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Correspondence

Defective oligodendrocyte differentiation by hypomyelinating leukodystrophy 13 (HLD13)-associated mutation of Hikeshi

Letter to the Editor

Hikeshi (also called C11orf73) plays a key role in translocating the 70-kDa heat shock protein (Hsp70) to the nucleus. Hsp70 possesses ATP-metabolizing enzymatic activities and Hikeshi functionally binds only to its ATP-bound form to protect nuclear proteins under stress conditions such heat shock stress. Thus, mutation(s) in Hikeshi is thought to cause nuclear damage and in turn cell damage in cell types highly expressing Hikeshi. The Cys4-to-Ser (C4S) [1,2] and Val54-to-Leu (V54L) [3] mutations of C11ORF73 are associated with infantile leukoencephalopathy and hypomyelinating leukodystrophy type 13 (HLD13), respectively, which are severe diseases in central nervous system myelin-forming oligodendroglial cells. Especially, it remains unclear how Hikeshi with the V54L mutation affects differentiation in oligodendrocytes, resulting in hypomyelinating phenotypes. Herein we describe that expression of Hikeshi proteins with the V54L mutation as well as well as C4S one in rat primary oligodendrocyte precursor cells inhibits their differentiation. This system is used as a reproducible system for the *in vitro* experiments [4]. Infection of retroviruses harboring Hikeshi with the V54L mutation, but not wild type, decreased the expression levels of differentiation marker proteins, myelin basic protein (MBP) and 2′, 3′-cyclic nucleotide 3′-phosphodiesterase (CNPase) (Fig. 1, A-C). In addition, the infection decreased the percentage of cells positive for MBP or adenomatous polyposis coli (APC) as a differentiation marker (Fig. 1, D-G), suggesting that HLD13-associated mutation of Hikeshi is linked to defective oligodendrocyte differentiation. Furthermore, Hikeshi with the V54L mutation inhibited heat shock-induced nuclear transport of Hsp70 (Fig. S1).

It is possible that myelination may become difficult due to the inability of oligodendrocytes to differentiate. Further studies may allow us to understand the detailed mechanism by which HLD13-associated V54L mutant proteins inhibit oligodendrocyte differentiation. Elucidation of the molecular pathogenesis mechanism for HLD13 may lead to the development of molecular target-specific drugs.

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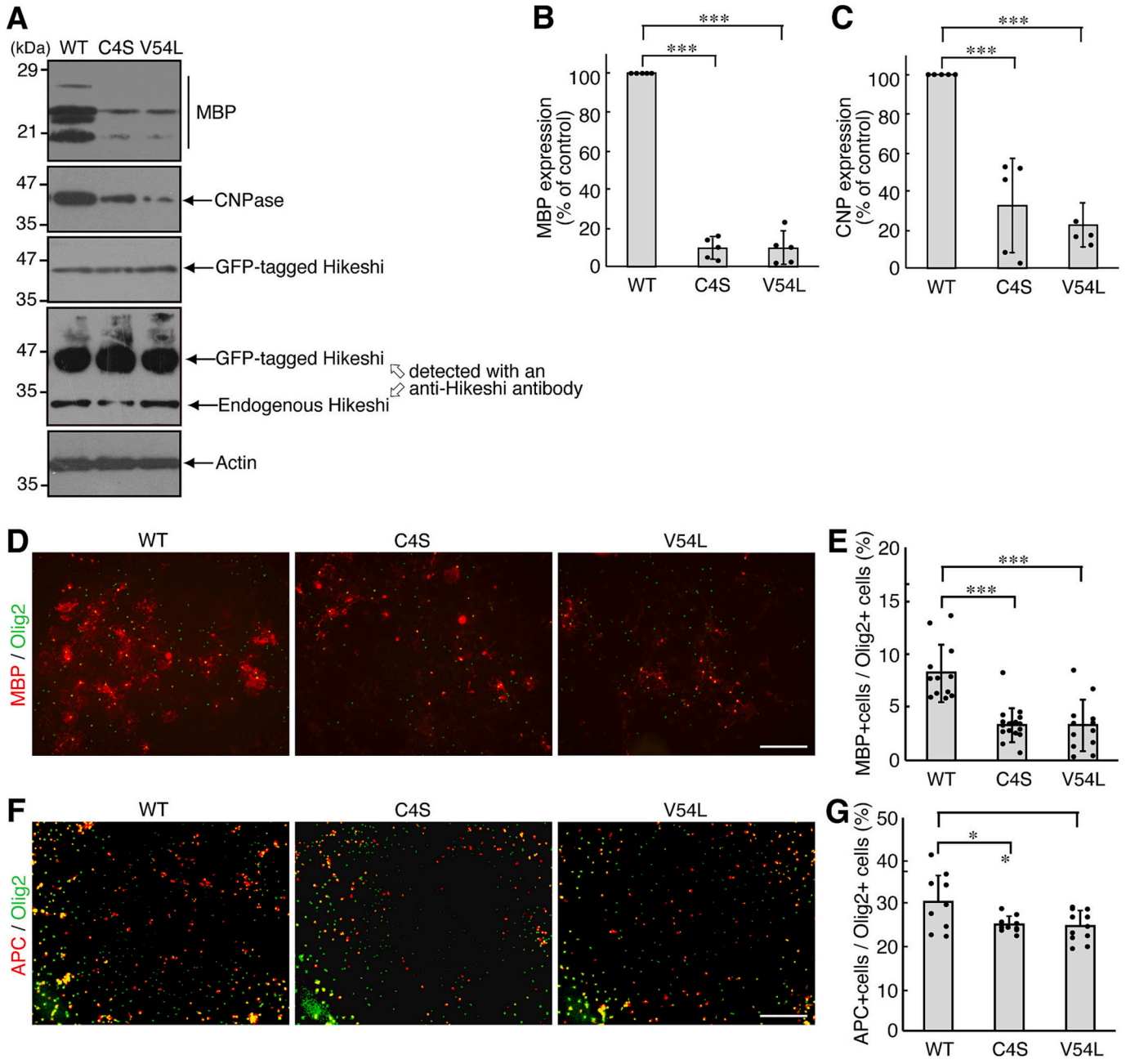

Fig. 1. Expression of the V54L mutant proteins of Hikeshi inhibits differentiation in rat primary oligodendrocyte precursor cells. (A-C) Oligodendrocyte precursor cells were isolated from embryonic day 15 (E15) Sprague-Dawley rats, infected using recombinant retroviruses (wild type [WT] Hikeshi or Hikeshi with the C4S or V54L mutation), and induced to differentiate. The lysates of their cells were subjected into SDS-PAGE and immunoblotted with an anti-MBP, CNPase, Hikeshi, or control actin antibody. Exogenous GFP-tagged Hikeshi proteins were detected with an anti-GFP antibody. Expression of GFP-tagged Hikeshi with the C4S mutation weakly decreased the expression levels of endogenous Hikeshi, suggesting that decreased expression levels of endogenous Hikeshi can affect differentiation. On the other hand, Expression of GFP-tagged Hikeshi with the V54L mutation did not significantly change the expression levels of endogenous Hikeshi, suggesting that its mutation itself may affect differentiation. Immunoreactive protein bands were statistically represented relative to protein bands in cells expressing the wild types (control bands) as 100% in graphs (*** $p < 0.001$; $n = 5$ blots). (D-G) Infected oligodendrocyte precursor cells were induced to differentiate and stained with anti-MBP or APC and Olig2 antibodies (red). Differentiating MBP- or APC-positive cells per Olig2-positive cells were represented as percentages in a graph (*** $p < 0.001$, * $p < 0.05$; $n = 10$ fields). Olig2 are expressed throughout all oligodendrocyte lineage cells. Although the efficiencies of differentiated cells such as MBP-positive cells for all Olig2-positive oligodendrocyte lineage cells were not high, Hikeshi with the C4S or V54L mutation indeed inhibited differentiation. Scale bar indicates 20 μm.

Declaration of Competing Interest

None.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgmr.2023.101017.

Data availability

No data was used for the research described in the article.

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