Conditional deletion of Id2 or Notch1 in oligodendrocyte progenitor cells does not ameliorate disease outcome in SOD1<sup>G93A</sup> mice

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A B S T R A C T

Oligodendrocytes are essential for structural and trophic support of motor axons. Their impairment has been implicated in amyotrophic lateral sclerosis (ALS), a neurodegenerative disorder of motor neurons. Oligodendrocyte progenitor cells fail to differentiate into mature oligodendrocytes and thereby jeopardize the health of motor neurons. Here, we report that oligodendrocytic ablation of inhibitor of DNA binding 2 (Id2) or Notch receptor 1 (Notch1), 2 negative master modulators of oligodendrocyte differentiation, fails to alleviate oligodendrocyte dysfunction or alter disease outcome in a murine model of ALS. Our data suggest that these inhibitors are not suitable targets for intervention in ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder with selective upper and lower motor neuron degeneration (Brown and Al-Chalabi, 2017; Swinnen and Robberecht, 2014). Although traditionally viewed as a motor neuron disease, non-neuronal supporting cells extensively contribute to motor neuron degeneration (Boillee et al., 2006; Ilieva et al., 2009). Oligodendrocytes are such contributing non-neuronal cells, as they degenerate during disease and are replaced by newly formed oligodendrocytes (Kang et al., 2013; Philips et al., 2013). However, in both ALS patients and mutant SOD1 mice, these newly formed oligodendrocytes are immature and dysfunctional, as they insufficiently generate myelin basic protein (MBP) and monocarboxylate transporter 1 (MCT-1) (Kang et al., 2013; Lee et al., 2012; Philips et al., 2013). Consequently, motor neurons lose an important source of structural and trophic support. Therefore, strategies to improve differentiation of oligodendrocyte progenitor cells (OPCs) toward mature and functional oligodendrocytes could be of therapeutic interest in ALS (Philips and Rothstein, 2017).

Considerable efforts were made to shed new light on a plethora of factors that regulate oligodendrocyte differentiation (Li et al., 2009). Different studies showed that Id2 and Notch1 are permissive for OPC expansion and inhibit their differentiation into functionally mature oligodendrocytes (Wang et al., 2001; Zhang et al., 2009), suggesting their potential involvement in the oligodendrocyte pathology documented in ALS mice (Kang et al., 2013; Philips et al., 2013).

In this study, we investigated whether oligodendroglial-specific deletion of these 2 master regulators of oligodendrocyte differentiation, maturation, and functioning could reduce oligodendrocyte pathology and improve disease outcome in ALS mice.

2. Methods

2.1. Transgenic mouse models

Human mutant (SOD1<sup>G93A</sup>) and wild-type (SOD1<sup>WT</sup>) SOD1 overexpression mice (#004435; https://www.jax.org/strain/004435 and #002297; https://www.jax.org/strain/002297) and Notch1<sup>Lox/Lox</sup>
mice were purchased from the Jackson Laboratory. ID2\textsuperscript{lox/lox} mice were described previously (Niola et al., 2012). Platelet-derived growth factor-\(\alpha\) receptor (PDGF\(\alpha\)-R)-CreER mice (Kang et al., 2010) were kindly donated by Dwight E. Bergles (Johns Hopkins School of Medicine, Baltimore). All mice were maintained on a C57BL/6J background, and littermate controls were used for this study. Cre-mediated recombination was induced at P60 by administering tamoxifen as described previously (Philips et al., 2013). The use and maintenance of all the mice used in this study was approved by the ethical committee of the University of Leuven, Belgium.

2.2. Motor performance, disease onset, and end-point determination

Motor performance and disease onset were assessed by the hanging grid test, as described previously (Staats et al., 2016). End-stage was determined as described previously (Van Hoecke et al., 2012).

2.3. Immunoblot analysis

Protein lysates (30 \(\mu\)g) were loaded on a 10% (for MCT-1) or 15% (for MBP) SDS-polyacrylamide gel (SDS-PAGE) and processed as described previously (Philips et al., 2013). Following primary antibodies were used: anti-MBP (goat, 1/10,000; Santa Cruz, RRID:AB_1113858), anti-MCT-1 (chicken, 1/1000; Millipore, RRID:AB_90565), and anti-GAPDH (mouse, 1/2000; Ambion, RRID:AB_437392).

3. Results

3.1. Disease outcome is not improved in ALS mice upon oligodendroglial deletion of Id2 or Notch1

To evaluate if oligodendroglial-specific deletion of Id2 or Notch1 could reduce oligodendrocyte pathology and improve disease outcome in ALS mice, we crossbred Id2\textsuperscript{lox/lox} or Notch1\textsuperscript{lox/lox} mice to PDGF\(\alpha\)-R-CreER and SOD1\textsuperscript{G93A} mice. The specificity of the PDGF\(\alpha\)-R promoter–driven gene excision and the recombination efficiency obtained with Id2\textsuperscript{lox/lox} or Notch1\textsuperscript{lox/lox} mice were proven to be as expected, and no significant upregulation of the other Id or Notch molecules was seen (see Supplementary Results and Supplementary Figures 1 and 2). In the triple transgenic offspring, motor performance, determined by the hanging grid test (Fig. 1A and D) and the rotarod test (Supplementary Figure 3A and C), and body weight (Supplementary Figure 3B and D) were evaluated throughout the course of disease. For none of these parameters, a significant improvement was observed upon deletion of Id2 or Notch1 compared to control SOD1\textsuperscript{G93A} mice. Neither was disease onset delayed (Fig. 1B and E), nor was survival affected (Fig. 1C and F). Overall these data implicate that oligodendrocytic ablation of Id2 or Notch1 could not improve disease outcome in the SOD1\textsuperscript{G93A} mouse model.

3.2. Oligodendroglial deletion of Id2 or Notch1 does not improve oligodendrocyte dysfunction in ALS mice

MCT-1 and MBP are generally known as markers to assess terminally differentiated and functional oligodendrocytes in ALS (Kang et al., 2013; Philips et al., 2013). We evaluated the expression of both proteins in the lumbar spinal cord of end-stage SOD1\textsuperscript{G93A} mice in which Id2 or Notch1 were selectively removed from the oligodendroglial cells and compared it to control SOD1\textsuperscript{G93A} mice and age-matched SOD1\textsuperscript{WT} mice. We found that deleting Id2 or Notch1 is insufficient to correct MBP and MCT-1 levels (Fig. 2). In contrast to what has been suggested before (Nonneman et al., 2014; Wang et al., 2001; Zhang et al., 2009), our data indicate that deleting the differentiation inhibitors Id2 or Notch1 from OPCs is not sufficient to correct differentiation and functioning of the oligodendrocytes, at least in this ALS mouse model.

![Fig. 1](image-url) Disease outcome is not altered upon oligodendroglial-specific deletion of Id2 or Notch1 in ALS mice. (A, D) Motor performance of SOD1\textsuperscript{G93A} mice assessed by the hanging grid test upon oligodendroglial deletion of (A) Id2 or (D) Notch1 (2-way ANOVA: \(p > 0.05\)). (B, E and C, F) Kaplan Meier curves displaying (B, E) disease onset determined by failure on the hanging grid test and (C, F) survival in SOD1\textsuperscript{G93A} mice with selective oligodendroglial deletion of (B, C) Id2 or (E-F) Notch1 compared to control SOD1\textsuperscript{G93A} mice (Log-rank Mantel-Cox test: \(p > 0.05\)). For Id2 deletion: G93A – SOD1\textsuperscript{G93A} (n = 30–32), Id2\textsuperscript{+/−} – Id2\textsuperscript{lox/lox}; PDGF\(\alpha\)-R-CreER SOD1\textsuperscript{G93A} (n = 15–16), and Id2\textsuperscript{−/−} – Id2\textsuperscript{lox/lox}. For Notch1 deletion: G93A – SOD1\textsuperscript{G93A} (n = 9), Notch1\textsuperscript{+/−} – Notch1\textsuperscript{lox/lox}; PDGF\(\alpha\)-R-CreER SOD1\textsuperscript{G93A} (n = 8–9), and Notch1\textsuperscript{−/−} – Notch1\textsuperscript{lox/lox}. Abbreviation: HW, hanging wire.
4. Discussion

Strong evidence suggests that degeneration of oligodendrocytes and improper replacement of the lost cells by newly formed immature and dysfunctional oligodendrocytes substantially contribute to the degeneration of the motor neurons (Kang et al., 2013; Philips et al., 2013). We evaluated the potential of Id2 and Notch1, 2 master modulators of oligodendrocyte differentiation, maturation, and functioning (Wang et al., 1998; Zhang et al., 2009), to overcome the oligodendrocyte pathology seen in ALS. Neither deleting Id2 nor Notch1 specifically in the OPCs and their progeny improved oligodendrocyte differentiation and functioning and consequently did not result in a beneficial effect on disease onset, motor performance, or survival in this ALS mouse model. It obviously remains possible that modulators of oligodendrocyte differentiation and maturation other than Id2 and Notch1, such as Wnt-signaling, Nogo-A, Id4, and human endogenous retroviruses, can be of particular importance to overcome the oligodendroglial pathology seen in ALS (Nonneman et al., 2014).

Disclosure statement

The authors declare no actual or potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.neurobiolaging.2018.03.026.

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