Dab2IP Regulates Laminar Organization in the Mammalian Brain

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Introduction
Reelin signaling pathway controls cell migration and cortical histogenesis during development (Roa and Curran, 2001; D'Arcangelo, 2006). Reelin functions by binding to lipoprotein receptors ApoR2 and VLDLR, and inducing tyrosine phosphorylation of intracellular adapter protein disabled 1 (Dab1) via Src family tyrosine kinases.

Previously, we found that Dab1 interacts with the Npxy motif in DCC/Dab Interacting Protein (Dab2IP), a new member of RasGTPase activating proteins (RasGAP). Several isoforms of Dab2IP have been identified thus far (Wang et al., 2002; Homayouni et al., 2003). Recently, we identified a novel mouse Dab2IP transcript variant, (Dab2IP-L), which contains a considerably longer pleckstrin homology (PH) domain than previously reported isoforms of Dab2IP.

In order to investigate the role of Dab2IP during brain development, we generated a mouse model in which the Dab2IP gene was disrupted by a retroviral promoter trap strategy. This targeting construct contained a LacZ reporter gene which allows for in situ analysis of gene expression in various tissues, such as the brain. Western blot and q-RT-PCR analysis showed that the expression of all except one isoform of Dab2IP was abolished in Dab2IP mutant mice.

In this study, we used the Dab2IP mutant mice to characterize the expression of Dab2IP during brain development and examine its role in neuronal migration and layer formation in the developing cortex. Since Dab2IP interacts with Dab1, it may play a role in Reelin signaling. Thus, we also investigated if Dab2IP protein levels are altered in reeler mice.

Dab2IP gene structure and knock-out strategy

Lexicon OSTM434525

Defective lamination in Dab2IP KO Mice

Fig. 1. Insertion of the retroviral gene trap cassette into the Dab2IP gene locus.

The mouse Dab2IP gene structure was examined using the UCSC Genome Browser (July 2007 builds). Dab2IP contains 18 exons spanning over 17500. At least 4 different variants of Exon 18 have been isolated from EST libraries.

To generate Dab2IP KO mice, blastocysts were injected with Creberalisis OSTM434525 ES cells (Lexicon Genetics, Inc.), which contained a retroviral gene trap inserted immediately after Exon 4 of the Dab2IP gene. The promoter-trap cassette encodes the β-galactosidase-neomycin (β-neo) flanked by 5′-splice acceptor site, which results in the β-gene under the control of the native Dab2IP promoter. The presence of the neo-flanked cassette was confirmed by PCR, promoter upstream of a splice donor site confers a selectable marker to identify targeted ES cells in culture.

Dab2IP expression is developmentally regulated

Fig. 2. Expression of the Dab2IP gene in the developing mouse brain. A. Western blot analysis of Dab2IP expression in cortex at various developmental ages. B. Sagittal sections of E12 and E14 embryos (a, b) or of brains from E12 to P30 (c–h), were processed for β-gal and counterstained with nuclear fast red. Scale bars: 1.0 mm. C. Higher-magnification images of c–h. Scale bars: 50 μm. D. Expression of Dab2IP in the hippocampus. Scale bar: 10 μm.

Fig. 4. Birthdating studies in the developing cortex. A. BrdU was injected into pregnant females at E16.5, and the brains were collected at post-natal day 21 (P21) and immunostained with anti-BrdU antibody. B. Quantification of BrdU-positive cells as a percentage in each of 10 bins relative to the total number of BrdU-positive cells in WT (blue line) and Dab2IP mutant (red line) brains. Data represent mean ± SD. n=13. Neurons which are born at E16.5 normally occupy layers III/IV in the cortex. However, in Dab2IP mutant mice, E16.5-born neurons occupy deeper layers.

Dab2IP regulates neuronal migration in late-generated neurons

Fig. 5. CSPG immunostaining of WT and Dab2IP mutant cortex at E16.5. Coronal sections from E16.5 WT or Dab2IP mutant embryos were immunostained with CSPG (red) along with a DAPI nuclear stain (blue). The marginal zone (MZ), cortical plate (CP) and subplate region (SP) are indicated. These results indicate that the preplate splits properly at E11.5 and that the cortical plate is forming correctly until E16.5.

Dab2IP levels accumulate in reeler cortex

Fig. 6. Dab2IP protein levels in reeler cortex. A. Dab2IP (red) and DAPI (blue) immunostaining in the cortex of wild-type and reeler (rlr) mice at postnatal day 30. Higher-magnifications are shown in the insets. Scale bars=100 μm. B. Immunoblot of Dab2IP and beta-actin from pooled cortices lysates from two wt and two rlr mice at various developmental ages. Dab2IP levels are higher in reeler mice, particularly at later developmental ages.

Summary and Conclusions
1. Dab2IP is a Ras and Rap1 GTPase activating protein which interacts with Dab1, an adapter molecule critical in Reelin signaling pathway (Homayouni et al., 2003; Kim and Homayouni, 2010).
2. Using the promoter trap LacZ marker, we show that Dab2IP is widely expressed during embryonic development. Unlike reeler mice, the Dab2IP mutant mice have normal gross cortical and cerebellar morphology.
3. Interestingly, Dab2IP mutants have defective laminations in the somatosensory cortex.
4. It appears that early events during cortical development are normal in Dab2IP mutant animals, whereas migration of later-born neurons that occupy layers III/IV of the cortex are disrupted by deletion of Dab2IP. Also, the marginal zone boundary is disrupted in Dab2IP mutants.
5. The levels of Dab2IP are higher in a subset of cortical neurons in reeler mice.
6. Our data suggest that Dab2IP may play an important role in some aspects of Reelin signaling during brain development.

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