論文の内容の要旨

論文題目： Study of the Regulation of NMDA Receptor Transport via KIF17 Molecular Motor by Transgenic Mouse Approach

和訳： KIF17 モータ分子による NMDA 受容体輸送の制御機構：トランスジェニックマウス法によるアプローチ

指導教官： 廣川 信隆　教授

東京大学大学院医学系研究科
平成 18 年 4 月入学
医学博士課程
分子細胞生物学専攻
氏名： 馮　雪

Introduction

In neurons, kinesin superfamily proteins (KIFs) are important molecular motors that directionally transport various cargoes to axon or dendrites. Among these KIFs, KIF17 transports N-methyl-D-aspartate (NMDA) receptor subunit 2B (NR2B)-containing vesicles together with the scaffolding protein Mint1 along microtubules in dendrites. Since NMDA receptors are pivotal in the regulation of synaptic function in neurons, regulation of NMDA receptor trafficking from cell body to synapses should be important to modulate synaptic function. A previous work demonstrated that Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) phosphorylates KIF17 C-terminal tail domain (CTD) on Ser1029 to trigger the release of NR2B-containing vesicle from KIF17(-/-)However, how the NMDA receptor transport is controlled in neurons in living mouse brain is still unclear. In this study, I generated various KIF17 transgenic mouse lines and analyzed these mice to investigate the functional significance of the regulation of NMDA receptor transport in learning and memory.

Methods and Results

1. Generation of transgenic mice

① I introduced two kinds of mutation (S1029A and S1029D) into Ser1029 of *kif17* CTD to mimic various phosphorylation states by PCR mutagenesis. KIF17 with S1029A mimics unphosphorylated form of KIF17(-/-)It binds to cargoes but does not release it. KIF17 with S1029D mimics a phosphorylated form, which does not bind to

cargoes. Using these mutated and wild-type (WT) *kif17* cDNA, I constructed three transgenic vectors designed to express (GEP)-fused KIF17s under the control *CaMKIIa* promoter.

② I microinjected these constructs into mouse pronucleus and established three transgenic mouse lines: TgA ((GEP)kif17(-/-)with S1029A), TgD ((GEP)kif17(-/-)with S1029D), and TgS ((GEP)-wild-type KIF17).

③ To investigate the function of these exogenous proteins, I crossed the transgenic mice with *kif17(-/-)* mice to generate three transgenic mouse lines with *kif17(-/-)* background: *TgSkif17(-/-)*$^{-/-}$, *TgA/kif17(-/-)* and *TgD/kif17(-/-)* mice.

2. Characterization of *Tg*$^{+}$*kif17(-/-)*$^{-/-}$ mice

① Western blot analysis showed the $^{(GEP)}$KIF17 fusion protein was expressed in the forebrain region.

② *Tg*$^{+}$*/kif17(-/-)* mice grew normally and their brains showed no obvious morphological abnormality.

③ Immunoprecipitation indicated that $^{(GEP)}$KIF17 and $^{(GEP)}$KIF17$_{A1029}$ were precipitated with the NR2B and Mint1 but $^{(GEP)}$KIF17$_{D1029}$ did not.

3. Analysis of *Tg*$^{+}$*/kif17(-/-)* mice

① Behavior experiments and electrophysiological analysis revealed that *kif17(-/-)* mice exhibited severe impairments in spatial memory and synaptic plasticity such as long-term potentiation (LTP) because of the disruption of KIF17-mediated NR2B transport (Yin et al., unpublished observation). Spatial memory and LTP were intact in *TgS/kif17(-/-)* mice, suggesting that the expression of wild-type $^{(GEP)}$KIF17 into *kif17(-/-)* mice rescued these impairments. However, *TgA/kif17(-/-)* and *TgD/kif17(-/-)* transgenic mice showed impairments in spatial memory and LTP. These results suggest that not only binding of NR2B to KIF17 but also release of NR2B fromkif17(-/-)is a critical step for NR2B transport *in vivo*.

② I further investigated the intracellular localization of NR2B in the transgenic mouse neurons by immunocytochemical analysis. Double-staining of hippocampal neurons with antibodies against NR2B and Golgi marker showed an increased accumulation of NR2B in Golgi apparatus in *TgDkif17(-/-)*$^{-/-}$ neurons. This result suggests that $^{(GEP)}$KIF17$_{D1029}$ is not capable of translocating NR2B from cell body to dendrites.

③ I examined localization of NR2B in dendrites by double-labeling with NR2B/Synaptophysin or NR2B/MAP2. The amount of synaptic NR2B was significantly decreased in *kif17(-/-)* neurons. *TgS/kif17(-/-)* mouse neurons showed normal level of

synaptic NR2B. In both *TgA/kif17(-/-)* and *TgD/kif17(-/-)* neurons, synaptic NR2B levels were decreased to almost the same level of *kif17(-/-)* mouse neurons. Interestingly, there is a marked difference between *TgAkif17(-/-)*$^{-/-}$ and *TgD/kif17(-/-)* mouse neurons. Level of NR2B in dendritic shafts (colocalized with MAP2) was decreased in *TgD/kif17(-/-)* mouse neurons, but not in *TgAkif17(-/-)*$^{-/-}$. Level of NR2A in dendrites was also decreased in *kif17(-/-)*, *TgA/kif17(-/-)*, and *TgD/kif17(-/-)* mouse neurons.

⑥ I used surface biotinylation assay to detect the surface level of NR2B in primary hippocampal neurons. The level of surface NR2B was significantly decreased in *kif17(-/-)* neurons. *TgS/kif17(-/-)* mouse neurons showed normal level of surface NR2B. In both *TgAkif17(-/-)*$^{-/-}$ and *TgD/kif17(-/-)* neurons, level of surface NR2B was decreased to almost the same level of *kif17(-/-)* mouse neurons.

⑨ Water maze training induces up-regulation of phosphorylated CREB and *nr2a/2b* mRNAs in hippocampal neurons, as revealed previously (Yin et al. unpublished observation). *Kif17(-/-)* , *TgA/kif17(-/-)*, and *TgD/kif17(-/-)* mice lacked this up-regulation. *TgS/kif17(-/-)* mice exhibited normal level of the up-regulation.

Summary

I generated three transgenic mouse lines; *TgS/kif17(-/-)*, *TgA/kif17(-/-)* and *TgD/kif17(-/-)* mice. Using them, I examined a role of motor (KIF17)-cargo (Mint1/CASK/VELI/NR2B) interaction in synaptic accumulation of NR2B, neuronal plasticity (LTP), and spatial learning. Expression of (GEP)-KIF17 in forebrain regions rescued defects of *kif17(-/-)* mice in synaptic accumulation of NR2B (*TgS/kif17(-/-)*). In *TgA/kif17(-/-)* mouse neurons, (GEP)-mutated KIF17(-/-)which mimics unphosphorylated form, binds to the cargo but does not release it. As a result, NR2B entered into dendritic shafts but did not localize to synapses. In *TgD/kif17(-/-)* mouse neurons, (GEP)-mutated KIF17(-/-)which mimics phosphorylated form, does not bind to the cargo. As a result, NR2B was retained in Golgi-regions in cell bodies. *TgA/kif17(-/-)* and *TgD/kif17(-/-)* mice exhibited defects in CREB activation, LTP, and spatial memory formation, similar to those seen in *kif17(-/-)* mice.

These results suggest that phosphorylation-based regulation of motor (KIF17)-cargo (Mint1/CASK/VELI/NR2B) interaction plays critical roles in the process of spatial learning and memory.