Neuroscience 336 (2016) 20–29

THE C-TERMINAL REGION OF REELIN IS NECESSARY FOR PROPER POSITIONING OF A SUBSET OF PURKINJE CELLS IN THE POSTNATAL CEREBELLUM

KOTA NAKAMURA, MAKO BEPPU, KAORI SAKAI, HAYATA YAGYU, SAORI MATSUMARU, TAKAO KOHNO AND MITSUHARU HATTORI *

Department of Biomedical Science, Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya, Aichi 467-8603, Japan

Abstract—In the normal cerebellum, Purkinje cells (PCs) are generated in a zone along the ventricular surface, migrate radially, and align to form a single-cell layer. However, in mice lacking the secreted protein Reelin or its downstream adaptor protein Dab1, the majority of PCs are located ectopically in the deep cerebellar mass. Nonetheless, how Reelin regulates migration and alignment of PCs remains incompletely understood. Reelin has a highly-conserved C-terminal region (CTR), which is required for its full activity. Here, we report an abnormality of the cerebellum in Reelin CTR-lacking knock-in (ΔC-KI) mice. In the ΔC-KI mice, cerebellar formation was largely normal, but some PCs in selected regions were found to be located ectopically and to frequently form clusters. Ectopic PCs contained a higher amount of Dab1 protein and functional Reelin receptors, including mainly very low-density lipoprotein receptor than correctly-aligned PCs. Decreasing Dab1 gene dosage exacerbated mislocalization of PCs and the cerebellar structure in Reelin ΔC-KI mice. These results indicate that ectopic PCs in ΔC-KI mice failed to receive sufficient Reelin signaling en route to their final destinations. Further, we also found that Reelin protein with intact CTR binds preferentially to PCs. Thus, it was suggested that the extent or quality of Reelin/Dab1 signaling that PCs require for correct positioning vary and that Reelin with intact CTR is required for that of a certain subset of PCs. **© 2016 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: Reelin, Purkinje cell, Dab1, knock-in mouse.

INTRODUCTION

The cerebellum plays a major role in the control and coordination of motor activity. More recently, its role in cognitive and emotional functions is also appreciated (Buckner, 2013). Therefore, its dysplasia or degeneration causes various types of pathological problems. Our understandings of cerebellar development and maintenance, however, are still far from perfect, and many devastating cerebellar diseases are neither foreseeable nor curable (Leto et al., 2015). Obviously, it is necessary to clarify the detailed molecular mechanisms of cerebellar development.

In the latter half of the previous century, important anatomical observations and molecular insights regarding cerebellar development were obtained by utilizing naturally-occurring mutant mice. In *Reeler* mice, one of the first such mutants, the Purkinje cells (PCs) fail to migrate properly and remain largely situated in ectopic clusters deep in the cerebellar anlage (Mikoshiba et al., 1980; Miyata et al., 2010). The protein that is deficient in *Reeler* mice, called Reelin, is a very large secreted glycoprotein with 3461 amino acid residues (D'Arcangelo et al., 1995). In humans, Reelin deficiency leads to severe lissencephaly and cerebellar hypoplasia (Hong et al., 2000). Reelin binds to the lipoprotein receptors apolipoprotein E receptor 2 (ApoER2) and very low-density lipoprotein receptor (VLDLR) (D'Arcangelo et al., 1999; Hiesberger et al., 1999), resulting in multiple tyrosine phosphorylation of the cytosolic adapter protein Dab1 (Howell et al., 2000; Arnaud et al., 2003; Bock and Herz, 2003). In turn, phosphorylated Dab1 binds to various downstream molecules and regulates neuronal dynamics.

Molecular cascades downstream of Reelin in forebrain development have been extensively investigated (Frotscher, 2010; Sekine et al., 2014), while studies on them in the cerebellum are relatively scarce. It is known that, in the developing cerebellum, Reelin is expressed by granule cell precursors in the external granule layer (EGL) (D'Arcangelo et al., 1995; Miyata et al., 1996; Jensen et al., 2002), while ApoER2, VLDLR, and Dab1 are expressed largely by PCs (Trommsdorff et al., 1999; Perez-Garcia et al., 2004), whereas how Reelin orchestrates the dynamics of PCs remains poorly understood (Leto et al., 2015). Miyata and colleagues performed detailed analyses regarding the role of Reelin on the earliest stage of PC migration and found that the first

*Corresponding author.
E-mail address: mhattori@phar.nagoya-cu.ac.jp (M. Hattori).

Abbreviations: AP, alkaline-phosphatase; ApoER2, apolipoprotein E receptor 2; CTR, C-terminal region; EDTA, ethylenediaminetetraacetic acid; EGL, external granule layer; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IP_3R1, inositol 1,4,5-trisphosphate receptor type 1; P, postnatal day; PBS, phosphate-buffered saline; PC, Purkinje cells; RR, Reelin repeats; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; VLDLR, very low-density lipoprotein receptor; WT, wild-type; ΔC-KI, Reelin C-terminal region-lacking knock-in.

http://dx.doi.org/10.1016/j.neuroscience.2016.08.039
0306-4522/© 2016 IBRO. Published by Elsevier Ltd. All rights reserved.

Reelin-dependent event is the "posture-change" step around embryonic day 14.5 (Miyata et al., 2010). After this step, normal PCs migrate toward the pial surface and eventually form a monolayer (PC layer) during the early postnatal days (Leto et al., 2015). However, it is unknown for which part of PC migration and alignment Reelin is required and how different receptor/downstream molecules are separately or commonly utilized.

The Reelin protein is composed of an N-terminal region, Reelin repeats (RR), and a C-terminal region (CTR) (D'Arcangelo et al., 1995; Ichihara et al., 2001). The CTR is necessary for efficient induction of Dab1 phosphorylation in primary cultured cortical neurons (Nakano et al., 2007). Reelin is proteolytically cleaved within the CTR by proprotein convertase family proteases, and this cleavage attenuates Reelin's ability to induce a dendrite-rich marginal zone-like structure when overexpressed in the subventricular zone of the cerebral cortex (Kohno et al., 2015). Moreover, in knock-in mice in which the CTR of Reelin had been deleted (ΔC-KI mice), the marginal zone of the cerebral cortex was found to be narrower than in wild-type (WT) mice and the CA1 pyramidal cell layer of the hippocampus was split into two layers (Kohno et al., 2015). It was thus revealed that the CTR is required for proper orientation and branching of the apical dendrites and that these events are critical for the maintenance of the superficial layers of the cerebral cortex. The ΔC-KI mice show some behavioral abnormalities but their motor ability and sense of balance appear normal (Sakai et al., 2016).

In this study, we found that ectopic PCs exist in selected regions of postnatal ΔC-KI mice and that they contain high amounts of Dab1 and VLDLR. Our results provide novel insights into the functions of Reelin in the migration and/or alignment of PCs.

EXPERIMENTAL PROCEDURES

Reagents

Anti-inositol 1,4,5-trisphosphate receptor type 1 (IP_3R1) rat monoclonal antibody 18A10 was kindly provided by Dr. Katsuhiko Mikoshiba (Brain Science Institute, Riken, Saitama, Japan). Anti-VLDLR and anti-ApoER2 rat monoclonal antibodies (Hirota et al., 2015) were kindly provided by Dr. Kazunori Nakajima (Keio University School of Medicine, Tokyo, Japan). The anti-Dab1 rabbit polyclonal antibody (Uchida et al., 2009) and rat monoclonal antibodies 4E12 and 4H11 (Onoue et al., 2014) have been described previously. Anti-β-actin antibody was purchased from Sigma Aldrich. Alexa Fluor 488- and Alexa Fluor 594-conjugated secondary antibodies were purchased from Thermo Fisher Scientific. Recombinant Reelin proteins fused with alkaline-phosphatase (AP) were prepared as described previously (Uchida et al., 2009; Kohno et al., 2015).

Animals

The generation of ΔC-KI mice has been previously described (Kohno et al., 2015). Dab1-deficient *yotari* mice (Sheldon et al., 1997; Kojima et al., 2000) were kindly provided by Dr. Katsuhiko Mikoshiba. WT and ΔC-KI mice were obtained by crossing heterozygous male and female mice. The mice were kept in a room with a 12-h light/dark cycle (lights on at 6:00 am), with access to food and water *ad libitum*. All the experimental protocols were approved by the Animal Care and Use Committee of Nagoya City University and were performed according to the guidelines of the National Institutes of Health of Japan. The day mice were born was designated as postnatal day 0 (P0). Both male and female mice were used for analyses.

Immunohistochemistry

The mice were transcardially perfused with 4% paraformaldehyde in phosphate-buffered saline (PBS) and the cerebellum was removed. The cerebellums were further fixed with 4% paraformaldehyde in PBS overnight at 4 °C, cryoprotected by immersion in 20% and 30% sucrose in PBS, embedded in OCT Compound (Sakura Finetek), and frozen on dry ice. The frozen cerebellums were sectioned at 14 μm using a cryostat (CM1850; Leica Microsystems, Wetzlar, Germany). Immunohistochemistry was performed as described previously (Kohno et al., 2015). The concentrations of primary antibodies were as follows: anti-IP_3R1 18A10, 0.5 μg/ml; anti-Dab1 rabbit polyclonal antibody, 0.5 μg/ml; anti-VLDLR, 1 μg/ml; anti-ApoER2, 1 μg/ml. Pictures were taken with a BZ-9000 fluorescence microscope (Keyence). The pictures shown are the representatives from at least five independent mice. For quantification of the amount of VLDLR, the total fluorescent intensity of the cell body of ectopic PCs and that of their nearest correctly-aligned PCs (5–6 cells) were obtained using ImageJ software (version 1.48v, National Institutes of Health, USA).

Immunoblotting

The dissected cerebellum was sonicated in lysis buffer (20 mM Tris–HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 0.1% H_2O_2, and 5 mM Na_3VO_4). Insoluble debris was removed by centrifugation (14,000 rpm, 10 min), and the supernatants were mixed with 4× sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) loading buffer (0.25 M Tris–HCl, pH 6.8, 40% (w/v) glycerol, 8% SDS, 20% (v/v) 2-mercaptoethanol, 0.05% bromophenol blue). The samples were separated by SDS–PAGE (8% separating gel). Immunoblotting was performed as described previously (Kohno et al., 2015) with rat monoclonal antibodies against Dab1 4E12 and 4H11 (0.1 μg/ml each) or β-actin (×10,000). Images were captured using a LAS4000 system (Fuji). Images were analyzed and quantified with ImageJ. The significance of differences was determined by the unpaired *t*-test.

AP staining

Staining of unfixed cerebellar slices with AP fusion probes was performed as previously described, with some modifications (Flanagan et al., 2000; Uchida et al., 2009). Briefly, freshly prepared cerebellum was cut with

Download English Version:

https://daneshyari.com/en/article/4337248

Download Persian Version:

https://daneshyari.com/article/4337248

Daneshyari.com