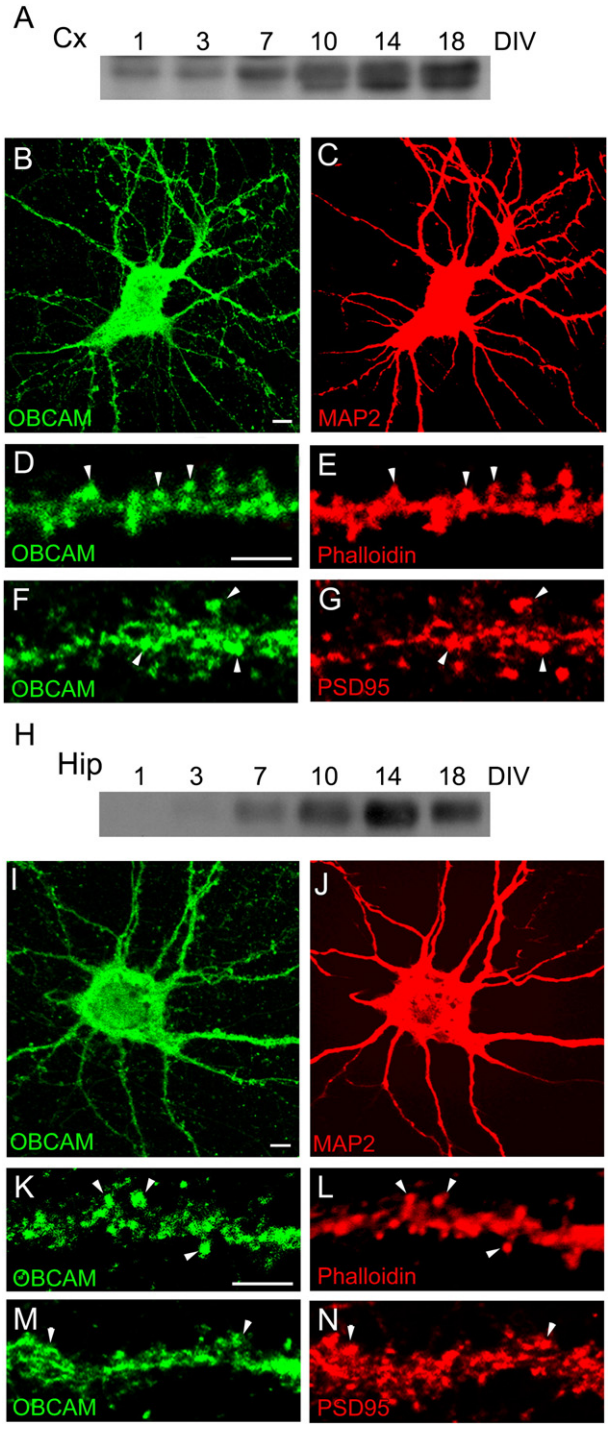

Fig. 1 – Polarized targeting of OBCAM to dendritic spines in cultured neurons. Expression of OBCAM in cortical and hippocampal neurons was analyzed by immunoblotting and immunocytochemistry. Expression of OBCAM was increased as neuronal culture development (A and H). Cortical (Cx, B–G) and hippocampal (Hip, I–N) neurons from 18 DIV were fixed and immunostained with the anti-OBCAM antibody. OBCAM expression was strong at MAP2-positive dendrites on 18 DIV (B, C, I, and J). High magnification view revealed OBCAM accumulation at spines (arrowheads in D–G and K–N) which were visualized with rhodamine–phalloidin or anti-PSD-95 antibody. Scale bars, 5 μm.