様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　６ 年　 ５ 月 ２４ 日現在

機関番号：１３９０１
研究種目：若手研究
研究期間：2022～2023
課題番号：２２Ｋ１５７０７

研究課題名（和文）Exploring the mechanisms of synaptic dysfunction related to loss-of-TDP-43 function in the pathogenesis of ALS/FTLD

研究課題名（英文）Exploring the mechanisms of synaptic dysfunction related to loss-of-TDP-43 function in the pathogenesis of ALS/FTLD

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交付決定額（研究期間全体）：（直接経費）　　3,600,000円

研究成果の概要（和文）：本研究では、ALS/FTLD病態におけるTDP-43の機能とシナプス病態に着目した。ニューロン特異的TDP-43ノックアウトマウス(TDP-43CKO)を作成し、Novel object recognition testを行い、TDP-43CKOマウスは記憶障害を認めることが判明した。病理学的検証を行い、TDP-43CKO マウスではTDP-43ノックアウトニューロンに変性は生じず、形態も保たれていたが、シナプス数が減少していることが観察された。次にニューロン特異的トランスクリプトーム解析を実施して、遺伝子発現を解析した。この解析により、シナプス機能関連遺伝子の変化が認められた。

研究成果の学術的意義や社会的意義
ALS/FTLDでは、シナプス損傷が特徴的な病理学的変化である一方、神経細胞におけるTDP-43タンパク質とシナプス可塑性との関係は依然として不明である。我々の研究では、TDP-43タンパク質の喪失がマウスにおいてシナプス喪失と機能障害を引き起こすことを明らかにした。この発見は、ALS治療への新たな可能性を示唆している。

研究成果の概要（英文）：Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease, which affects both upper and lower motor neuron. Synaptic damage is observed in the early events, followed by neurodegeneration in ALS/FTLD. This study investigates the role of TDP-43 in synapse dysfunction during ALS progression. We created mice with conditional knockout of TDP-43 in neurons (TDP-43cKO). These mice exhibited reduced synapse numbers without obvious neuronal death and axonal damage. We isolated control and TDP-43 knockout neurons and analyzed their gene expression (RNAseq). This analysis revealed changes in genes associated with synapse function. Additionally, TDP-43cKO mice displayed memory deficits in novel object recognition test.

研究分野：内科学

キーワード：TDP-43　ALS　synapse　neuron

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Synapse formation and elimination are crucial for both the development of the central nervous system (CNS) and the pathogenesis of neurodegenerative diseases like amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) (Cardozo et al., 2019). TDP-43, a major contributor to neurodegeneration in ALS/FTLD, binds to RNA targets involved in synaptic function (Alami., et al., *Neuron* 2014). This suggests a role for TDP-43 in regulating synaptic plasticity through RNA transport and local translation. Recent evidence suggests that synaptic damage precedes neurodegeneration in ALS/FTLD (Darch et al., 2015; Qiu et al., 2014). Despite these insights, the exact role of neuronal TDP-43 in synaptic plasticity remains to be fully elucidated.

２．研究の目的

This study aims to investigate the specific roles of neuronal TDP-43 in regulating synaptic function within the context of ALS/FTLD pathogenesis. Unraveling this critical link between TDP-43 and synaptic plasticity holds immense promise for the development of targeted therapeutic strategies. By gaining a deeper understanding of how TDP-43 disrupts the finely tuned balance of synapse formation and elimination, we may be able to develop interventions that slow or even prevent the progression of ALS and FTLD.

３．研究の方法

(1) Generation of neuron-specific TDP-43 knockout mouse:

This study employed a genetically modified mouse model with a conditional knockout of TDP-43 specifically in neurons (TDP-43cKO).

(2) Analysis of synaptic function in TDP-43cKO Mouse:

Compared to control mice, a comprehensive analysis of synaptic function was conducted in the brains of TDP-43cKO mice. This involves a combination of techniques including immunohistochemistry, Western Blot, real-time PCR, and Golgi's staining.

(3) Identifying TDP-43-regulated synaptic factors:

This stage aims to identify the specific factors regulated by neuronal TDP-43 that control synaptic function. This was achieved by using fluorescence-activated cell sorting (FACS) and RNA sequencing (RNAseq).

(4) Behavioral assessment of TDP-43cKO mouse:

The functional consequences of TDP-43 loss on behavior were evaluated using the novel object recognition test.

４．研究成果

This study explores the impact of neuronal TDP-43 on synaptic function in the context of ALS/FTLD. To investigate this connection, we generated a neuron-specific TDP-43

knockout mouse model (TDP-43cKO) by crossing TDP-$43^{flox/flox}$ mouse with $CamKII^{Cre/-}$ mouse. This approach selectively removes TDP-43 in neurons, enabling us to isolate its effects on synaptic function. At 3 months of age, we observed a significant decrease in synaptophysin-positive synapses in the brains of TDP-43cKO mice compared to controls (TDP-$43^{flox/flox}$) (Figure 1). This finding was corroborated by Golgi staining, which revealed altered synaptic morphology. Notably, NeuN and neurofilament expressions remained unchanged, suggesting that the observed synapse loss was not due to neuronal or axonal death. To assess the functional consequences of these synaptic alterations, we employed the novel object recognition test. TDP-43cKO mice displayed impaired memory compared to controls, strongly suggesting that the observed synaptic damage contributes to cognitive decline (Figure 2).

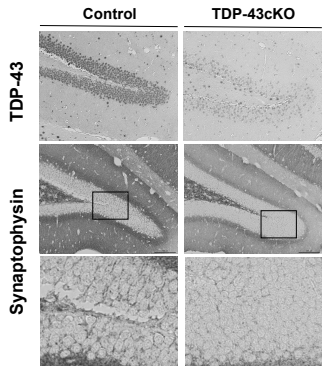

Figure 1. Synaptophysin+ signal was less detected in TDP-43cKO mouse compared with control mouse at the age of 3 months.

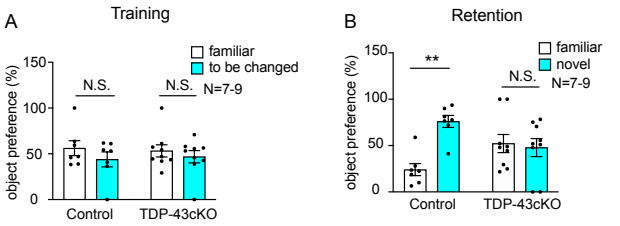

Figure 2. Novel object recognition test in TDP-43cKO mouse and control mouse.

To identify factors potentially regulated by neuronal TDP-43 and critical for synaptic function, we utilized green fluorescent protein (GFP)-tagged adeno-associated virus (GFP-flex-AAV) injected into the dentate gyrus of both control and TDP-43cKO mice. One month later, GFP-positive neurons were isolated from brain lysates, allowing for the purification of both normal and TDP-43 knockout neurons. RNAseq performed on RNA isolated from these purified neurons revealed several genes potentially involved in synaptic regulation (Figure 3). Future studies will be crucial to elucidate the specific roles of these genes in the context of TDP-43 and synaptic function.

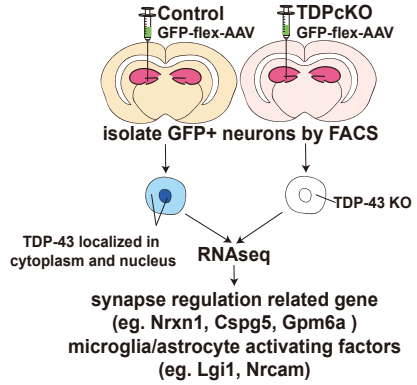

Figure 3. Flow-chart of analysis identifying the factors regulating synapse formation and elimination down-stream of neuronal TDP-43.

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５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　2件／うちオープンアクセス　2件）

１．著者名 Tsujikawa Koyo、Hamanaka Kohei、Riku Yuichi、Hattori Yuki、Hara Norikazu、Iguchi Yohei、Ishigaki Shinsuke、Hashizume Atsushi、Miyatake Satoko、Mitsuhashi Satomi、Miyazaki Yu、Kataoka Mayumi、Jiayi Li、Yasui Keizo、Kuru Satoshi、Koike Haruki、Kobayashi Kenta、Sahara Naruhiko、Ozaki Norio、Yoshida Mari、et al.	４．巻 8
２．論文標題 Actin-binding protein filamin-A drives tau aggregation and contributes to progressive supranuclear palsy pathology	５．発行年 2022年
３．雑誌名 Science Advances	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.1126/sciadv.abm5029	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Iguchi Yohei、Takahashi Yuhei、Li Jiayi、Araki Kunihiko、Amakusa Yoshinobu、Kawakami Yu、Kobayashi Kenta、Yokoi Satoshi、Katsuno Masahisa	４．巻 223
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３．雑誌名 Journal of Cell Biology	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.1083/jcb.202302048	査読の有無 有
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〔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

共同研究相手国	相手方研究機関