学 位 論 文 の 要 旨

氏名　　LIU XINLANG

学 位 論 文 名　　UBE3A Controls Axon Initial Segment in the Cortical Pyramidal Neurons

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著 者 名　　Xinlang Liu, Zhuqian Jiang, Yoshinori Otani, Xiaowei Zhu, Yanyan Yu, Tarif Abu Md Mamun, Ferdousy Raihana Nasrin, Tatsuya Kishino, Masashi Fujitani

論 文 内 容 の 要 旨

INTRODUCTION

The axon initial segment (AIS) is a critical regulator of neuronal excitability and serves as the initiation site for action potentials (APs). Alterations in structural features of AIS, such as length and position, have been shown to influence neuronal function. *UBE3A* gene encodes the E3 ubiquitin ligase enzyme (UBE3A), crucial for protein degradation in neurons. In mature neurons, *UBE3A* expression is uniquely regulated by genomic imprinting, in which only the maternal allele is active, and the paternal allele is silenced. Disruptions in maternally expressed *UBE3A* cause the disorder called Angelman syndrome (AS), a neurodevelopmental disorder characterized by severe cognitive impairment, ataxia, speech difficulties, and frequent seizures. However, the UBE3A function is still fully elusive. We analyzed the AIS length in the four different brain area of four genotypes of mice, wild type (WT), heterozygous *Ube3a* maternal-deficient mKO, heterozygous *Ube3a* paternal-deficient (pKO), and homozygous *Ube3a* biallelic-deficient mice (fKO). We found a specific elongation of the AIS in the prelimbic cortex (PrL) in mKO and fKO mice, but not in the somatosensory cortex (SC) or motor cortex (MC).

Additionally, we analyzed the cell-autonomous functions of UBE3A in cultured cortical neurons obtained from *Ube3a*-floxed (*Ube3/*$^{flox/flox}$) mice.

MATERIALS AND METHODS

Ube3a mutant mice were generated by deleting part of exons 15 and 16 from the C57BL6/J background. The mice brains were cut into 50 μm coronal sections, and the AIS length in PrL, MC, SC was detected by immunofluorescence staining. In addition, we generated *Ube3a/*$^{flox/flox}$ mice by mating *Ube3a*$^{tm1a(KOMP)Wtsi}$ and B6-Tg (CAG-FLPe) mice. After establishing a culture of cortical neurons from *Ube3a/*$^{flox/flox}$ E14.5 mice, we infected neurons with AAV-EGFP as a control or AAV-Cre-eGFP to ablate the Ube3a gene. Cultured neurons were immunostained for MAP2 as a neuronal marker and Ankyrin-G to visualize AIS. All images were obtained using FV-1000D and FV-3000D confocal microscopes with 60X (NA=1.30) oil immersion and 40X (NA=1.25) objectives, and appropriate laser excitation and filters. All 3D images were measured automatically using the Simple Neurite Tracer plugin in ImageJ after manually specifying the start and end points of the AIS in Z-stack images. All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University.

RESULTS AND DISCUSSION

To assess the AIS length, we first aimed to clearly visualize the three-dimensional morphology of pyramidal neurons (PyNs) in the cortex using confocal 3D imaging. We evaluated this method using NeuroTrace® as a marker for the whole cell bodies of PyNs, CTIP2 for layer V PyNs, and Ankyrin-G for AIS. We quantified the AIS length in the layer V PyNs of WT, mKO, pKO, and fKO mice. mKO mice are commonly used as AS models. Compared to WT mice, we observed an increase in the AIS length of layer V PyNs of PrL only in mKO and fKO mice, but no abnormalities were observed in the AIS of pKO mice. The cumulative frequency distribution plot of individual AIS lengths showed a rightward shift with no change in shape in layer V of the PrL in mKO and fKO mice. However, pKO mice did not show any shift compared to WT mice. These results indicate the paternal UBE3A protein does not influence the development of PyNs in the

cortex and importance of the maternal allele of the Ube3a gene for the regulation of AIS in PrL.

We subsequently investigated whether AIS abnormalities were present in layer V PyNs in the primary motor cortex (M1), secondary motor cortex (M2), and SC of WT mice. We found that PyNs in layer V of M2 have significantly longer AIS than M1 or SC. We further compared the results between WT and Ube3a-deficient mice. We found no significant differences in AIS length between WT and any other mutant mice in M1, M2 and SC.

To investigate whether abnormal AIS length in the PrL occurs cell-autonomously or via neurocircuit-dependent non-cell-autonomous mechanisms, we infected neurons of *Ube3a*$^{flox/flox}$ mice with AAV-EGFP as a control or AAV-Cre-eGFP to ablate the Ube3a gene. Immunohistochemical analysis at 6 days after post-AAV infection showed approximately 3.5μm significant increase in AIS length in *Ube3a*$^{flox/flox}$ neurons that received AAV-Cre-eGFP infection compared to that *Ube3a*$^{flox/flox}$ cortical neurons received AAV-EGFP control vector. These results indicated that *Ube3a* deficiency altered the length of the AIS in vitro.

CONCLUSION

In conclusion, this study showed that the AIS lengths of the layer V PyNs in the PrL of mKO and fKO mice were specifically altered through cell- and non-cell-autonomous mechanisms. A more detailed examination is necessary to reveal the mechanisms underlying neurocircuit abnormalities and the molecular mechanisms in *Ube3a*-deficient mice.