Neuroscience 275 (2014) 259–271

CYTOARCHITECTURE OF THE OLFACTORY BULB IN THE *LAGGARD* MUTANT MOUSE

J. YUNUS,[a] T. SETSU,[a] S. KIKKAWA,[a] T. SAKISAKA[b] AND T. TERASHIMA[a]*

[a] *Division of Developmental Neurobiology, Department of Physiology and Cell Biology, Kobe University Graduate School of Medicine, Kusunoki-cho 7-5-1, Chuo-ku, Kobe 650-0017, Japan*

[b] *Division of Membrane Dynamics, Department of Physiology and Cell Biology, Kobe University Graduate School of Medicine, Kusunoki-cho 7-5-1, Chuo-ku, Kobe 650-0017, Japan*

Abstract—The *laggard* (*lag*) mutant mouse, characterized by hypomyelination and cerebellar ataxia, is a spontaneously occurring mutant mouse caused by mutation in the *Kif14* gene. In this mutant mouse, the laminated structures such as the cerebral and cerebellar cortices and the dentate gyrus are cytoarchitecturally abnormal. Macroscopically, the olfactory bulb of the *lag* mutant mouse is smaller in size and more transparent than the normal counterpart. Hematoxylin–eosin staining reveals that the mutant olfactory bulb has normal lamination in general, but detailed analysis has demonstrated that olfactory periglomerular cells and granule cells are reduced in number. In the mutant, olfactory glomeruli are cytoarchitecturally disorganized and mitral cells are arranged in multiple cell layers instead of being arranged in a single layer. The rostral migratory stream in the mutant becomes gradually thinner or obliterated during early postnatal days. Some of mitral cells and periglomerular cells are multinucleated, suggesting that *Kif14* mutation leads to an abnormal cell division. In the mutant, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive cells in the subventricular zone of the lateral ventricle are increased in number, especially at perinatal age, suggesting that the decreased population of granule cells in the *lag* mutant mouse is caused by the increased apoptotic cell death. The olfactory input appears to be intact, as indicated by anterograde labeling of olfactory nerves with an injection of wheat germ agglutinin-horseradish peroxidase (WGA-HRP) into the olfactory mucosa. In conclusion, the olfactory bulb of the *lag* mutant mouse is cytoarchitecturally affected, suggesting that the causal gene for *lag* mutation, i.e., *Kif14*, has multiple effects on the development of laminated structures in the central nervous system in addition to the myelin formation.

Key words: *laggard*, *Kif14*, olfactory bulb, development, apoptosis.

INTRODUCTION

The olfactory bulb is a laminated structure which consists of seven layers, i.e., olfactory nerve layer (ONL), glomerular layer (GL), external plexiform layer (EPL), mitral cell layer (MCL), internal plexiform layer (IPL), granule cell layer (GCL), and subependymal-ependymal layer (SEL) (Shipley et al., 2004; Kosaka and Kosaka, 2009; Hellwig et al., 2012). As in other brain regions, there are two types of neuronal populations in the olfactory bulb: projection neurons and local circuit neurons. Projection neurons consist of mitral cells and tufted cells, whereas local circuit neurons consist of granule cells, periglomerular cells, and short-axon cells (Kosaka and Kosaka, 2009). Mitral cells and tufted cells are locally originated from the ventricular zone of the developing olfactory bulb, whereas periglomerular cells and granule cells are generated from the anterior subventricular zone of the lateral ventricle (Dellovade et al., 1998; Chazal et al., 2000; De Marchis et al., 2007; Lledo et al., 2008). From this neurogenic region of the lateral ventricle, immature neurons migrate tangentially in a chain-like organization into the olfactory bulb along a restricted pathway called the rostral migratory stream (RMS) (Corbin et al., 2001; Pencea and Luskin, 2003; Courtès et al., 2011). Within the olfactory bulb, immature neurons detach from the RMS, ascend radially into the GCL and GL, and differentiate into granule cells and periglomerular cells, respectively (Hack et al., 2002; Sun et al., 2010).

Kif14 is a mammalian kinesin that plays important roles in cell division (Nakagawa et al., 1997; Miki et al., 2001). Strong siRNA-mediated silencing of *Kif14* can induce cytokinesis failure, resulting in the formation of double-nucleated cells. Less efficacious silencing of *Kif14* induces apoptosis of cells that occurs upon entry into mitosis, while attempting cytokinesis, or after mitotic exit (Carleton et al., 2006). *Kif14* is essential for efficient cytokinesis via its interaction with citron kinase (citron-K) and protein regulator of cytokinesis 1 (PRC1) (Gruneberg et al., 2006). Both citron kinase and PRC1 localize to the cleavage furrow during mitosis.

*Corresponding author. Tel: +81-78-382-5320; fax: +81-78-382-5328.
E-mail address: ttera@med.kobe-u.ac.jp (T. Terashima).

Abbreviations: BSA, bovine serum albumin; DAB, 3,3′-diaminobenzidine; EPL, external plexiform layer; GCL, granule cell layer; GFAP, glial fibrillary acidic protein; GL, glomerular layer; HE, hematoxylin-eosin; MBP, myelin basic protein; MCL, mitral cell layer; ONL, olfactory nerve layer; PB, phosphate-buffered solution; PBS, phosphate-buffered saline; PRC1, protein regulator of cytokinesis 1; RMS, rostral migratory stream; TH, tyrosine hydroxylase; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; WGA-HRP, wheat germ agglutinin-horseradish peroxidase.

http://dx.doi.org/10.1016/j.neuroscience.2014.06.011
0306-4522/

Interference in interactions of these proteins with *Kif14* can obstruct cell division and potentially lead to apoptosis (Basavarajappa and Corson, 2012).

The *laggard* (*lag*) mutant mouse is a spontaneously occurring mutant mouse caused by mutation in the *Kif14* gene (Fujikura et al., 2013). The mutation is inherited as an autosomal recessive trait. Mice homozygous for the *lag* mutation suffer from ataxia and show measurable decrease in body weight in the second postnatal week, followed by premature death. The brain and spinal cord of this mutant mouse are reduced in size. Moreover, certain parts such as the cerebral cortex, cerebellum, and olfactory bulb are disproportionately smaller compared with the normal counterpart. The *lag* mutant mouse also displays a severe CNS hypomyelination, which is most presumably caused by the disruption of oligodendrocyte maturation.

At present, there is no clear evidence about the involvement of *Kif14* in the olfactory bulb development. The present study was conducted to determine whether the olfactory bulb development is also altered in the *lag* mutant mouse. In this report, we describe the effect of mutation in *Kif14* to the olfactory bulb development. A more detailed understanding of the mechanism of *Kif14* action and its roles (if any) will offer insights into the olfactory bulb development.

EXPERIMENTAL PROCEDURES

Animals

All homozygous mutants (*lag*/*lag*) and normal littermates (+/+ or +/*lag*) were generated from a breeding colony maintained at the Institute for Experimental Animals, Kobe University Graduate School of Medicine. In the present study, 34 *lag* mutant mice and 29 normal littermates were used. All procedures were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animal (NIH Publications No. 80-23), revised in 1996, and approved by the Committee on Animal Care and Welfare, Kobe University Graduate School of Medicine. Mice were housed in a 12-h light–dark cycle and given free access to food and water. For the morphological analysis, the mice were sacrificed at embryonic day (E) 15.5, E17.5, postnatal day (P) 0, P3, P5, P7, and P10. For embryonic studies, the detection of a vaginal plug in the next morning after mating was considered as E0.5. Mice were anesthetized with sodium pentobarbital (5 mg/100 g body weight) via intraperitoneal injection.

Hematoxylin-eosin (HE) staining and immunohistochemistry

The mice were deeply anesthetized as described above and transcardially perfused with 0.1 M phosphate-buffered solution (PB) containing 0.9% NaCl (phosphate-buffered saline (PBS)) for 5 min at room temperature, followed by perfusion of 4% paraformaldehyde in PB for 15 min at 4 °C. The brains were removed from the skulls and postfixed in 4% paraformaldehyde for 2 h at 4 °C. Then, the brains were dehydrated in a graded ethanol series, cleared in xylene, and embedded in paraffin. The sagittal or coronal sections at 4 μm in thickness were prepared using a sliding microtome (SM2000R; LEICA) and mounted on a MAS-coated glass slide (Matsunami Glass; Osaka, Japan). The sections were counterstained with HE, dehydrated in a graded ethanol series, cleared in xylene, and coverslipped with HSR solution (Sysmex; Kobe, Japan).

For the immunohistochemistry of paraffin-embedded sections, the sections adjoining to the HE-stained sections were first washed in 0.1 M PBS and immersed in blocking buffer (0.1 M PBS containing 1% bovine serum albumin (BSA)) for 1 h. The sections were then incubated with anti-Calretinin (rabbit polyclonal, Millipore; 1:1000 dilution), anti-glial fibrillary acidic protein (GFAP) (rabbit polyclonal, Dako; 1:4 dilution), anti-myelin basic protein (MBP) (rabbit polyclonal, Nichirei Biosciences, Tokyo, Japan; 1:1 dilution), anti-Reelin (mouse monoclonal, Millipore; 1:1000 dilution), anti-S100B (mouse monoclonal, Abcam, Tokyo, Japan; 1:10 dilution), and anti-tyrosine hydroxylase (TH) (mouse monoclonal, Millipore, Tokyo, Japan; 1:400 dilution) antibodies for 1 h at room temperature, and then overnight at 4 °C. The sections were washed three times in PBS, incubated in biotinylated anti-mouse IgG (Vector, Burlingame, CA, USA; 1:500 dilution) or biotinylated anti-rabbit IgG (Vector; 1:500 dilution), and then in the VECTASTAIN ABC Kit (Vector). They were reacted in 0.05% 3,3′-diaminobenzidine (DAB) and 0.01% H_2O_2. After washing in 0.1 M PBS, the sections were dehydrated in a graded ethanol series, cleared in xylene, and coverslipped with HSR solution.

For the frozen sections, after removing the brains and postfixing in 4% paraformaldehyde for 2 h at 4 °C, the brains were cryoprotected in 20% sucrose PB overnight. The frozen sections were cut sagittally on a freezing microtome (Yamato Koki) at 40 μm in thickness. The sections were washed in 0.1 M PBS and immersed in blocking buffer (0.3% Triton in PBS (PBST) containing 1% BSA and 0.05% azide) for 1 h. They were then incubated in anti-PSA-NCAM (mouse monoclonal, Millipore; 1:400 dilution) for 1 h at room temperature, and then overnight at 4 °C. After incubation with primary antibody, they were incubated in biotinylated anti-mouse IgM (Vector; 1:500 dilution), and then in the VECTASTAIN ABC Kit (Vector). They were reacted in 0.05% DAB and 0.01% H_2O_2. After washing in 0.1 M PBS, the sections were mounted on glass slides pretreated with gelatin and coverslipped with HSR solution.

For fluorescence imaging, after blocking for 1 h at room temperature with 1% BSA, the sections were incubated with anti-Reelin (mouse monoclonal, Millipore; 1:1000 dilution) and anti-TH (mouse monoclonal, Millipore; 1:400 dilution) antibodies for 1 h at room temperature, and then overnight at 4 °C. Then, the sections were incubated for 1 h at room temperature with secondary antibody (Alexa Fluor™488 goat anti-mouse IgG; Molecular Probes, Eugene, OR, USA; 1:1000 dilution) and counterstained with DAPI. Slides were examined under the Olympus AX80 fluorescence microscope.