[課程－2]

審査の結果の要旨

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An organelle's subcellular localisation is closely related to its functions. Early endosomes require localisation to somatodendritic regions to enable their functions in neuronal morphogenesis, polarised sorting and signal transduction to occur. However, it is not known how the somatodendritic localisation of early endosomes is determined. Here, we show that Kinesin superfamily protein 16B (KIF16B) is essential for correct localisation of endosomes in neurons. Loss of KIF16B function induced aggregated endosomes, while, interestingly, in neurons expressing a stalkless KIF16B, endosomes were mistransported to axons. The binding between the motor domain of KIF16B and microtubules was inhibited by the stalk domain, which mediates a self-adjusting regulation mechanism that directs the protein to its correct destination. Thus, "the stalk inhibition" mechanism of KIF16B determines the somatodendritic localisation of endosomes in neurons.

1. KIF16B binds to early endosomes in neurons. First, we immunostained hippocampal neurons at 8 div with an anti-KIF16B antibody. As a result, KIF16B was observed to co-localise with early endosomes in somatodendritic regions. When GFP-KIF16B was expressed in neurons, its localisation was similar to that of endogenous KIF16B, significantly localised in the somatodendritic regions of hippocampal neurons. GFP-KIF16B was not localised in axons. EEA1-positive early endosomes, cargos of KIF16B, are also specifically localised in the cell body and dendrites. Thus, KIF16B is a somatodendrite-specific motor protein binding to EEA1-positive early endosomes in neurons.
2. Knockdown of KIF16B leads to accumulation of early endosomes in somatodendritic regions. To identify the function of KIF16B in neurons, KIF16B was knocked down using RNA interference (RNAi), and the localisation of early endosomes was observed. When KIF16B was knocked down, early endosomes formed aggregates in the cell body and dendrites, probably due to lack of the function of KIF16B. Thus, KIF16B is required for evenly punctuate somatodendritic localisation pattern of early endosomes in neurons.
3. KIF16B lacking the 3rd coiled-coil domain specifically accumulated in axonal tips. We made a PX-mutated KIF16B (GFP-KIF16BPXmut), similar to the localisation of control GFP, GFP-KIF16BPXmut was diffused while the full-length KIF16B localised on endosomes in somatodendritic regions. When the PX domain was deleted from KIF16B, the same result was obtained.. However, when the 3rd coiled-coil domain was deleted (GFP-KIF16B936), the diffuse localisation was totally changed and GFP-KIF16B936 accumulated in the tips of axons.
4. KIF16B lacking the 3rd coiled-coil domain missorts early endosomes to axonal tips. GFP-KIF16B810, which does not contain the 3rd coiled-coil, was also located to the tips of axons. We next generated a construct that lacked the third coiled-coil domain, but has the PX domain. This protein also moved along axons and accumulated in axonal tips. If the association and dissociation between KIF16B and early endosomes determine the somatodendritic localisation of early endosomes, KIF16B810-PX, which mislocalises to axons, should not change the somatodendritic localisation of early endosomes. Contrary to our hypothesis, early endosomes were mislocalised to axonal tips in neurons expressing KIF16B810-PX. Mislocalised endosomes co-localised well

axons. Taken together, the 3rd coiled-coil domain of KIF16B regulates not only the localisation of KIF16B itself but also the somatodendritic localisation of early endosomes.

5. Binding between the motor domain and the inhibitory domain. To clarify the molecular mechanism of how the 3rd coiled-coil domain controls the localisation of early endosomes, we performed biochemical assays. Two deletion mutants, KIF16B810 and KIF16B1096, were tested because the former lacks the 3rd coiled-coil and specifically accumulated in axonal tips while the latter contains the 3rd coiled-coil and did not accumulate in any region. First, the binding of deletion mutants to microtubules was tested, more than half of KIF16B810 co-sedimented with microtubules and the remaining fraction was cytosolic. However, KIF16B1096, which possesses the 3rd coiled-coil domain, did not co-sediment with microtubules and localised to the cytoplasmic fraction. Immunoprecipitation was performed to test whether the 3rd coiled-coil domain binds directly to the motor domain. The result showed that the 3rd coiled-coil domain binds directly to the motor domain of KIF16B. To confirm that the 3rd coiled-coil domain regulates the localisation of endosomes, COS-7 cells that were transfected with control GFP, endosomes, revealed by EEA1 staining, were evenly distributed throughout the cytoplasm. When the 3rd coiled-coil domain was expressed in COS-7 cells, endosomes were significantly accumulated in the centre of cells.

In conclusion, in this study, we show that KIF16B is a somatodendrite-specific motor that transports early endosomes. Here, for the first time we show that the stalk domain of a motor protein determines the localisation of molecular motors and its cargos. Results suggest that the motor activity of KIF16B is negatively regulated by the 3rd coiled-coil domain. This mechanism inhibits the motor activity of KIF16B in axons and determines the precise localisation of KIF16B in neurons. Our data suggest that intra-molecular regulation of KIF16B, rather than cargo binding, determines the localisation of early endosomes. Although, we do not rule out a potential role of kinesin associated proteins or other covalent modifications in regulation of kinesin motility and directivity. There are more yet to be clarified about KIF16B, including roles of associated proteins or any post-translational modification such as glycosylation, phosphorylation or acetylation involved in the regulation mechanism introduced in our work for the motility of KIF16B. It would be also interesting to examine the KIF16B malfunction in diseases related to dysfunction of endocytosis.