様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）Investigating roles of exosome microRNAs related to interactions between oligodendrocyte and neuron for ALS pathogenesis

研究課題名（英文）Investigating roles of exosome microRNAs related to interactions between oligodendrocyte and neuron for ALS pathogenesis

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研究成果の概要（和文）：筋萎縮性側索硬化症（ALS）では核タンパク質であるTDP-43が核から消失し、細胞質において凝集体として蓄積し細胞死に至る。ALS病態におけるニューロンとOLGの相互連関は過去にほとんど解析されていない。本研究で作成したニューロン特異的TDP-43ノックアウト(TDPcKO)マウスはニューロンが保たれていたが、特に海馬領域でミエリンの減少が確認された。ニューロン特異的mRNAを用いたRNAseq解析により、ニューロンに発現しOLG分化・ミエリン誘導を促進する分子を複数同定した。同定された因子の中の分子Aはミエリンを回復させることが確認できた。

研究成果の学術的意義や社会的意義
ALS病態解析研究はニューロンやミクログリア、アストロサイトを用いた解析で進められてきたが、OLGとニューロンの関連は未解明な点が多い。本研究ではニューロンにおけるTDP-43がミエリンの誘導を制御していること、ALS病態ではその制御が破綻している可能性が示唆された。ALS病態におけるミエリン-OLGの障害は更なるニューロン-軸索障害を引き起こす”負の連鎖”に陥っている可能性がある。本研究によりALS病態の理解を深めることは学術的に意義があり、本研究成果はALSの治療薬開発にも寄与しうるため社会的意義も高いと考えられる。

研究成果の概要（英文）：In ALS, nuclear TDP-43 is mislocalized to cytoplasm and forms aggregates in neurons and oligodendrocytes. Previous studies suggest that loss of TDP-43 function in motor neurons induces and facilitates neurodegeneration in ALS pathogenesis. However, mechanisms of neuron-oligodendrocyte interaction in ALS are still poorly understood. In this study, we generated neuron-specific TDP-43 knockout mice and analyzed the neuron-OLG relationship in the TDP-43 loss-of-function conditions. In the pathological analysis, there was no obvious neuronal loss, but a decrease in myelin formations was observed, especially in the hippocampal region of TDPcKO mice. RNAseq analysis using neuron-specific mRNA identified several molecules expressed in neurons that promote OLG differentiation and myelin formation. Factor A among the identified factors was introduced into the hippocampus of TDPcKO mice in a neuron-specific manner, and restoration of myelin was confirmed.

研究分野：内科学

キーワード：ALS　TDP-43　neuron　oligodendrocyte　myelin

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様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by the degeneration of upper and lower motor neuron. The progressive paralysis usually endanger life within 3-5 years, and more than 90% of ALS are sporadic with no known cause. TDP-43 is an RNA binding protein that plays important role in regulating pre-mRNA splicing, RNA processing, RNA stability. Physiologically, TDP-43 shuttles between nucleus and cytoplasm, however, in ALS nuclear TDP-43 is mislocalized to cytoplasm and forms aggregates in neuron and oligodendrocyte. Loss of TDP-43 function in motor neurons induces and facilitates neurodegeneration in ALS pathogenesis (Iguchi et al, 2013). Although the impact of oligodendrocyte dysfunction and myelin damage has progressively received more attention and is now considered to be one of the major contributing factors to ALS (Traiffort et al., 2020), mechanisms of neuron-oligodendrocyte interaction in ALS are still poorly understood. In the preliminary study, we found that degenerations of oligodendrocyte and loss of myelination in neuron-specific TDP-43 knockout mouse (TDPcKO; $TDP^{flox/flox}$::$CamKII^{cre/-}$). This finding suggests that TDP-43 in neurons is indispensable for myelin formation.

２．研究の目的

Although ALS research has been focused on the pathophysiology of ALS using neurons, microglia, and astrocytes, the relationship between OLG and neurons remains largely unexplored. In this study, we focus on the interconnection between TDP-43-associated neurons and OLG, and aim to identify new therapeutic targets for ALS by elucidating the pathophysiology of the disease.

３．研究の方法

(1) Neuron specific TDP-43 knockout (TDPcKO) mouse was generated and used in this study.

(2) OLG and neuronal changes were analyzed by immunohistochemistry, Western Blot, and real time PCR.

(3) AAV-flex system was applied to express a specific molecule with the presence of Cre expression.

(4) Factors regulated by cytoplasmic TDP-43 were identified by FACS and RNAseq.

(5) RNA stability assay, RNA-IP and RNA pull-down assay were used to clarify the mechanism that neuronal cytoplasmic TDP-43 regulating myelination.

(6) Behavioral tests including open field test, novel object recognition test and elevated plus-maze test were performed in TDPcKO and control mice.

４．研究成果

We found that myelin markers such as CNPase and OSP were downregulated in the dentate gyrus of TDPcKO mice, and they were recovered by neuron-specific cytoplasmic TDP-43 expression. No significant neuronal death and oligodendrocyte decrease was detected in TDPcKO mouse.

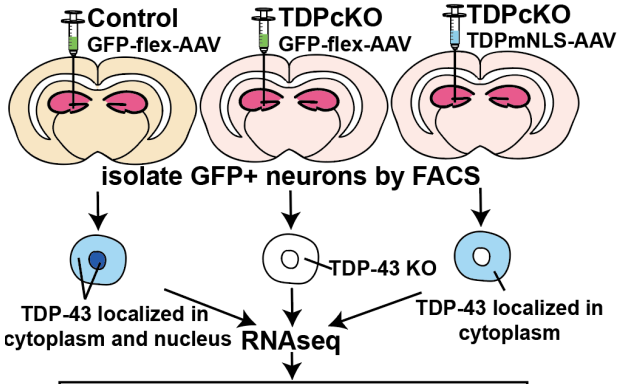

Figure 1. Flow-chart of analysis identifying the factors of myelination regulated by neuronal TDP-43.

To address the mechanism(s) of myelin change regulated by neuronal TDP-43 we injected GFP-flex-AAV and TDPmNLS-GFP-flex-AAV to control and TDPcKO mouse. GFP-positive cells were sorted by fluorescence-activated cell sorting (FACS), and RNAseq was done with the RNAs extracted from the sorted

neurons. Factors that match the following conditions were selected: (1) significantly down- or up-regulated in the neurons of TDPcKO mouse compared to CamKII-Cre mouse; (2) compensated the expression change in (1) by the cytoplasmic expression of TDP-43 in the neurons of TDPcKO mouse compared to TDPcKO mouse; (3) known to regulate myelin formation (Figure 1). One of the identified factors is Factor A, a presynaptic cell-adhesion protein. Factor A also regulates myelination and oligodendrocyte differentiation. The validation was done by qPCR and immunohistochemistry, and it was confirmed that cytoplasmic TDP-43 regulates Factor A mRNA expression.

Next, mRNA stability assay was done with primary neurons from TDPcKO mouse. As a result, the depletion of TDP-43 facilitated Factor A mRNA degradation, suggesting that cytoplasmic TDP-43 stabilizes Factor A mRNA. Then, RNA-IP with TDP-43 antibody or IgG was performed. Real time RT-PCR show that Factor A mRNA could bind to TDP-43. Finally, we proved that TDP-43 could bind 3'UTR of Factor A mRNA with RNA pull-down assay.

Furthermore, we generated Factor A-flex-GFP-AAV and injected it into the hippocampus of TDPcKO mouse. The myelin formation was successfully recovered by the supplementation in the injection site of the hippocampus (Figure 2) This result highly suggests that neuronal TDP-43 promotes myelination through regulation of Factor A expression.

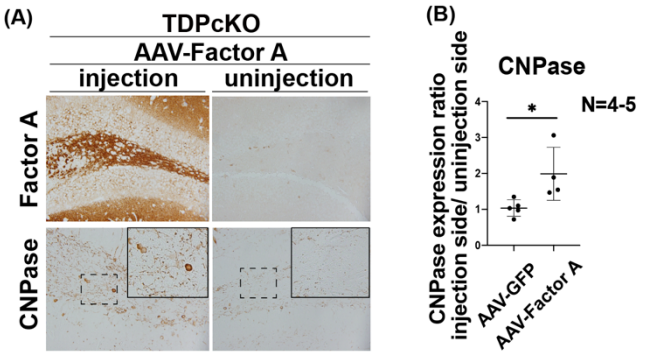

Figure 2. Factor A and CNPase immunohistochemistry result shows that CNPase+(myelin) staining area is increased in AAV-Factor A injected hippocampus of TDPcKO mouse.

<Reference>

Iguchi Y, Katsuno M, Niwa J, Takagi S, Ishigaki S, Ikenaka K, Kawai K. Loss of TDP-43 Causes Age-Dependent Progressive Motor Neuron Degeneration. Brain: A Journal of Neurology. 2013;136:1371–1382.

Traiffort E, Kassoussi A, Zahaf A, Laouarem Y. Astrocytes and Microglia as Major Players of Myelin Production in Normal and Pathological Conditions. Front Cell Neurosci. 2020; 14:79.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

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