小脳と前頭前野形態形成に置ける神経投射と ニューレグリン−１の役割

NRG-1 dependent neural projections play critical roles in the formation of normal cerebellum and prefrontal cortex structure

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Abstract —The brain structure was systematically compared between neuregulin-1 (NRG-1) knock out mice (KO) and wild type (WT) mice. The results show that there are significantly histological changes especially in two parts of KO mice brain, the cerebellum and prefrontal cortex. The size of cerebellum of KO mice was significantly reduced in several parts compared with that of WT, while the classical three layers of cortical organization was intact. On the other hand, KO mice present an outstanding phenotype in cerebellar cortex structure. The intercrural fissure separating lobule VI was much shallower in KO mice than that of WT. This phenotype was accompanied by cellular abnormalities, such as a reduced population of cells in granule cell layer (especially in lobule VI), Purkinje cell layer, and molecular layer. However, the numbers of ErbB4 positive Bergmann glial cells were largely increased in the molecular layer of adult KO cerebellum. In Bergmann glial cells, the redial extensions were completely misaligned in lobule VI or sparse in other lobules of cerebellum in KO mice. Furthermore, the cortical organization of prefrontal cortex in KO mice was totally disorganized, while the cells in WT prefrontal cortex showed a systemically radial alignment.

***Keywords* — Neural Network, Neurotrophic factors, Schizophrenia, projection, ErbB**

1. Introduction

Schizophrenia, once considered to be a neurodegenerative disorder but now most commonly viewed as a disorder of development, is a serve psychiatric disorder, which typically produces life-long disability. This disease is a highly heritable mental disorder characterized by chronic psychotic symptoms and cognitive deficits that are extremely difficult to model in animals. The neuregulins are a family of four genes (NRG-1-4), encoding proteins that share an epidermal growth factor-like domain, activate ErbB receptor tyrosine kinase. NRG-1 is the most well characterized member of the family, and it is known to be important in many organs, including heart, breast, and nervous system.

Within the nervous system, NRG-1 has diverse functions, such as neuronal migration, specification and neurite outgrowth, regulation of acetylcholine, $GABA_A$ and glutamate receptor expression, as well as oligodendrocyte proliferation and development. Interestingly, these development processes are thought to be involved, directly or indirectly, in schizophrenia. A psychiatric relevance of NRG-1 has emerged with the increasing evidence that it is a leading susceptibility gene for schizophrenia. There is now substantial but not incontrovertible evidence that genetic variation in NRG-1 is associated with schizophrenia. Even an overexpression of NRG-1 regarded as a genetic animal model for schizophrenia, the nature of the disorders in the animal models are still not fully understood, especially at the neuropathologic, neurochemical, and neuroanatomical levels. Further examination of the NRG-1-ErbB pathway by genetic and molecular methods, and by the use of animal models, may lead to a deeper understanding of the roles of these molecules in normal development and in the pathogenesis of this devastating disease. In this report, we investigate the possible roles of NRG-1 in heterozygous NRG-1 knockout mouse with disrupting Ig-L domain. Our results provide some evidence that NRG-1 plays roles in maintaining the normal cytoarchitectural structure of cerebellum and prefrontal cortex, and functional neural circuits.

2. Results

The structure of whole cerebellum was observed systematically in WT and NRG-1 KO mice. The

size of cerebellum in KO mice was significantly reduced in several parts of cerebellum compared with that of WT, while the classical three layers of cortical organization was intact. Brain serial sections of WT and KO mice were stained with Nissl staining (0.1% cresyl violet) and observed under microscope. On the structure of cerebellum, the intercrural fissure separating lobule VI was much shallower in KO mice than that of WT mice. The structure of lobule VI was examined with a higher magnification. The cell numbers in Purkinje cell layer of cerebellar lobule VI was reduced in KO mice. The cell numbers in granule cell layer was largely reduced especially in lobule VI of KO mice. To compare the number of granule cells, the brain slices (fixed with 4% PFA and permeabilized with 0.1% Triton x-100) from WT and KO mouse brain were stained with anti-α6GABA receptor, and counted cells under 400X confocal microscope within 100 μm x 100 μm area. The cell numbers in granule cell layer of lobule IV/V did not show significant difference between WT and KO mice, while the cells in only lobule VI was reduced in KO mice ($P < 0.05$, $n = 7$). Next, the cell numbers in cerebellar molecular cell layer was examined in KO mice. The brain slices from WT and KO mice were stained with both anti-GFAP (1:500) and anti-erbB4 antibodies (1:200). The ErbB4 positive cells were displayed in molecular layer around different lobules by labeling with NRG receptors, ErbB4 antibodies. In KO mice, ErbB4 positive cells remained in molecular layer even in adult mice compared to WT. The both GFAP and erbB4 stained cells in molecular cell layer could be detected in the cerebellar molecular layer in KO mice, while there was no such cells in WT mice cerebellar molecular layer, which should be located at Purkinje cell layer at adult stage. Bergmann glia radial extensions were completely misaligned in lobule VI or sparse in other lobules of cerebellum in KO mice. The radial extensions of Bergmann glial cells were much sparser in lobule IV in KO mice compared with WT mice. However, the extensions were misaligned in lobule VI in KO mice, while the extensions in WT mice were well organized. The difference between KO and WT mentioned the above seems to be dependent on the difference of neural projection from pontine nuclei by experiments of fluorescence tracer dye, DiI.

Secondly, the structure of cerebral cortex is examined in KO and WT mice. In KO mice, the cytoarchitectural structure of some parts of cerebral cortex was totally disorganized, The cells in the same parts of WT mice were well organized in radial direction outward to the pial surface, while the arrangement of cells in KO mice was a big mess and lost the direction. Next the projection into cerebra cortex was examined by DiI labeling (DiI was injected into Lobule VI, curs I and II after fixation to know the neural circuit between cerebellum and cerebrum). In KO mice the member of projection was decreased in the special areas. The abnormality in cerebra cortex caused from the difference of the projection through pontine nuclei from cerebellum. In the relay point, pontine nuclei, the cell fate was controlled by NRG-1. In KO mice, the neural cells decreased at postnatal day 9 dramatically. From these results we defined that the center of area is prefrontal cortex (PFC) of mice and the area (PFC) was examined extensively. The cell density in WT mice PFC was much higher than that in KO mice. The radial aliment of cell disappeared in KO mice PFC.

3. Conclusion

1) NRG-1 plays critical roles in the formation of normal structure of mouse brain cerebellum and prefrontal cortex.

2) The total numbers of cells in molecular cell layer, granule cell layer and Purkinje cell layers of cerebellum are largely reduced in NRG-1 KO mice.

3) The development of Bergmann glial cell in cerebellum are affected in NRG-1 KO mice. More Bergmann glial cells are stuck in cerebellar molecular cell layer rather than in the normal location in Purkinje cell layer. And the Bergmann glial cell's scaffold is disorganized in cerebellar lobule VI.

4) The cerebellar lobule VI is specifically affected in KO mice indicates that the formation of different folia of mouse cerebellum might undergo special mechanisms and folia singular regulation.

5) The cortical organization of prefrontal cortex in KO mice was disorganized, while the cells in WT showed a radial alignment.

6) The neural projection to prefrontal cortex through pontine nuclei from cerebellum (lobule VI, crus1 and II) decreased in KO mice.

Our results suggested some hints to explain the relation of development after birth and the mechanism of brain information processing in schizophrenia.

4. References

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