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Research Report

Laser capture microdissection and cDNA array analysis for identification of mouse KIAA/FLJ genes differentially expressed in the embryonic dorsal spinal cord

Tomoyuki Masuda[a,*], *Nobuyuki Kai*[b], *Chie Sakuma*[a], *Kazuto Kobayashi*[b], *Hisashi Koga*[c], *Hiroyuki Yaginuma*[a]

[a]*Department of Anatomy, Fukushima Medical University School of Medicine, 1 Hikarigaoka, Fukushima 960-1295, Japan*
[b]*Department of Molecular Genetics, Institute of Biomedical Sciences, Fukushima Medical University School of Medicine, 1 Hikarigaoka, Fukushima 960-1295, Japan*
[c]*Laboratory of Medical Genetics, Department of Human Genome Research, Kazusa DNA Research Institute, 2-6-7 Kazusa-Kamatari, Kisarazu, Chiba 292-0108, Japan*

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ABSTRACT

During early development, centrally projecting dorsal root ganglion (DRG) neurons extend their axons toward the dorsal spinal cord. We previously reported that this projection is achieved by dorsal spinal cord-derived chemoattraction. However, the molecular nature of the chemotrophic cue is not yet fully understood. To identify novel genes differentially expressed in the dorsal spinal cord in the embryonic day 10.5 mouse, we used the Kazusa cDNA array system comprising approximately 1700 mouse KIAA/FLJ (mKIAA/mFLJ) cDNA clones and laser capture microdissection (LCM) in combination with PCR-based cDNA amplification. We observed that a certain population of genes showed significantly increased expression in the dorsal spinal cord. *In situ* hybridization analysis verified the expression of mRNAs of 6 genes (*Hip1r*, *Nav2*, *Fstl5*, *Cacna1h*, *Bcr*, and *Bmper*) in the cells that constitute the dorsal spinal cord. The dorsal spinal cord-specific genes identified in this study provide a basis for studying the molecular nature of the neural development including the axonal guidance of DRG neurons. These results also demonstrate that the combined use of LCM coupled with the Kazusa cDNA array technology will be useful for the identification of large proteins expressed in the restricted small regions of embryos.

1. Introduction

Growing axons navigate along specific appropriate pathways to reach their targets. Axonal guidance involves the coordinate action of attractive and repulsive guidance cues (reviewed in Tessier-Lavigne and Goodman, 1996; Masuda and Shiga, 2005). In higher vertebrates, dorsal root ganglion (DRG) neurons extend their axons toward a restricted region of the dorsal spinal cord called the "dorsal root entry zone (DREZ)" at embryonic day (E) 10.5 in the mouse embryo (see Fig. 1A; Ozaki and Snider, 1997). Previously, we reported that the dorsal spinal cord exerts a chemoattractive signal toward

* *Corresponding author*. Fax: +81 24 549 8811.
E-mail address: tmasu@fmu.ac.jp (T. Masuda).

Abbreviations: DRG, dorsal root ganglion; LCM, laser capture microdissection; DREZ, dorsal root entry zone; RT, reverse transcriptase; DAPI, 4′6-diamidino-2-phenylindole

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DRG axons (Masuda et al., 2007). However, the molecular mechanism of this guidance process is largely unknown. As the first step to elucidate the molecules involved in this guidance, it is necessary to search for sets of genes that have a unique expression pattern in the dorsal spinal cord at just the time when DRG axons are being projected toward the DREZ.

The mouse KIAA/FLJ (mKIAA/mFLJ) cDNA microarray ver. 2.0 (GEO DataSets #GPL2536) is a cDNA array system comprising 1,707 mKIAA/mFLJ cDNA clones (Koga et al., 2004; Yamamoto et al., 2004). Most mKIAA/mFLJ genes are long cDNA clones that encode high molecular weight proteins. To date, approximately one-third of the clones spotted on this array are novel ones whose expression and biological function are unknown (http://www.kazusa.or.jp/rouge/index.html).

In the present study, we isolated dorsal spinal cord tissues by using laser capture microdissection (LCM), and prepared hybridization probes to identify genes differentially expressed in the dorsal spinal cord. Using the mKIAA/mFLJ cDNA array, we found candidate genes with statistically significant difference in expression between dorsal spinal cord and control tissues. Furthermore, we verified dorsal spinal cord-specific expression of these genes by *in situ* hybridization.

2. Results

2.1. *Verification of dorsal spinal cord-specific expression of genes by in situ hybridization of E10.5 mouse embryos*

In the present study, we used LCM to isolate dorsal and intermediate spinal cord tissues from mouse embryos at E10.5 (Fig. 2). By means of cDNA microarrays, we investigated gene expression profiles and compared these profiles between these 2 tissues. We obtained 43 candidate genes according to the difference in their average Z-score. At E10.5, the DREZ is localized in the dorsolateral edges of the spinal cord, which receive the axonal projection from the DRGs (Figs. 1A, 3A). To search for genes having a unique expression pattern around the DREZ and/or dorsal spinal cord, we performed *in situ* hybridization for the candidate genes to verify the expression of their mRNAs in the dorsal spinal cord. None of these genes

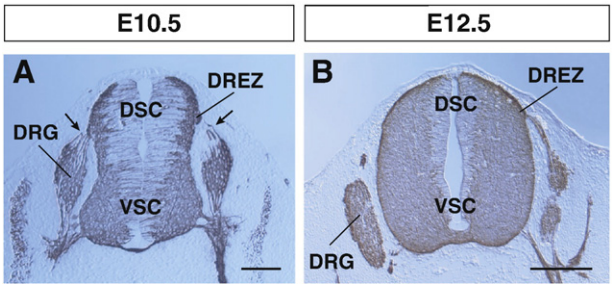

Fig. 1 – DRG axonal trajectories visualized by Tuj1 staining. Transverse sections of the mouse spinal cord at E10.5 (A) and E12.5 (B) were stained by using anti-Tuj1 antibody. The neural network outside of the spinal cord including DRG axonal trajectories is visualized. Arrows indicate DRG axons projecting toward the DREZ. DSC, dorsal spinal cord; VSC, ventral spinal cord. Scale bars, 100 μm (A), 300 μm (B).

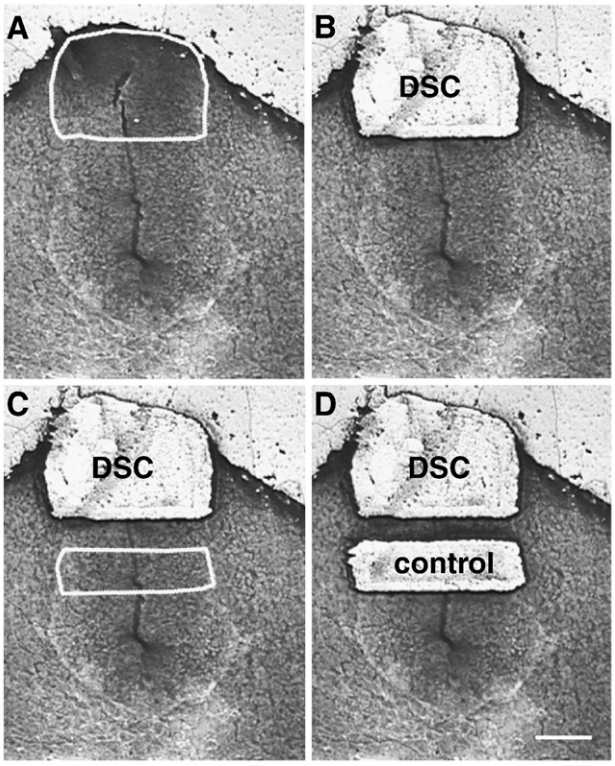

Fig. 2 – Laser capture microdissection of embryonic dorsal and intermediate (control) spinal cord tissues. (A–D) Toluidine blue-stained sections of the mouse spinal cord at E10.5 before (A) and after (B–D) the sequential dissection of dorsal and intermediate spinal cord tissues. White lines indicate the traces of the laser beam. DSC, dorsal spinal cord. Scale bar, 100 μm.

except for *mKIAA1965* (*Bmper*) had been previously reported to be expressed in the mouse embryonic spinal cord. *In situ* hybridization analysis confirmed that 19 genes displayed low expression in various regions of the dorsal spinal cord at E10.5 and that 6 genes displayed high expression in the dorsal spinal cord at that time (Table 1). The *mKIAA0655* gene (*Hip1r*) was strongly expressed in the dorsal third of the spinal cord including the DREZ and the roof plate (Fig. 3B). This gene was also expressed in a part of the DRG and in motor neurons (Fig. 3B). Strong expression of the *mKIAA3015* gene (*Nav2*) was found in the dorsolateral part of the spinal cord including the DREZ, but not in the roof plate (Fig. 3C). This gene was also expressed in DRG neurons, the dermamyotome, and in the subpopulation of motor neurons (Fig. 3C). The mRNA of the *mKIAA1263* gene (*Fstl5*) was detected in the dorsolateral edges of the spinal cord, especially in the DREZ (Fig. 3D). Its expression was also detected in motor neurons, DRG neurons, and in the floor plate (Fig. 3D). The mRNA of the *mKIAA4255* gene (*Cacna1h*) was detected in the lateral region of the spinal cord, including the DREZ, motor neurons, the floor plate, and DRG neurons (Fig. 3E). The *mKIAA3017* gene (*Bcr*) was expressed in the lateral region of the spinal cord, including the DREZ and motor neurons (Fig. 3F). The mRNA of the *mKIAA1965* gene (*Bmper*) was detected in and around the roof plate (Fig. 3G). Its expression was also detected in the