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Research Report

Hes3 expression in the adult mouse brain is regulated during demyelination and remyelination

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ABSTRACT

Hes3 is a component of the STAT3-Ser/Hes3 Signaling Axis controlling the growth and survival of neural stem cells and other plastic cells. Pharmacological activation of this pathway promotes neuronal rescue and behavioral recovery in models of ischemic stroke and Parkinson's disease. Here we provide initial observations implicating Hes3 in the cuprizone model of demyelination and remyelination. We focus on the subpial motor cortex of mice because we detected high Hes3 expression. This area is of interest as it is impacted both in human demyelinating diseases and in the cuprizone model. We report that Hes3 expression is reduced at peak demyelination and is partially restored within 1 week after cuprizone withdrawal. This raises the possibility of Hes3 involvement in demyelination/remyelination that may warrant additional research. Supporting a possible role of Hes3 in the maintenance of oligodendrocyte markers, a Hes3 null mouse strain shows lower levels of myelin basic protein in undamaged adult mice, compared to wild-type controls. We also present a novel method for culturing the established oligodendrocyte progenitor cell line oli-neu in a manner that maintains Hes3 expression as well as its self-renewal and differentiation potential, offering an experimental tool to study Hes3. Based upon this approach, we identify a Janus kinase inhibitor and dbcAMP as powerful inducers of Hes3 gene expression. We provide a new biomarker and cell culture method that may be of interest in demyelination/remyelination research.

1. Introduction

Hairy and Enhancer of split 3 (Hes3) is a member of the Hes/hey family of basic helix-loop-helix (bHLH) transcriptional repressors (Hatakeyama et al., 2004; Hirata et al., 2000, 2001; Imayoshi and Kageyama, 2014). genetic deletion experiments demonstrate that it opposes precarious differentiation of neural precursors during development, in cooperation with other members of the Hes3/hey family (Hirata et al., 2000; Hirata et al., 2001). in neural stem cells (NSCs)/precursor cells, Hes3 expression is regulated by the STAT3-Ser/Hes3 signaling axis, a signaling pathway that involves a non-canonical branch of the notch signaling pathway and phosphorylation of STAT3 on serine 727.

In vitro, pharmacological activation of this pathway promotes NSC survival and growth (Androutsellis-Theotokis et al., 2006, 2008, 2009; Ohta et al., 2012; Salewski et al., 2012). Hes3 may also be involved in the direct reprogramming of adult cells into induced NSCs as successful reprogramming correlates with Hes3 gene transduction (Cassady et al., 2014). In vivo, pharmacological activation improves behavioral scores in models of ischemic stroke and Parkinson's disease (Androutsellis-Theotokis et al., 2006, 2009).

Abbreviations: Hes3, Hairy and Enhancer of Split 3; MBP, myelin basic protein; NSC, neural stem cell; OPC, oligodendrocyte precursor cell; GFAP, Glial Fibrillary Acidic Protein; JAK, Janus kinase; STAT3, Signal Transducer and Activator of Transcription

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Here we provide early evidence of the involvement of Hes3 in demyelination/remyelination. For in vivo work we utilized the cuprizone model of demyelination/remyelination (Blakemore, 1973; Denic et al., 2011; Kipp et al., 2009; Matsushima and Morell, 2001; Skripuletz et al., 2008; Torkildsen et al., 2008; Zendedel et al., 2013). We focus on the subpial motor cortex (spmCTX) because we observed high expression of Hes3 and because this area is also affected in this model as well as in patients with demyelinating diseases (Bo et al., 2003; Skripuletz et al., 2011; Wegner et al., 2006). We show that Hes3 expression is reduced at peak demyelination and is partially restored within 1 week after cuprizone withdrawal. This raises the possibility of Hes3 involvement in demyelination/remyelination that may warrant additional research. Supporting a possible role of Hes3 in the maintenance of oligodendrocyte markers, a Hes3 null mouse strain (Hirata et al., 2000, 2001) shows lower levels of myelin basic protein (MBP) in undamaged adult mice, compared to

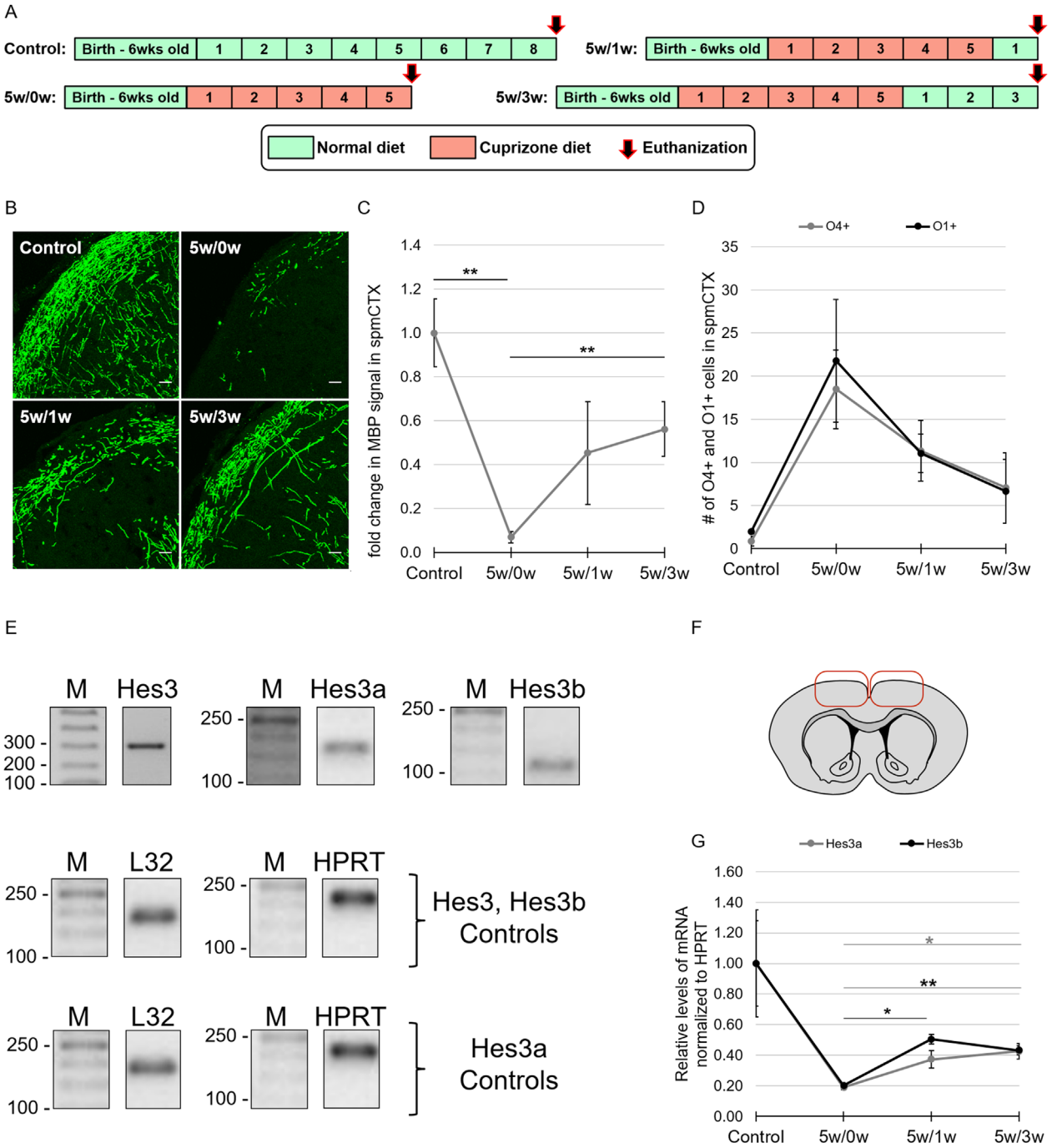

Fig. 1. Hes3 expression in the adult mouse spmCTX is regulated in different stages of the cuprizone model of demyelination and remyelination. (A) Schematic diagram of the experimental animal groups used: control mice (8 weeks of control diet); mice placed on a cuprizone-containing diet for 5 weeks and euthanized immediately after (5w/0w); mice placed on a cuprizone-containing diet for 5 weeks, then placed on a normal diet for 1 or 3 weeks and euthanized immediately after (5w/1w and 5w/3w, respectively). (B) Immunohistochemical analysis of MBP signal in the spmCTX in different animal groups. (C) Quantification of MBP signal (measured as coverage area times intensity) from the images in (B) (Error bars: SEM, $N=4$, $^{**}p<0.00025$, within Bonferroni limits). (D) Number of O4+ and O1+ cells in the spmCTX (data from both hemispheres were pooled together) at the different stages of the cuprizone model. (E) PCR detection of Hes3 mRNA in the spmCTX (both hemispheres pooled together; "M": molecular weight markers; L32 rRNA and hypoxanthine phosphoribosyltransferase/HRPT are used as internal standards). (F) Diagram showing the dissected spmCTX area used for the analyses. (G) Quantitative PCR analysis of Hes3a and Hes3b mRNA in samples from the dissected spmCTX. (Error bars: SEM, $N=4$, $^{*}p<0.0083$, $^{**}p<0.0016$, within Bonferroni limits).