Prenatal chemical exposures induce the hyperplasia of lobule in developing cerebellar cortex and defection of granule cell migration

胎生期の化学物質投与は小脳小葉の過形成と顆粒細胞移動の異常を誘発する

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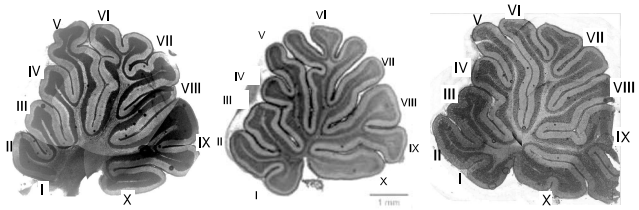
Abstract

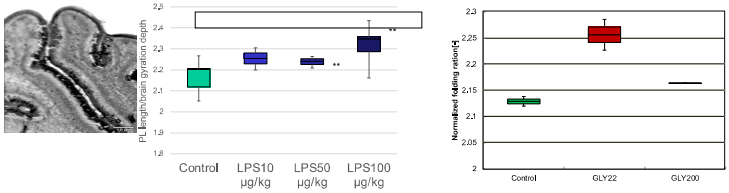
Chemical exposure in utero has potential effects on developmental neurotoxicity (DNT). Multiple chemicals, for example, sodium valproate (VPA), chlorpyrifos (CPF), or lipopolysaccharide (LPS), have been associated with an increased risk of ASD. We have observed that VPA-administrated rats showed excess folding, hyperplasia between the V to VI lobules of cerebellar vermis within two weeks after birth with dose-dependent and administrated-period-dependent manner. Cerebellar excess development is maintained in adults, and its alteration is similar to the human early ASD cerebellum. The irregular folding of vermis appeared in butyrate (BA)- and LPS-administrated rat cerebellum in early weeks, but not in CPF-administrated one. This irregular folding of lobules appeared even in the P5 cerebellum of the VPA-administrated rat. Recently, some reports presented that the selective knockdown of liver kinase B1 (LKB1), an activator of AMPK, induced cerebellar hyperplasia. In the VPA- or BA-administrated cerebellum, LKB1 expression disappeared in the inner EGL, whereas LKB1 was expressed in the control. In the VPA-, BA-, or LPS-administrated cerebellum, the granule cell migration sometimes defected, and the expressions of H3K9me were decreased in the molecular layer. We suggest that the epigenetic alteration of developing cerebellum with chemical exposure would induce the aberrant differentiation of granule cells, preventing the pruning of irregular neuronal development to hyperplasia.

Background



Valproate, or HDAC inhibitors, Trichostatin A and butyric acid, induce hyperplasia of develop-mental cerebellar vermis, and neurodevelopmental alteration.

Excess folding in the primary fissure (b/w lobule V and VI) is observed in utero HDAC inhibitor-administrated rat cerebellum.



Additionally, embryonic high-dose lipopolysaccharide (LPS) –administrated offspring and low-dose chronic glyphosate-administrated offspring showed temporal hyperplasia and neurodevelopmental alteration.

Ryan et. al. and Men et. al. reported LKB1 Regulates Cerebellar Development, and LKB1 knock out induced hyperplasia in Cerebellum.

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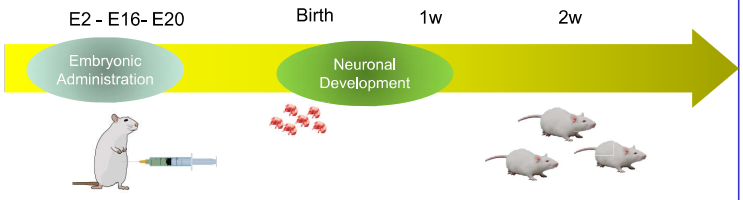
・HDAC inhibitors or above chemicals decrease the expression of LKB1 or not?
・Decrease of LKB1 expression induces malformation and abnormal development of cerebellum or not?

Methods



600 mg/kg valproate (VPA), 1 mg/kg Trichostatin A (TSA), or 100 mg/kg LPS was administrated to GD15 pregnant rat. VPA; p.o., TSA; p.o., LPS; i.p.

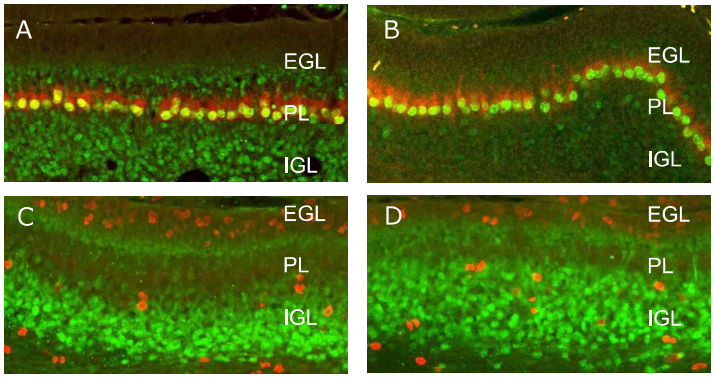
1.5 mg/kg/day glyphosate was chronically administrated to pregnant rat from GD 2 to 20 p.o.

Offspring of each administrated rat were fixed at postnatal day 7, and their cerebellum was observed immunohistochemically.

LKB1 is a primary upstream kinase of adenosine monophosphate-activated protein kinase (AMPK), a necessary element in cell metabolism that is required for maintaining energy homeostasis. It is known as a regulator of cell polarity and a tumor suppressor.

Result 1 : Alteration of LKB1 expression

LKB1-expression in granule cells in VPA-administrated offspring was decreased.

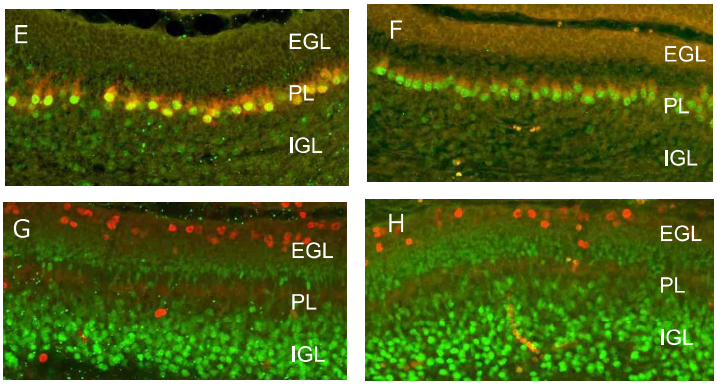


LKB1 and Calbindin D-28k expression in Control (A) or VPA-administrated (B) rat cerebellum at postnatal 7 day. green: LKB1, red: Calbindin D-28k. NeuN and Phospho-Histone H3 (Ser10) expression in Control (C) or VPA-administrated (D). green: NeuN, red: Phospho-Histone H3.

However, granule cell differentiation with NeuN expression did not relate to LKB1 expression.

Result 2 : Cases of TSA or LPS administration

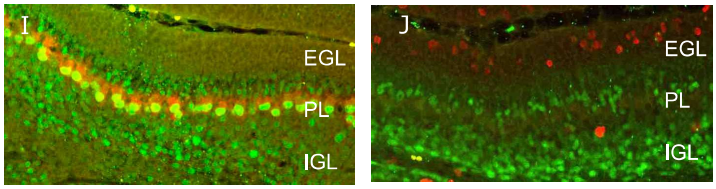
In TSA or LPS-administrated offspring, LKB1-expression of granule cells was similarly decreased.



LKB1 and Calbindin D-28k expression in TSA- (E) or LPS-administrated (F) rat cerebellum at postnatal 7 day. green: LKB1, red: Calbindin D-28k. NeuN and Phospho-Histone H3 (Ser10) expression in TSA- (G) or LPS-administrated (H). green: NeuN, red: Phospho-Histone H3.

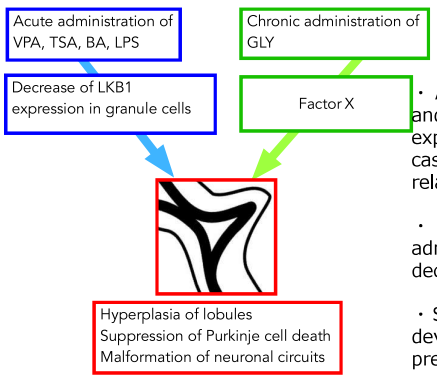
Result 3 : Case of chronic GLY administration

In GLY-administrated offspring, however, LKB1-expression of granule cells was not decreased.



LKB1 and Calbindin D-28k expression in GLY-administrated (I) rat cerebellum at postnatal 7 day. green: LKB1, red: Calbindin D-28k. NeuN and Phospho-Histone H3 (Ser10) expression in GLY-administrated (J). green: NeuN, red: Phospho-Histone H3.

Conclusion and Discussion



・Administration of HDAC inhibitors and LPS induced the decrease of LKB1 expression in granule cells. In these cases, LKB1 decrease might be related to hyperplasia.

・Administration of chronic GLY administration did not induce the decrease of LKB1.

・Several pathway to hyperplasia of developing cerebellum would be present.

・LKB1 expression has less correlation with granule cell differentiation.