論　文　内　容　の　要　旨
Synopsis of Thesis

氏　名 Name	張　素香
論文題名 Title	The newly identified migration inhibitory protein regulates the radial migration in the developing neocortex (新規に同定した精神発達遅滞原因候補遺伝子MINPによる皮質神経細胞放射状移動制御機構の解明)

論文内容の要旨

〔目　的(Purpose)〕

Rare copy number variations (CNVs) in the chromosome 16p13.11 have been reported to be associated with several neuropsychiatric disorders such as epilepsy, mental retardation and attention deficit hyperactivity disorder. There were only 8 genes located on the 16p13.11 region but most of their functions in the nervous system (except NDE1 gene) still remain unclear. In this study, we focused on a gene MINP, which was one of the candidate genes of 16p13.11 CNV, hoping to clarify its functions in the developing brain.

〔方法ならびに成績(Methods/Results)〕

We firstly investigated the expression patterns of the MINP gene using in situ hybridization and RT-PCR. We found that MINP gene was highly expressed in the central and peripheral nervous system such as brain, spinal cord and dorsal root ganglion. In the developing mouse brain, the MINP gene was abundantly expressed in the cerebral cortex, and was specifically distributed in the intermediate zone and the cortical plate, suggesting that MINP is expressed in post-mitotic neurons, but not in precursor cells. In order to elucidate the function of MINP in the developing cortex, we performed gain or loss of function analysis of MINP through *in utero* electroporation of E14.5 mice using MINP siRNA, MINP shRNA, or MINP-overexpressing vectors. We found that the radial migration was accelerated by MINP knockdown and reduced by MINP overexpression but the neuronal morphology, final layer positioning and differentiation were not affected. We then performed *in vitro* transwell migration assay using primary-cultured neurons. The result showed that MINP knockdown also increased neural migration *in vitro*, indicating that MINP regulates neuronal migration in a cell-autonomous manner. We supposed that MINP is associated with modulating cytoskeletal structures or the motilities of neurons. We examined whether perturbation of the actin assembly-disassembly system affects MINP-mediated neuronal migration using cytochalasin D (CytoD), an inhibitor of actin polymerization. However, the accelerated migration induced by MINP knockdown was not apparently affected by CytoD treatment. Then we investigated if MINP is a potential regulator of microtubule dynamic stability. We examined the change of tyrosinated tubulin, detyrosinated tubulin, and △2-tubulin in MINP-suppressed neurons. The result of western blot showed that △2-tubulin, a stable tubulin assembly, was significantly decreased, suggesting that MINP knockdown influences microtubule stability. Finally, to better understand how MINP regulates microtubule, we carried out co-immunoprecipitation using Myc-tagged MINP expressing vector. More endogenous tubulin was detected in MINP overexpressed-cell lysate, indicating that MINP can interact with tubulin either directly or indirectly.

〔総　括(Conclusion)〕

In this study, we identified the role of a novel gene MINP in the developing neocortex. We found that MINP gene was highly expressed in the cerebral cortex, especially in post-mitotic neuronal area. Acute knockdown of MINP accelerated the radial migration of cortical neurons, but no significant abnormalities were observed in neuronal morphology and cortical lamination. Finally, MINP interacted with tubulin and the downregulation of MINP affected microtubule stability that could lead to the alteration of neuronal migration.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

申請者は16p13.11領域のコピー数変異が、様々な脳発達障害と関わりがあるという事実を元に研究を進めてきた。この小さな遺伝子領域内に脳発達を制御する遺伝子が複数あると考え、8つある全ての遺伝子のmRNA発現を確認した。その中で、発達時期の新生神経細胞特異的に高発現が認められる遺伝子MINPに着目し、その機能解析を行った結果、MINP遺伝子が神経細胞の放射状移動を負に制御する新規分子であることを見いだした。また、神経細胞移動の分子メカニズムを明らかにする為、細胞骨格であるアクチンと微小管重合との関与を調べたところ、MINPがTubulinと相互作用し、微小管安定性を制御することが示唆された。本研究は、新規神経発達疾患関連遺伝子機能を解明したという点で、今後の治療研究に新たな展開をもたらすものであり、学位論文に値すると考えられる。