Cellular Distribution of Dab2IP Isoforms in the Developing Cerebellum

Shuhong Qiao, Sun-Hong Kim, Hemachand Tummala, Ramin Homayouni
Department of Biology, University of Memphis, Memphis, TN

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Introduction

The Reelin signaling pathway plays an important role in neuronal positioning as well as dendritic maturation during brain development. Reelin functions by binding to lipoprotein receptors ApoER2 and VLDLR, and inducing tyrosine phosphorylation of intracellular adapter protein disabled-1 (Dab1) via Src family tyrosine kinases.

Using a two-hybrid screen, we found that Dab1 interacts with the NPXY motif in DOC2/Dab2 Interacting Protein (Dab2IP), a new member of Ras GTPase activating proteins (RasGAP). Several different isoforms of Dab2IP have been identified thus far (Wang et al.,2002; Homayouni et al.,2003). We have identified a novel mouse Dab2IP transcript variant, (Dab2IP-L) that contains a considerably longer pleckstrin homology (PH) domain than previously reported isoforms of Dab2IP.

In order to investigate the role of Dab2IP-L during brain development, we generated Dab2IP-L KO mice using commercially available mouse ES cells carrying a retroviral promoter trap insertion of LacZ reporter gene in the Dab2IP gene locus. Using β-galactosidase and immunoblot assays, the developmental expression profile of Dab2IP was examined.

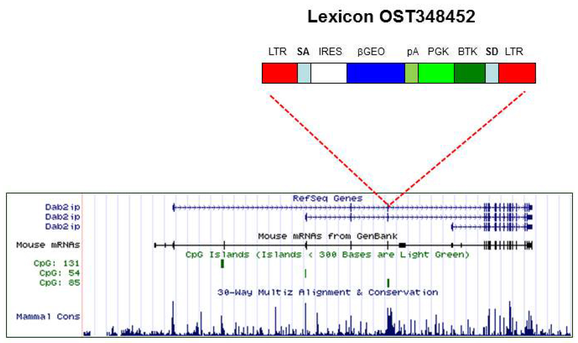
Using immunohistochemistry and immunofluorescence microscopy, we examined the expression of Dab2IP in different cell populations in the developing cerebellum. Both Dab2IP isoforms appear to be expressed in Purkinje cells (PCs). Targeted deletion one of the isoforms appears to affect PC dendrite maturation, synapase formation and spontaneous firing behavior in the adult.

Dab2IP Gene Structure and KO Strategy

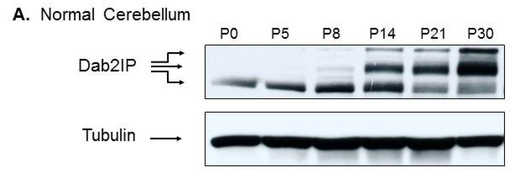
The mouse *Dab2IP* gene structure was examined using the UCSC Genome Browser (July 2007 build). Dab2IP contains 18 exons spanning over 175Kb. At least 4 different variants of Exon 18 have been isolated from EST libraries.

To date, 3 isoforms of Dab2IP have been reported. The longest isoform, termed Dab2IP-L, contains an additional 5' exon containing an alternative translation site. Dab2IP-L contains a complete PH-domain, while the shortest isoform (Dip1/2) does not.

To generate Dab2IP KO mice, blastocysts were injected with Omnibank® OST348452 ES cells (Lexicon Genetics, Inc.), which contained a retroviral gene-trap inserted immediately after Exon 4 of *Dab2IP* gene. The promoter-trap cassette encodes the β-galactosidase-neomycin (β-neo) gene flanked by 5'-splice acceptor site, which renders the β-geo gene under the control of native Dab2IP promoter. The presence of BTK under the control of PGK promoter upstream of a splice donor site confers a selectable marker to identify targeted ES cells in culture.

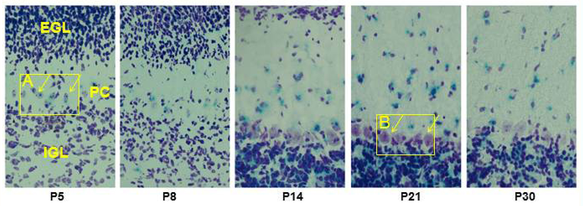


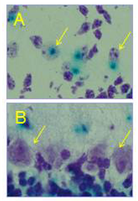
Dab2IP Protein levels in Developing Cerebellum



Western blot of normal **(A)** and Dab2IP-KO **(B)** Cerebellum lysates from various developmental ages probed with rabbit anti-Dab2IP polyclonal antibody. Dab2IP appears to have 3 major isoforms in the adult, ranging from 110-150 KDa (*Left Panel*). The Gene trap significantly affects the two lower isoforms (*Right Panel*).

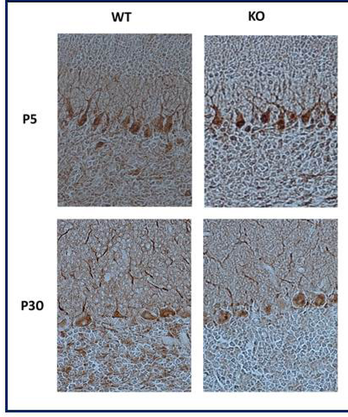
Dab2IP-L Expression in Cerebellum





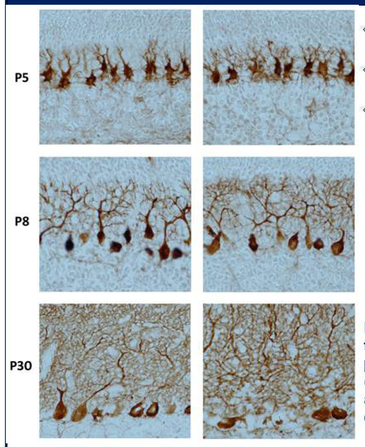
Dab2IP gene expression was examined in sagittal cerebellar sections at various developmental ages (P5,P8,P14,P21,P30) by β-galactosidase assays and Nissl staining. Dab2IP gene expression was detected at postnatal day 0 (P0) and increased gradually to peak levels by P14 and P21. Early during development, Dab2IP is expressed in the external granule layer cells (EGL), Purkinje cells (PC) and internal granule layer IGL) cells. From P21, there is no Dab2IP expression in Purkinje Cells.

Dab2IP Protein Expression in Cerebellum



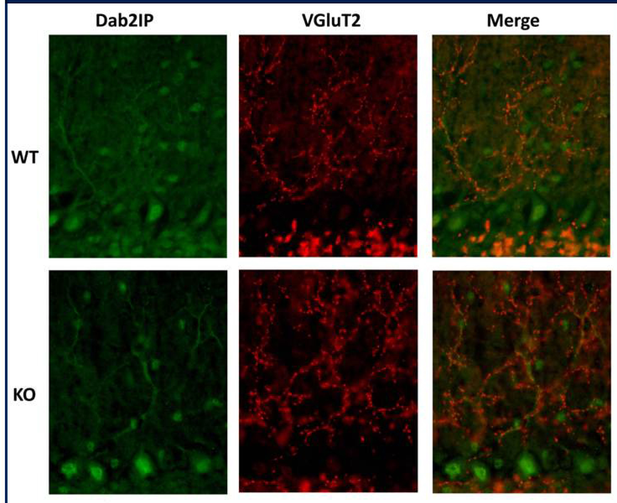
Dab2IP labeling in the wild-type (WT) and DAb2IP knock-out (Dab2IP$^{-/-}$) littermates at various developmental ages (P5 and P30). In the WT mice, Dab2IP is expressed in PC soma and dendrites, and some cells in the granule cell layer. In the Dab2IP$^{-/-}$ mice, Dab2IP is expressed only in the PCs soma and dendrite.

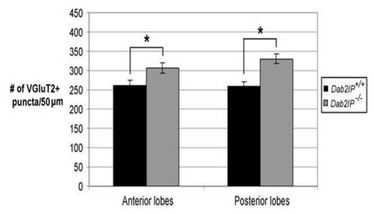
Purkinje Cell Morphology of Dab2IP$^{-/-}$ Mice



Expression of Calbindin in the Wild-type and Dab2IP$^{-/-}$ littermates at postnatal day 5 (P5), 8 (P8) and 30 (P30). The PCs in Dab2IP -/- mice appear to have more branches compared with wild-type littermates.

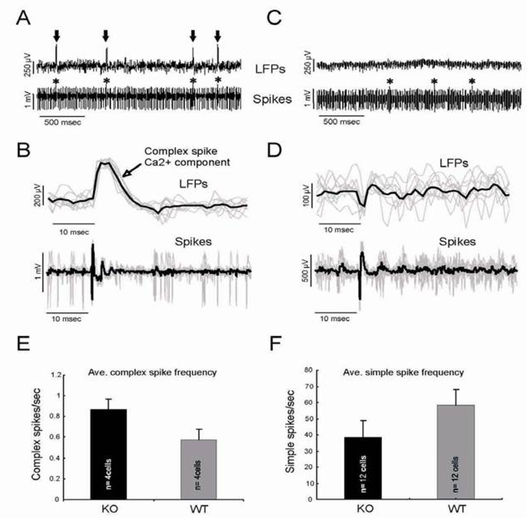
VGluT2 Expression in *Dab2IP$^{-/-}$* Cerebellum





Fluorescent images of cerebella from P30 wild-type and Dab2IP$^{-/-}$ littermates double immunolabeled with anti-Dab2IP antibody (green) and anti-VGluT2 antibody (red) for labeling climbing fiber terminals. The density of the VGluT2-immnopositive puncta is increased in Dab2IP$^{-/-}$ mice compared to wild-type mice.

Extracellular Recordings of Wild-type and Dab2IP-/- Cerebella



Extracellular recordings of local field potential (LFP) revealed that the slow, long lasting Ca^{2+}-dependent depolarization is missing in the Dab2IP-/- Purkinje cells. This finding may account for the lack of characteristic CF-evoked all-or-none complex spikes in Dab2IP KO Purkinje cells, since slow component of complex spikes is shown to be Ca^{2+}-dependent. In addition, Dab2IP -/- mice show longer inter-spike interval durations and less frequent simple spike frequency of Purkinje cell firing in Dab2IP KO mice.

Summary and Conclusions

1. Dab2IP is a brain-enriched novel GTPase activating protein which interacts with Dab1, an adapter molecule in the Reelin signaling pathway (Homayouni et al., 2003).
2. Dab2IP appears to have two different promoters and also undergoes differential splicing during brain development.
3. Only one Dab2IP isoform is detected in cerebellum and hippocampus at P0 and additional isoforms appear later in development.
4. Our gene trap strategy targeted only one Dab2IP-/- promoter and resulted in downregulation of two of the three major Dab2IP isoforms.
5. Dab2IP-/- mice exhibited abnormal PC firing patterns compared to their wild-type littermates.
6. Dab2IP-/- mice showed longer and more elaborate PC dendrites, concomitant with an increase in VGlut2 climbing fiber synaptic marker staining.
7. Our results demonstrate that Dab2IP isoforms are differentially regulated during development and play an important role in dendrite maturation.

Acknowledgement

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