

Abstract of Doctoral Thesis

Title : Novel functions of cytoskeletal protein ezrin in nervous system.

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Establishment of neural circuits in the central nerve system requires generation and development of multiple dendrites and single axon. Cultured neurons that showed a sequence of morphological changes have been well studied for neuronal development. Ezrin/Radixin/Moesin (ERM) proteins link between membrane proteins and actin cytoskeleton, and contribute to maintenance of cellular function and morphology. In cultured hippocampal neurons, suppression of both radixin and moesin showed deficits in growth cone morphology and neurite extensions. On the other hand, down-regulation of ezrin using siRNA caused impairment of netrin-1-induced axon outgrowth in cultured cortical neurons. However, roles of ezrin in the neuronal morphogenesis of the cultured neurons have been poorly understood. In this study, we performed detailed studies on the roles of ezrin in the cortical neurons *in vitro* and *in vivo* using ezrin knockdown (*Vil2^{kd/kd}*) mice.

We demonstrated that the cultured cortical neurons prepared from the *Vil2^{kd/kd}* mice embryo exhibited impairment of neuritogenesis, not neurite and axon outgrowth. In *Vil2^{kd/kd}* neurons, RhoA activity and phosphorylation of MLC2 were increased compared with wild-type neurons. Moreover, treatment of Y-27632 or blebbistatin, inhibitors specific for Rho kinase and myosin II, respectively, rescued the abnormalities in neuritogenesis in the *Vil2^{kd/kd}* neurons. These data suggest a novel role of ezrin in the neuritogenesis via down-regulation of RhoA/Rho kinase/MLC2 pathway.

Next, we examined the roles of ezrin in the pyramidal neurons of adult *Vil2^{kd/kd}* mouse cerebral cortex. Golgi staining showed that length of apical dendrites and numbers of basal dendrites of layer V pyramidal neurons in *Vil2^{kd/kd}* mice were obviously reduced. Thus, these results suggest that ezrin is an important element in neuritogenesis *in vitro* and *in vivo*.

We also observed that ezrin was increased in the hippocampus by intrastriatal injection of neurotoxin 6-hydroxydopamine (6-OHDA).

These results suggest that ezrin is one of the key players for neuritogenesis in cortical neurons and nervous system.