Molecular Genetics and Metabolism Reports 17 (2018) 80–82

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Correspondence

Effects of HLD-associated POLR1C mutant proteins on cellular localization and differentiation

Hypomyelinating leukodystrophis (HLDs) are composed of a group of congenital central nervous system (CNS) neuropathies, which resemble prototypic Pelizaeus-Merzbacher disease (PMD, also called HLD1). They display myelin dysfunction by causing repeated demyelination and remyelination, leading to severe demyelination [1–5]. CNS myelin is derived from morphologically differentiated oligodendrocyte plasma membranes. It plays an essential role in propagation of saltatory conduction and in protecting neuronal axons from physical and physiological stresses [6–8].

The *polr1c* gene encodes one subunit of nuclear RNA-catabolizing enzymes. RNA polymerase I and III subunit C (POLR1C) contributes to transcription of ribosomal RNA (rRNA), transfer RNA (tRNA), and other small RNAs. Two missense mutations Asn-32-to-Ile (N32I) and Asn-74-to-Ser (N74S) of the *polr1c* gene are associated with HLD11 (OMIN No. 616494) [9]; however, it is still unknown whether their mutations indeed affect intracellular localization of POLR1C proteins and/or cell morphological differentiation.

The plasmid encoding each human POLR1C construct or the wild type was introduced into oligodendrocyte cell line FBD-102b [10,11]. In the Fig. 1, the wild type was localized in the nuclei. In contrast, the N32I mutant proteins were primarily localized in punctate structures of the extranuclear region. Similar results were observed in the case of the N74S mutant (Fig. S1). The structures partially corresponded to the ones stained with an antibody against the lysosome marker LAMP1 (Fig. S2). Cells harboring the N32I or N74S mutant failed to exhibit differentiated phenotypes with myelin web-like structures along inner processes (Fig. S3).

The N32 and N74 positions are closely placed either on an amino acid sequence (Fig. S4) or on a predicted three-dimensional structure (Fig. S5). But, they are different from missense mutation positions in Treacher-Collins syndrome 3 (OMIN No. 248390). Further analyses will allow us to promote our understanding of the relationship between POLR1C mutations and the cellular effects.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgmr.2018.11.002.

https://doi.org/10.1016/j.ymgmr.2018.11.002
Received 9 November 2018; Accepted 9 November 2018
Available online 16 November 2018

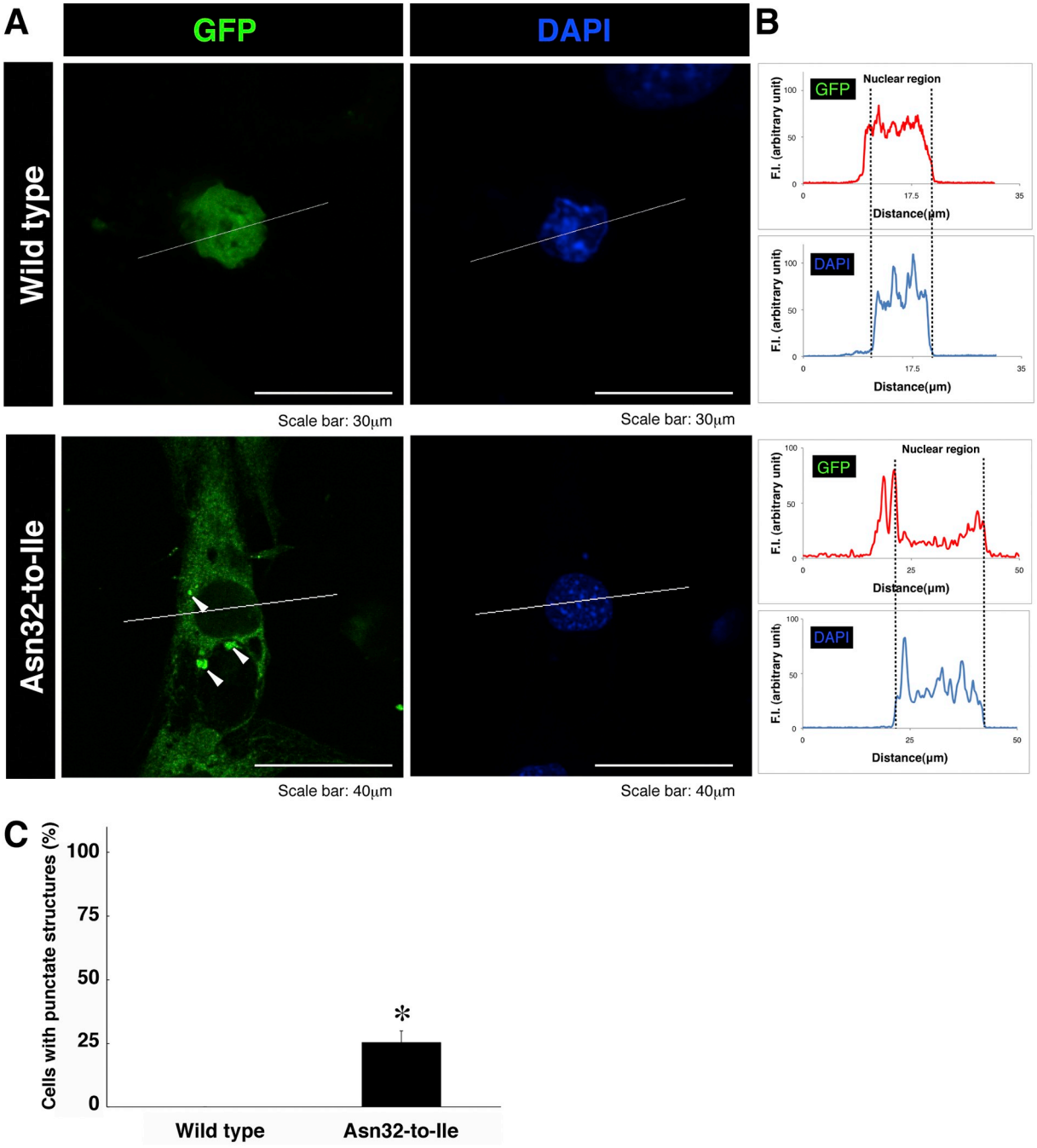

Fig. 1. The Asn-32-to-Ile missense mutant proteins of POLR1C exhibit extracellular localization with punctate structures. (A) FBD-102b cells were transfected with the plasmid encoding the wild type or the Asn-32-to-Ile mutant of GFP-tagged POLR1C. Representative GFP-fluorescence (green) and DAPI-fluorescence (blue) images are shown. The white arrowheads indicate the representative aggregation-like punctate structures. (B) The line plot along a white line (from the left to right direction) of the respective images is shown. F.I. indicates fluorescent intensities. (C) Cells with punctate structures of GFP-tagged POLR1C are statistically shown (*, $p < .01$ of Student's *t*-test for the control; $n = 3$ fields). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

References

[1] J. Garbern, F. Cambi, M. Shy, J. Kamholz, The molecular pathogenesis of Pelizaeus-Merzbacher disease, Arch. Neurol. 56 (1999) 1210–1214.
[2] A.S. Dhaunchak, D.R. Colman, K.A. Nave, Misalignment of PLP/DM20 transmembrane domains determines protein misfolding in Pelizaeus-Merzbacher disease, J. Neurosci. 31 (2011) 14961–14971.
[3] W. Lin, Y. Lin, J. Li, A.G. Fenstermaker, S.W. Way, B. Clayton, S. Jamison, H.P. Harding, D. Ron, B. Popko, Oligodendrocyte-specific activation of PERK signaling protects mice against experimental autoimmune encephalomyelitis, J. Neurosci. 33 (2013) 5980–5991.
[4] K. Inoue, Cellular pathology of Pelizaeus-Merzbacher disease involving chaperones associated with endoplasmic reticulum stress, Front. Mol. Biosci. 4 (2017) 7.
[5] M. Abu-Rub, R.H. Miller, Emerging cellular and molecular strategies for enhancing central nervous system (CNS) remyelination, Brain Sci. 8 (2018) E111.
[6] M. Simons, D.A. Lyons, Axonal selection and myelin sheath generation in the central nervous system, Curr. Opin. Cell Biol. 25 (2013) 512–519.
[7] P.D. Morton, N. Ishibashi, R.A. Jonas, V. Gallo, Congenital cardiac anomalies and white matter injury, Trends Neurosci. 38 (2015) 353–563.
[8] A.S. Saab, K.A. Nave, Myelin dynamics: protecting and shaping neuronal functions, Curr. Opin. Neurobiol. 47 (2017) 104–112.
[9] I. Thiffault, N.I. Wolf, D. Forget, K. Guerrero, L.T. Tran, K. Choquet, M. Lavallée-Adam, C. Poitras, B. Brais, G. Yoon, L. Sztriha, R.I. Webster, D. Timmann, B.P. van de Warrenburg, J. Seeger, A. Zimmermann, A. Máté, C. Goizet, E. Fung, M.S. van

der Knaap, S. Fribourg, A. Vanderver, C. Simons, R.J. Taft, J.R. Yates 3rd., B. Coulombe, G. Bernard, Recessive mutations in POLR1C cause a leukodystrophy by impairing biogenesis of RNA polymerase III, Nat. Commun. 6 (2015) 7623.
[10] Y. Miyamoto, J. Yamauchi, J.R. Chan, A. Okada, Y. Tomooka, S. Hisanaga, A. Tanoue, Cdk5 regulates differentiation of oligodendrocyte precursor cells through the direct phosphorylation of paxillin, J. Cell Sci. 120 (2007) 4355–4366.
[11] Y. Urai, M. Yamawaki, N. Watanabe, Y. Seki, T. Morimoto, K. Tago, K. Homma, H. Sakagami, Y. Miyamoto, J. Yamauchi, Pull down assay for GTP-bound form of Sar1a reveals its activation during morphological differentiation, Biochem. Biophys. Res. Commun. 503 (2018) 2047–2053.

Yuri Hiraoka[a], Kohei Hattori[a], Yu Takeuchi[a], Minami Yamawaki[a], Natsumi Watanabe[a], Naoto Matsumoto[a], Keiichi Homma[b], Yuki Miyamoto[a,c], Junji Yamauchi[a,c,*]
[a] *Laboratory of Molecular Neuroscience and Neurology, Tokyo University of Pharmacy and Life Sciences, Hachioji, Tokyo 192-0392, Japan*
[b] *Department of Life Science and Informatics, Maebashi Institute of Technology, Maebashi, Gunma 371-0816, Japan*
[c] *Department of Pharmacology, National Research Institute for Child Health and Development, Setagaya, Tokyo 157-8535, Japan*
E-mail address: yamauchi@toyaku.ac.jp (J. Yamauchi).

* Corresponding author: Laboratory of Molecular Neuroscience and Neurology, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan.