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機関番号：17102

研究種目：挑戦的研究(萌芽)

研究期間：2017～2018

課題番号：17K19663

研究課題名(和文) 肥厚性硬膜炎の世界初の動物モデルの樹立とTGF- β 1を標的とした画期的新規治療法開発研究課題名(英文) Establishment of world-premier pachymeningitis animal model and development of new therapeutic option targeting TGF- β 1.

研究代表者

吉良 潤一(Kira, Jun-ichi)

九州大学・医学研究院・教授

研究者番号：40183305

交付決定額(研究期間全体)：(直接経費) 5,000,000円

研究成果の概要(和文)：肥厚性硬膜炎(Hypertrophic pachymeningitis; HP)は原因不明の神経難病である。私たちはIgG4関連疾患のモデル動物であるLATY136Fマウスにおいて、硬膜炎と線維性肥厚が起こることを発見した。硬膜病変ではT細胞、B細胞、マクロファージ、形質細胞、好中球が浸潤し、IgG1(ヒトIgG4に対応)陽性細胞も多数みとめた。TGF- β 1/SMADシグナルが肥厚硬膜で亢進し、抗TGF- β 1作用を持つイルベサルタンを経口投与したところ、硬膜炎と肥厚が完全に抑制された。LATY136FマウスはHPの新規モデルであり、TGF- β 1/SMADシグナル抑制が有用である可能性が示唆された。

研究成果の学術的意義や社会的意義

肥厚性硬膜炎の根本的原因は不明であり、これまで動物モデルの報告も無く病態機序の検討や新規治療法開発といった基礎研究は全く進められていなかった。今回、私たちはLATY136Fマウスが世界初の肥厚性硬膜炎動物モデルとなることを報告し、さらにTGF- β 1/SMADシグナルが硬膜炎や線維性肥厚に対する治療標的となることをはじめて証明した。肥厚性硬膜炎の基礎研究を推進し、新規バイオマーカー探索や治療法開発を見据えた本研究の学術的・社会的意義は十分に高いと考えられる。

研究成果の概要(英文)：IgG4-related disease is a major cause of hypertrophic pachymeningitis (HP). HP lacks an animal model. We longitudinally evaluated dura in mice with a mutation (Y136F) in the linker for activation of T cells (LAT), which induced Th2 cell proliferation and IgG1 (IgG4 human equivalent) overexpression. Mice were administered daily oral irbesartan. Human IgG4-related, ANCA-related, and idiopathic HP dura were also examined. LATY136F mice had massive infiltration of B cells, IgG1+ cells, plasma cells, T cells, macrophages, and neutrophils in the dura, followed by marked fibrotic thickening. In dural lesions, TGF- β 1 was produced in B cells and macrophages while TGF- β 1 receptor I (TGF- β 1RI) was upregulated on fibroblasts. A similar upregulation of TGF- β 1RI and pSMAD2/3 was present in autopsied dura of all three types of human HP. Irbesartan abolished dural inflammation and fibrotic thickening in treated LATY136F mice. TGF- β 1/SMAD pathway is a potential novel therapeutic target for HP.

研究分野：神経免疫

キーワード：肥厚性硬膜炎

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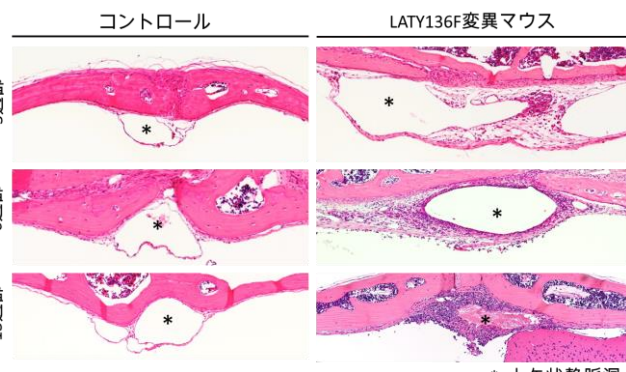


図1 LATY136F変異マウスは硬膜炎症細胞浸潤を呈する

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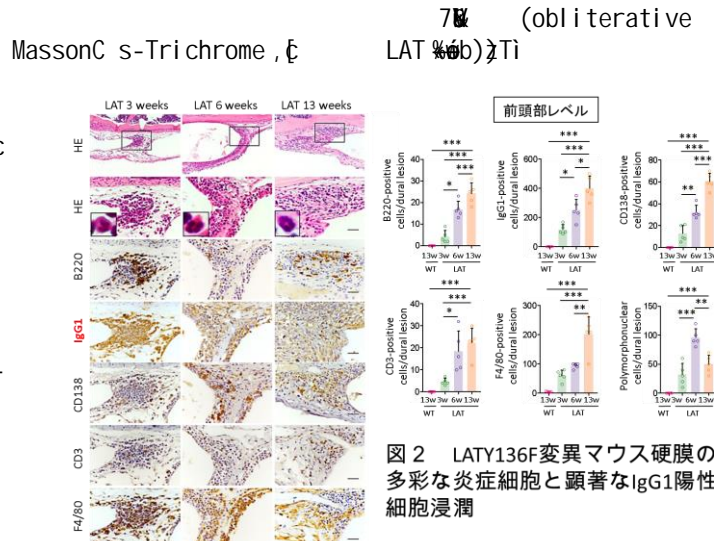


図2 LATY136F変異マウス硬膜の多彩な炎症細胞と顕著なIgG1陽性細胞浸潤

64 HP H*bs6 ANCA 64 HP H*x16 HP H*b%6y TGF-x SMAD2/SMAD3

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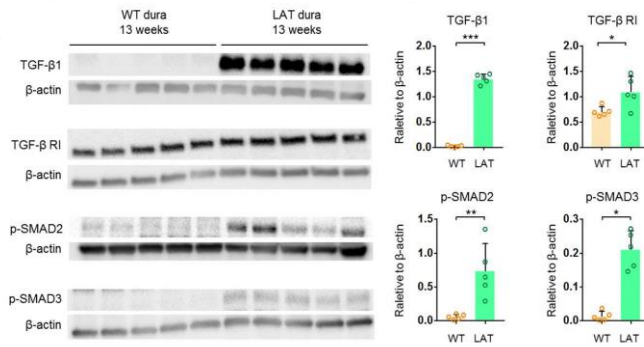


図3 LATY136F変異マウス硬膜でのTGF-β1, TGF-β受容体の発現亢進 SMAD2/SMAD3のリン酸化

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1) Cui Y, Masaki K, Zhang X, Yamasaki R, Fujii T, Ogata H, Hayashida S, Yamaguchi H, Hyodo F, Eto H, Koyama S, Iinuma K, Yonekawa T, Matsushita T, Yoshida M, Yamada K, Kawano M, Malissen M, Malissen B, Kira JI. A novel model for treatment of hypertrophic pachymeningitis. *Ann Clin Transl Neurol*. 2019 Jan 15; 6(3): 431-444. doi: 10.1002/acn3.715.

2) Zhang X, Fujii T, Ogata H, Yamasaki R, Masaki K, Cui Y, Matsushita T, Isobe N, Kira JI. Cerebrospinal fluid cytokine/chemokine/growth factor profiles in idiopathic hypertrophic pachymeningitis. *J Neuroimmunol*. 2019 May 15; 330: 38-43. doi: 10.1016/j.jneuroim.2019.01.010.

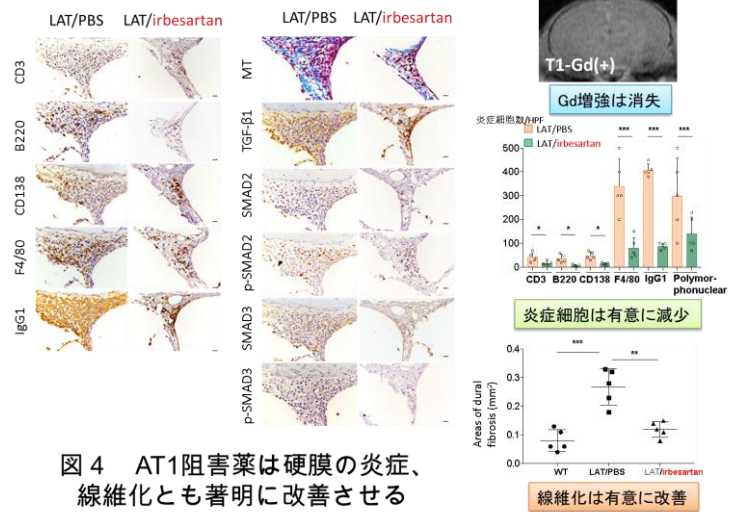


図4 AT1阻害薬は硬膜の炎症、線維化とも著明に改善させる

3) Kobayakawa Y, Masaki K, Yamasaki R, Shiraiishi W, Hayashida S, Hayashi S, Okamoto K, Matsushita T, Kira JI. Downregulation of Neuronal and Dendritic Connexin36-Made Electrical Synapses Without Glutamatergic Axon Terminals in Spinal Anterior Horn Cells From the Early Stage of Amyotrophic Lateral Sclerosis. *Front Neurosci*. 2018 Nov 28; 12: 894. doi: 10.3389/fnins.2018.00894.

4) Fang M, Yamasaki R, Li G, Masaki K, Yamaguchi H, Fujita A, Isobe N, Kira JI. Connexin 30 Deficiency Attenuates Chronic but Not Acute Phases of Experimental Autoimmune Encephalomyelitis Through Induction of Neuroprotective Microglia. *Front Immunol*. 2018 Nov 7; 9: 2588. doi: 10.3389/fimmu.2018.02588.

5) Nakamura Y, Gaetano L, Matsushita T, Anna A, Sprenger T, Radue EW, Wuerfel J, Bauer L, Amann M, Shinoda K, Isobe N, Yamasaki R, Saida T, Kappos L, Kira JI. A comparison of brain magnetic resonance imaging lesions in multiple sclerosis by race with reference to disability progression. *J Neuroinflammation*. 2018 Sep 5; 15(1): 255. doi: 10.1186/s12974-018-1295-1.

6) Fujita A, Yamaguchi H, Yamasaki R, Cui Y, Matsuoka Y, Yamada KI, Kira JI. Connexin 30 deficiency attenuates A2 astrocyte responses and induces severe neurodegeneration in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride Parkinson's disease animal model. *J Neuroinflammation*. 2018 Aug 13; 15(1): 227. doi: 10.1186/s12974-018-1251-0.

7) Fujii T, Yamasaki R, Iinuma K, Tsuchimoto D, Hayashi Y, Saitoh BY, Matsushita T, Kido MA, Aishima S, Nakaniishi H, Nakabeppu Y, Kira JI. A Novel Autoantibody against Plexin D1 in Patients with Neuropathic Pain. *Ann Neurol*. 2018 Aug; 84(2): 208-224. doi: 10.1002/ana.25279.

8) Watanabe M, Shiraiishi W, Yamasaki R, Isobe N, Sawatsubashi M, Yasumatsu R, Nakagawa T, Kira JI. Oral phase dysphagia in facial onset sensory and motor neuronopathy. *Brain*

9) Maimaitijiang G, Shinoda K, Nakamura Y, Masaki K, Matsushita T, Isobe N, Yamasaki R, Yoshikai Y, Kira JI. ~~WFLDWLRQ'HFHDMG3HEHWDJHRI997800V~~
With Disease Severity in Multiple Sclerosis. Front Immunol. 2018 Apr 10;9:748. doi: 10.3389/fimmu.2018.00748.

10) Li G, Yamasaki R, Fang M, Masaki K, Ochi H, Matsushita T, Kira JI. Novel disease-modifying anti-rheumatic drug iguratimod suppresses chronic experimental autoimmune encephalomyelitis by down-regulating activation of macrophages/microglia through an NF-~~%SDWKZD\~~ Sci Rep. 2018 Jan 31;8(1):1933. doi: 10.1038/s41598-018-20390-5.

11) Fujita A, Ogata H, Yamasaki R, Matsushita T, Kira JI. Parallel fluctuation of anti-neurofascin 155 antibody levels with clinico-electrophysiological findings in patients with chronic inflammatory demyelinating polyradiculoneuropathy. J Neurol Sci. 2018 Jan 15;384:107-112. doi: 10.1016/j.jns.2017.11.035.

12) Shinoda K, Matsushita T, Nakamura Y, Masaki K, Yamasaki R, Yamaguchi H, Togao O, Hiwatashi A, Kira JI. HLA-DRB1*04:05 allele is associated with intracortical lesions on three-dimensional double inversion recovery images in Japanese patients with multiple sclerosis. Mult Scler. 2018 May;24(6):710-720. doi: 10.1177/1352458517707067.

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3) Ryo Yamasaki, Yiwen Cui, Katsuhisa Masaki, Shotaro Hayashida and Jun-ichi Kira. Establishing a Hypertrophic Pachymeningitis Model Mice and Successful Treatment with Tumor Growth Factor-beta Blockade. The 142nd Annual Meeting American Neurological Association. October 15-17, 2017.

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