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Research paper

Filamin A interacting protein plays a role in proper positioning of callosal projection neurons in the cortex

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HIGHLIGHTS

- FILIP is involved in neuronal cell positioning.
- The localization of callosal neurons was disturbed by insufficiency of FILIP.
- Positioning of *Plxnd1*-expressing callosal neurons was altered in *Filip*-knockout mice.

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ABSTRACT

The callosal connections between the two hemispheres of the neocortex are altered in certain psychiatric disorders including schizophrenia. However, how and why the callosal connection is impaired in patients suffering from psychiatric diseases remain unclear. Filamin A interacting protein (FILIP), whose alteration through mutation relates to schizophrenic pathogenesis, binds to actin-binding proteins and controls neurotransmission. Because cortical excitatory neurons, including callosal projection neurons, migrate to the cortical plate during development, with the actin-binding proteins playing crucial roles during migration, we evaluated whether FILIP is involved in the development of the callosal projection neurons by histological analysis of *Filip*-knockout mice. The positioning of the callosal projection neurons, especially those expressing *Plxnd1*, in the superficial layer of the cortex is disturbed in these mice, which suggests that FILIP is a key molecule that links callosal projections to the pathogenesis of brain disorders.

1. Introduction

The callosal projection neurons connect the two cerebral hemispheres via the corpus callosum [1] and play a role in associative and cognitive functions [2,3]. Callosal connections are decreased in the brains of patients with schizophrenia [4–6]. Development of the callosal projection neurons in the cerebral cortex requires complex and precise control of neuronal cell migration [7]. Excitatory neurons, including callosal projection neurons, are produced from neuronal progenitor cells in the ventricular zone and subventricular zone and migrate radially toward their final position [8,9]. Cytoskeletal control plays an important role in the regulation of neuronal migration, and impairment of this neuronal migration results in a variety of disorders, including gross brain abnormalities such as periventricular heterotopia and lissencephaly [10]. In addition to such disorders, migration impairment is thought to underlie several psychiatric diseases. It has been argued that the vulnerability to or etiology of schizophrenia can be comprehended in the context of neurodevelopmental processes including neuronal migration [11].

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We previously identified a novel molecule, filamin A interacting protein (FILIP, or FILIP-1 in humans), as a potential candidate for control of radial migration from the ventricular zone through interaction with the actin-binding protein, filamin A (FLNA) [12]. We have also shown that FILIP binds to non-muscle myosin heavy chain IIb (Myosin-10) and functions as a modulator for such actin-binding proteins [13]. As FLNA and Myosin-10 are involved in neuronal migration [14–16], we considered that FILIP is important for brain development and that the deletion of FILIP would result in cytoarchitectural disorganization in the brain. In the analysis of gene disruption in schizophrenia, a *de novo* missense mutation in *FILIP* (*FILIP-1*) was previously reported [17]. In the present study, we investigated how FILIP functions *in vivo* using *Filip*-knockout mice and revealed a novel role for FILIP in the development of callosal neurons in the mouse cortex.

2. Materials and methods

2.1. Animals

Mice were maintained in the animal room at the Division of Laboratory Animal Resources of University of Fukui. All experiments were conducted in accordance with the Guidelines for Animal Experiments of University of Fukui. The Animal Research Committee of University of Fukui approved the experiments. We ensured minimal pain and discomfort of the animals. The day that the presence of a vaginal plug was confirmed was defined as embryonic day 0.5 (E0.5). The day of birth was designated P0. Details of the generation of *Filip*-knockout mice have been previously reported [13].

2.2. Histological examination

For conventional histological examination, whole brains were fixed with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (pH 7.4) at 4 °C. Brains were cryoprotected with 30% sucrose in 0.1 M phosphate buffer (pH 7.4) and frozen in powdered dry ice. Coronal sections were cut at 14-μm thickness with a cryostat. Sections were mounted on 3-aminopropyltriethoxysilane-coated glass slides (Matsunami Glass Ind., Kishiwada, Japan) and stained with thionine. For immunohistochmical analyses, the sections were incubated overnight at 4 °C with the antibody-dilution buffer containing the anti-Cux-1 antibody (1:50, Santa Cruz Biotechnology, Santa Cruz, CA). The signals were visualized with Alexa Fluor 488-conjugated anti-rabbit IgG (Life Technologies Corporation, Grand Island, NY).

2.3. 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) injection

On postnatal day 2, the mice were deeply anesthetized by hypothermia. After perfusion with 4% PFA in phosphate-buffered saline (PBS), the brain was dissected out and fixed in 4% PFA in PBS. DiI crystals were inserted into the left occipital cortex of the

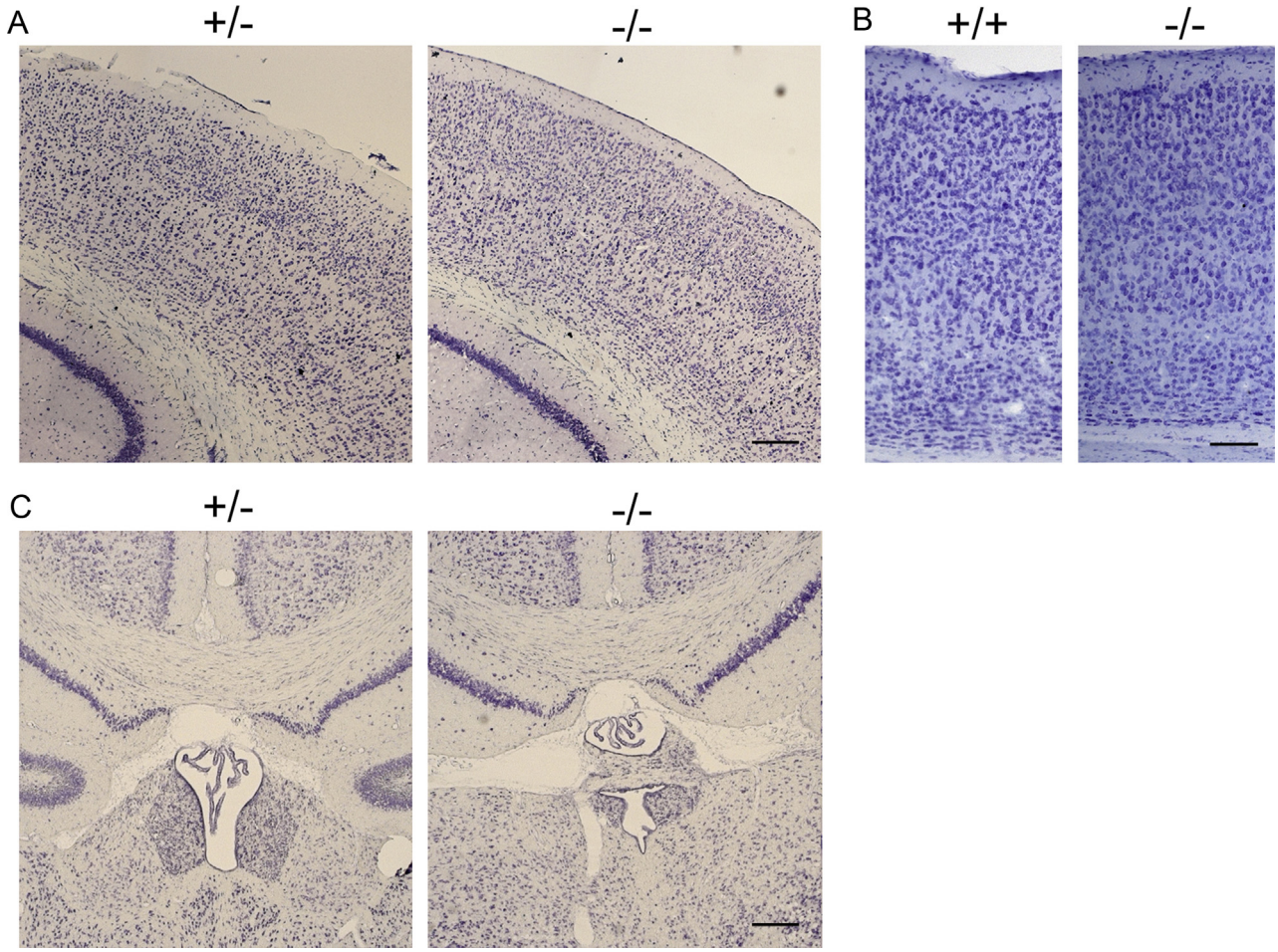

Fig. 1. No apparent abnormalities are recognized in *Filip*$^{-/-}$ mice (−/−) with Nissl staining.
The sensorimotor (A) and occipital (B) cortices and corpus callosum (C) of control (*Filip*$^{+/+}$ (+/+) and *Filip* $^{+/-}$ (+/−)) littermates and *Filip*$^{-/-}$ mice are shown. Scale bar = 200 μm (A, C) and 100 μm (B).