

[課程－2]

## 審査の結果の要旨

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**On Wednesday January 24th, 2018**, I presented my PhD research work to the committee of my dissertation defense. In my PhD research project, KIF3B role in brain development and wiring was examined. Both in vitro and in vivo data were interpreted. Histology, cytology, biochemistry and molecular biology techniques were used through the experiments. This study considered as the first direct test, to our knowledge, of KIF3B contribution to brain wiring and NMDARs trafficking, targeting and stability. In this study we showed that KIF3B is considered to play a pivotal role in neuronal development and function both as a transporter of NR2A and as a regulator of the canonical Wnt pathway. It was decided that my results were accepted and were appropriate to get the PhD degree.

### **Summary of discussion:**

#### **1- KIF3B role in brain development and wiring**

- *Kif3b*<sup>+/-</sup> mice exhibited a sudden enlargement of the brain during the pruning period from P7 to P14. Brain overgrowth might be partially induced by the overexpression of  $\beta$ -catenin because of the dysregulated APC. Indicates a critical role of KIF3B as a regulator of the  $\beta$ -catenin level in the brain.
- There was a significant modification of axons, dendrites and spine morphology during brain development in *Kif3b*<sup>+/-</sup> mouse brain. These modifications may be caused by many possible mechanisms, such as the altered regulation of cytoskeletal actin and tubulin, which might be a result of the dysregulated APC.
- *Kif3b*<sup>+/-</sup> neurons were shown to exhibit a loss of dendritic branches and spine density, and the morphology of the mushroom-like spines was selectively modified, potentially as a homeostatic response to enhance the connectivity level and contribute to synaptic plasticity in the *Kif3b*<sup>+/-</sup> mouse brain.

- In the *Kif3b*<sup>+/-</sup> brain, the anomalous development of hippocampal neurons might evoke an imbalance of brain activities during development, causing a failure in neuronal wiring through impairments in the synapse consolidation and patterning processes.
- The elimination of the unwanted synapses in the *Kif3b*<sup>+/-</sup> mouse brain was impaired, coinciding with the decrease in NR2A activity and increase in the stabilization of all synapses displayed by stable mature spines with wide heads in *Kif3b*<sup>+/-</sup> hippocampal neurons. Suggesting that KIF3B- dependent APC activities might mediate the proteosomal-dependent degradation of NMDARs in the spines, confirming the fundamental role of KIF3B in modulating synapse development and plasticity in the brain.
- KIF3B impacts the structural plasticity of the synaptic network and spine dynamics by regulating both the transportation of NR2A and the function of the APC protein in an activity-dependent manner by leading NR2A and APC to their specific target destinations.

## **2- Transport of NR2A by the KIF3 complex**

- A reduction in KIF3B expression led to an impaired transport of NR2A but not NR2B.
- KIF3B deficiency altered the turnover of NR2B, caused an accelerated degradation of NR2B in the dendrites of *Kif3b*<sup>+/-</sup> mouse neurons.
- We present evidence supporting a possible interaction among molecules in the KIF3-NR2A transporting complex. KIF3B forms a complex with NR2A, APC, and PSD95.
- KIF3B forms a heterodimer with KIF3A, and the KIF3A/3B complex binds to KAP3 to form a heterotrimeric protein complex. KAP3 interacts with APC through the armadillo repeat sequence, and APC connects to the PDZ domain of PSD95 in the PSD95-NR2A complex. Suggest that this protein complex is the basis of the transport of NR2A along microtubules in dendrites.
- The KIF3 complex mediates the connection between APC and PSD95, suggesting that KIF3B is not involved in NMDAR clustering around the assembly points and that scaffolding proteins such as PSD95 regulate NR2A assembly and recruit signalling proteins for NR2A trafficking as reported previously.

- Our data supports what was suggested in previous studies, that the dynamics of the trafficking of the different NMDAR subunits are differentially regulated, but the subunits need to form complexes with one another to be stabilized.
- The interaction between the NR2A/KIF3 complex and APC is controlled by the KIF3 motor protein and might play a crucial role in the synaptic Tagging theory as the trafficking and targeting of NR2A and APC to synapses is functionally regulated by an activity-dependent cargo-release around the activated synapses.
- APC-PSD95-NR2A cargos are selectively transported along dendrites and delivered by the KIF3 complex to the tagged synapses for NR2A-dependent synaptic activity during information acquisition and retention, suggests an involvement of KIF3B in the molecular mechanism of short-term memory (STM) and long-term memory (LTM) acquisition and formation.

### **3- APC/ $\beta$ -catenin in Wnt signalling pathway is regulated by the KIF3 complex**

- *Kif3b*<sup>+/-</sup> hippocampal neurons exhibited abnormal neuronal phenotypes at both molecular and cellular levels
- The levels of NR2A and the plasticity-related proteins (PRPs) (APC, PSD95, NR2B) were lower in KIF3B mutant neurons.
- NMDAR subunits 2A and 2B were influenced by KIF3B attenuation in the brain; the plus-end-directed transport of NR2A and the stability of NR2B clusters along dendrites were inhibited in the *Kif3b*<sup>+/-</sup> cells.
- Our observations provide clear evidence supporting the critical role of KIF3B in synaptic function, structure and plasticity not only through its regulation of the transportation and localization of NR2A but also through its regulation of the level of  $\beta$ -catenin in the synapses.
- In *Kif3b*<sup>+/-</sup> mice, the axonal GCs exhibited abnormal cytoskeletal tubulin and actin structure; microtubule and actin filaments were mismanaged and overlapped in the LE, causing an expansion in the axonal GC size and a depression in filopodia elongation and quantity, suggesting a functional deficiency in the APC/  $\beta$ -catenin complex, which may have partly stimulated the abnormal growth cone organization of the cytoskeleton. Confirming our finding in this study that KIF3B is vital for the functional regulation of

APC.

- In the *Kif3b*<sup>+/-</sup> mouse brain,  $\beta$ -catenin-targeted transportation to the proper synapses was altered. Overexpressed  $\beta$ -catenin may not be accurately integrated and may be influenced by the partial genetic disruption of KIF3B.

#### **4- Molecular pathology in the *Kif3b*<sup>+/-</sup> mouse brain**

- The reduction in the level of synaptic NR2A, which is considered to contribute to dysregulation of NMDA receptor-dependent forms of synaptic plasticity. The dysregulation of synaptic plasticity may be a pathophysiological basis for the phenotypes of these model animals.
- The alteration of the function of the APC complex; the APC level is decreased, and the  $\beta$ -catenin level is increased in the *Kif3b*<sup>+/-</sup> mouse brain compared to the expression in the *Kif3b*<sup>+/+</sup> brain. The APC- $\beta$ -catenin complex is an essential regulator of the Wnt signalling pathway, which is critical for the formation of neuronal circuits.

#### **Conclusion**

Our data suggest that the abnormalities of *Kif3b*<sup>+/-</sup> mutant mice are caused by a combination of two pathophysiological mechanisms: a reduction in the transport of NR2A and an impairment of the canonical Wnt pathway.