Dab2IP Transcript Variants and Promoter Methylation during Cerebellar Development

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Introduction

The Reelin signaling pathway plays an important role in neuronal positioning as well as dendrite 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.

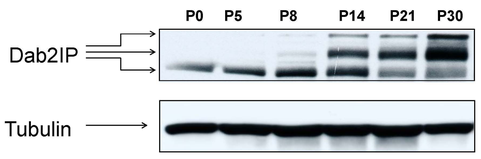
Using a two-hybrid screen, we found that Dab1 interacts with the NPXY motif in DOC2/Dab2 Interacting Protein (Dab2IP), a new member of Ras GTPase activating proteins (RasGAP). Several different isoforms of Dab2IP have been identified thus far (Wang et al.,2002; Homayouni et al.,2003). We have identified a novel mouse Dab2IP transcript variant, (Dab2IP-L) that contains a considerably longer pleckstrin homology (PH) domain than previously reported isoforms of Dab2IP (Kim and Homayouni, 2010).

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.

In addition, using a combination of RT-PCR and qRT-PCR strategies, we have investigated the expression of various 5' Dab2IP Exons during brain development. Lastly, we have examined the correlation between DNA methylation of several CpG islands within Dab2IP gene and expression of different Dab2IP transcripts during brain development.

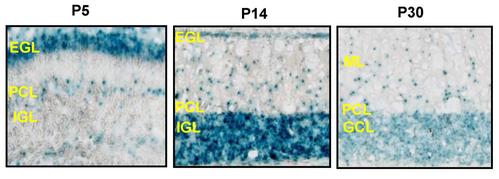
Dab2IP Protein Levels in Developing Cerebellum

A. Western Blot



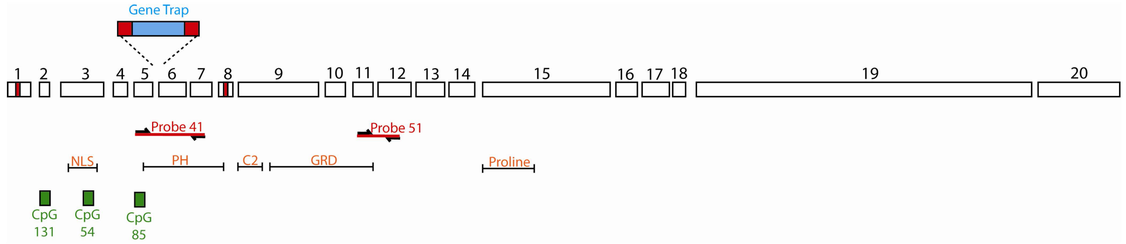
Western blot of normal Cerebellum lysates from various developmental ages probed with rabbit anti-Dab2IP polyclonal antibody. Dab2IP appears to have 3 major isoforms in the adult, ranging from 110-150 KDa.

B. β-gal staining



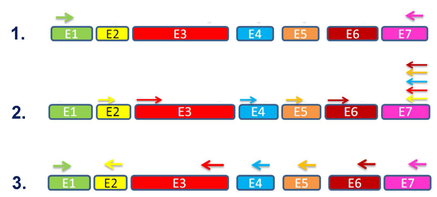
β-gal staining of saggittal sections obtained from Dab2IP-/+ mice shows that the Dab2IP is expressed in specific cell types in the EGL, PCL, and IGL. Dab2IIP expression in the GCL reaches a peak at P14 then decreases by P30. EGL, external granule cell layer. PCL, Purkinje cells layer. IGL, internal granule cell layer. ML, molecular layer. GCL, granule cell layer.

Dab2IP Gene Structure



The mouse *Dab2IP* gene structure was examined using the UCSC Genome Browser (July 2007 build). Dab2IP contains 20 exons spanning over 175Kb. Dab2IP-L protein contains a putative nuclear localization signal (NLS), complete PH-domain (PH), C2 domain (C2), Gap Related Domain (GRD), and a poly proline rich (Proline) region. Three CpG islands (CpG131, CpG54, and CpG 85) are located in the region spanning exons 2 and 5. Multiple translation start sites have been identified in various EST clones (eg. Exon 1 and Exon8) which result in two major protein isoforms. The main distinction between these two isoforms is the presence of the PH domain, which influences Dab2IP GAP specificity. To distinguish between these two major isoforms, different TaqMan probes were utilized (Probe 41 and Probe 51). The position of the gene trap cassette utilized to make Dab2IP KO mice is between exons 5 and 6 as indicated in the figure.

PCR Strategy for Identification of Dab2IP Splice Variants



Three different PCR strategies were developed to examine the expression of 5' Dab2IP exons during cerebellar development.

Expression of Dab2IP Isoforms are Developmentally Regulated

A.

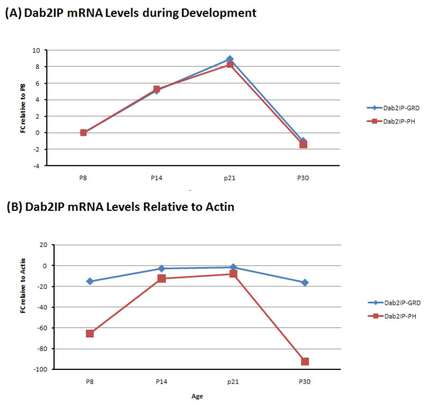
	A	B	C	D
E1	+	+	+	0
E2	0	0	0	0
E3	+	+	0	+
E4	+	+	0	+
E5	+	+	+	+
E6	+	+	0	0
E7	+	0	+	+

B.

	A	B	C	D
P 8	✓	✓	✓	
P21		✓	✓	✓

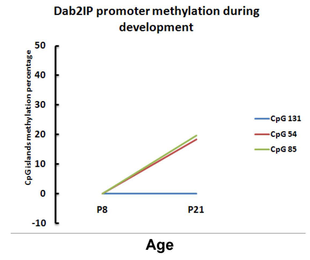
Using the PCR strategy described above, we identified at least 4 different transcript variants of Dab2IP (designated A-D). Exon 2 is lacking in all 4 variants found in the cerebellum. Two isoforms (B and C) appeared to be expressed at P8 and P21, whereas isoform A was expressed only at P8 and isoform D was expressed only in P21 cerebellum.

Dab2IP transcript levels in the Developing Cerebellum



Quantitative real-time PCR was performed using TaqMan probes which targeted either exons 5-7 (Dab2IP-PH) or exons 11-12 (Dab2IP-GRD). **(A)** Both transcripts increased from P8 to P21 and exhibited a sharp decrease by P30. **(B)** The actin-normalized levels of PH-domain containing isoform was 4-fold or 6-fold lower compared to GRD-Dab2IP (total transcripts) at P8 and P30, respectively.

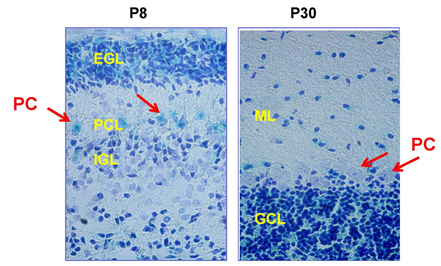
CpG islands associated with Dab2IP gene are Methylated during Development



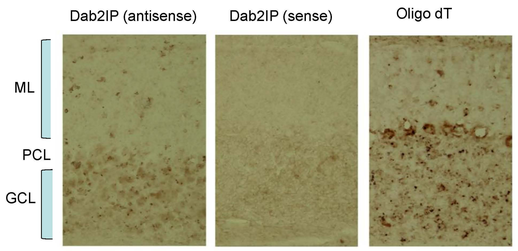
CpG islands methylation was examined at P8 and P21 by bisulfite sequencing approach. During cerebellar development, CpG131 was not methylated at either P8 or P21. In contrast, 18.8% and 19.6% of the cytosines were methylated in CpG54 and CpG85, respectively.

Cellular Distribution of Dab2IP Isoforms

A. β-gal and Nissl Staining



B. In Situ Hybridization



Dab2IP gene expression was examined in sagittal cerebellar sections at P8 and P30 by β-galactosidase assay and Nissl staining. **(A)** Dab2IP PH domain is expressed in EGL and PC at P8, and ML interneurons L and GCL at P30. **(B)** Non-radioactive *in situ* hybridization was performed on P30 Cerebllum using DIG labeled 48-mer antisense probes to Dab2IP GRD domain, which identifies both major Dab2IP isoforms. The sense sequence was used as negative control, and oligo dT probe was used as positive control.

Summary and Conclusions

1. Dab2IP gene appears to have two different promoters and also undergoes differential splicing during brain development. Three major Dab2IP protein isoforms were detected by Western blot analysis and 4 different 5' splice variants were detected by RT-PCR strategy.
2. In Cerebellum, the expression Dab2IP isoforms transiently increase between P8 and p21, and decrease dramatically by p30.
3. PH-domain containing Dab2IP isoform makes up a small proportion of the total Dab2IP transcripts at P8 and P30. Using the Lac Z reporter assay we show that this isoform is expressed in PC at P8 and is completely absent in PC at P30.
4. Two of the three CpG islands located in the 5' region of Dab2IP gene are methylated at P21 compared to P8. This increase in DNA methylation may be responsible for the rapid decrease in Dab2IP transcript levels during cerebellar development.

Acknowledgement

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