Expression and localization of the LGN protein in the mouse brain with reference to its relationship with $G_{\alpha_{i2}}$

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Abstract  It has been suggested that the LGN protein is associated with $G_{\alpha_{i2}}$ by the yeast two-hybrid system and in vitro pull-down assay. To determine the functions of LGN in the central nervous system, we examined the expression and localization of LGN in mouse brain by immunoblotting and immunofluorescence microscopy. By immunoblotting, almost similar amounts of LGN were detected in the olfactory bulb, cerebral cortex, hippocampus, and cerebellum of the adult mouse brain, and the levels of the postnatal LGN expression in the whole brain were fairly constant. Immunofluorescence microscopy showed that LGN is localized in nuclei of the neurons in the olfactory bulb, cerebral cortex, and hippocampus, but in both nuclei and cytoplasm of Purkinje cells in the cerebellum. On the other hand, $G_{\alpha_{i2}}$ was distributed throughout the neuronal elements except for the nuclei. Thus, LGN and $G_{\alpha_{i2}}$ were colocalized in the cytoplasm of Purkinje cells, but not in other neurons examined. These results suggest that LGN may be involved not only in the $G_{\alpha_{i2}}$-mediated signaling but also in other signaling pathways.

Key words  LGN · $G_{\alpha_{i2}}$ · Mouse brain · Immunoblotting · Immunofluorescence microscopy

Introduction

The guanine nucleotide binding proteins (G-proteins) play important roles in the signal transduction pathways for proliferation and differentiation by coupling membrane receptors and intracellular signal-transducing molecules.$^{12}$ Heterotrimeric G proteins are composed of $\alpha$, $\beta$, and $\gamma$ subunits and are divided into four major classes according to their $\alpha$-subunits: $G_{\alpha_i}$, $G_{\alpha_s}$, $G_{\alpha_q}$, and $G_{\alpha_{i2}}$. $G_{\alpha_{i2}}$ has been reported to be involved in inhibition of adenylyl cyclase, stimulation of inwardly rectifying and ATP-sensitive $K^+$ channels, stimulation of the MAP kinase pathway, and regulation of neonatal growth and development.$^{3-6}$ $G_{\alpha_{i2}}$ is also known to be involved in malignant transformation. Rat-1 cells transfected with the $gip2$ oncogene, which encodes a mutant $G_{\alpha_{i2}}$, form tumors in nude mice.$^7$ This mutant $G_{\alpha_{i2}}$ is inactive as GTPase and thus functions as a constitutively active mutant. Furthermore, GTPase-inactivating $G_{\alpha_{i2}}$ mutants have been found in ovarian and adrenal human tumors.$^8$ Mice deficient for $G_{\alpha_{i2}}$ display growth retardation and develop a lethal diffuse colitis that closely resembles ulcerative colitis in human, including the development of adenocarcinoma of the colon.$^9$

In an attempt to identify proteins that interact with $G_{\alpha_{i2}}$, Mochizuki et al.$^{10}$ performed the yeast two-hybrid system using $G_{\alpha_{i2}}$ as bait and identified LGN, a mosaic protein with 10 repeats of Leu-Gly-Asn (LGN) at its amino-terminus and 4 repeats of Asp-Asp-Gln-Arg (DDQR) at its carboxy-terminus. Although LGN was shown to interact with $G_{\alpha_{i2}}$ in vitro, their actual binding and interaction in vivo have not been investigated, and the function of LGN is not known. In the present study, we prepared anti-LGN antibody and examined its expression and localization in the mouse brain, with special reference to its relationship with $G_{\alpha_{i2}}$.

Materials and methods

Antibodies

A fragment of human LGN (amino acids 119–352) was prepared according to the method of Smith and Johnson.$^{11}$ Polyclonal rabbit anti-LGN antibody was produced by immunizing rabbits with the human LGN fragment fused to
glutathione S-transferase (GST). Antibody was purified by affinity chromatography using columns to which antigens used for immunization had been linked. Polyclonal rabbit antihuman G\textsubscript{\alpha}i\textsubscript{2} antibody was purchased from Santa Cruz Biotechnology (California, CA, USA). According to the manufacturer’s instructions, the antigen domain used for the production of anti-G\textsubscript{\alpha}i\textsubscript{2} antibody differs from corresponding mouse sequence by two amino acids.

Immunoblotting

Brain (whole brain, olfactory bulb, cerebral cortex, hippocampus, and cerebellum), heart, lung, liver, and kidney of the 8-week-old male ICR mice and whole brains of 2-, 7-, 13-, 23-, and 55-day-old mice were subjected to immunoblotting analysis. Dissected tissues were lysed with a dounce homogenizer in buffer A \[10\text{mM Tris-HCl (pH 8.0)}, 140\text{mM NaCl, 1 mM EDTA, 5 mg/ml leupeptin, 5 mg/ml aprotinin}\] containing 1% Triton X-100, and the lysates were resolved by SDS/PAGE and transferred to a poly(vinylidene difluoride) membrane filter (Immobilon P; Millipore, Bedford, MA, USA). The blot was subjected to immunoblotting analysis with anti-LGN antibody and alkaline phosphatase-conjugated mouse antirabbit IgG (Promega, Madison, WI, USA).

Immunofluorescence microscopy

Immunofluorescence microscopy was performed as described previously\textsuperscript{12} with slight modifications. Serial frozen sections (5\textmu m) of the mouse brain fixed with 4% paraformaldehyde at 4°C for 3 h were pretreated with 3% bovine serum albumin (BSA) for 15 min. Alternate sections were incubated with anti-LGN antibody diluted to 1 : 100 or anti-G\textsubscript{\alpha}i\textsubscript{2} antibody diluted to 1 : 20 for 60 min, followed by incubation with biotinylated antirabbit IgG antibody, and then with avidin–biotin complex (Vector, Burlingame, CA, USA). The immunoreaction was visualized by incubating with 0.01% H\textsubscript{2}O\textsubscript{2}-containing diaminobenzidine solution. The sections were examined with a light microscope (BH-2; Olympus, Tokyo, Japan). Control sections were incubated with antigen-preabsorbed anti-LGN antibody or normal rabbit serum.

Results and discussion

We generated antibody to the LGN protein by immunizing rabbits with a fragment of human LGN (amino acids 119–352) fused to GST. Immunoblotting analysis showed that anti-LGN antibody specifically reacted to GST-LGN but not to GST alone, and detection of this protein was inhibited by preincubation of the antibody with the antigen used for immunization (data not shown). When we subjected a lysate from mouse tissues to immunoblotting...