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Research Report

The U-box-type ubiquitin ligase PRP19β regulates astrocyte differentiation via ubiquitination of PTP1B

Takeyuki Yamada[1], *Yumiko Urano-Tashiro*[1], *Yoshimi Hashi*, *Marimu Sakumoto*, *Hirotada Akiyama*, *Fumio Tashiro*[*]

Department of Biological Science and Technology, Faculty of Industrial Science and Technology, Tokyo University of Science, Niijuku 6-3-1, Katsushika-ku, Tokyo 125-8585, Japan

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ABSTRACT

U-box protein PRP19β, a splicing variant of PRP19α, suppresses neuronal differentiation and conversely promotes astrocyte differentiation as a neuron/glia switch molecule. However, the mechanistic basis of PRP19β in astrocyte differentiation is not well understood. Here, we demonstrated that PRP19β regulates the stability of protein tyrosine phosphatase 1B (PTP1B) via ubiquitination during N^6,2′-O-dibutyryl cyclic AMP (cAMP)-induced astrocyte differentiation of C6 cells. Only overexpression of PRP19β conferred astrocyte properties at a certain level, and induced more astrocyte markers, glial fibrillary acidic protein (GFAP) and S100β, in the presence of cAMP, whereas its down-regulation by antisense RNA showed a suppressive effect. In addition, ectopic expression of PRP19β led to robust phosphorylation of signal transducer and activator of transcription 3 (STAT3) accompanying the reduction in PTP1B stability during astrocyte differentiation. Immunological analysis revealed that PRP19β interacted with PTP1B and ubiquitinated PTP1B via its U-box region. Forced expression of the U-box deletion mutant of PRP19β resulted in inhibition of astrocyte differentiation. Moreover, down-regulation of PTP1B by short hairpin (sh)RNA enhanced astrocyte differentiation, while forced expression of PTP1B showed an inhibitory effect. Thus, these results indicate that PRP19β activates the gp130/Janus kinase (JAK)/STAT signaling pathway during astrocyte differentiation of C6 cells via PTP1B ubiquitination.

1. Introduction

PRP19α is involved in a number of diverse pathways, including pre-mRNA splicing (Grillari et al., 2005), DNA repair (Mahajan and Mitchell, 2003), ubiquitination (Hatakeyama et al., 2001), lipid droplet biogenesis (Cho et al., 2007), and early development in mice (Fortschegger et al., 2007). Previously, we reported that PRP19α expression was enhanced

Abbreviations: cAMP, N^6,2-O-dibutyryl cyclic AMP; GAPDH, glyceraldehyde 3′-phosphate dehydrogenase; GFAP, glial fibrillary acidic protein; IL-6, interleukin-6; JAK, Janus kinase; PTP1B, protein tyrosine phosphatase 1B; RA, retinoic acid; SHP2, Src homology domain 2 (SH2)-containing protein phosphatase; STAT3, signal transducer and activator of transcription 3; pSTAT3, phosphorylated STAT3; Ub, ubiquitin; WB, Western blotting; CHX, cycloheximide

[*]*Corresponding author*. Fax: +81 3 5876 1639.
E-mail address: ftashir@rs.noda.tus.ac.jp (F. Tashiro).
[1]These authors contributed equally to the work.

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during retinoic acid (RA)-induced neural differentiation of P19 cells and that PRP19α overexpression led to the conversion of P19 cells into neurons without RA, accompanying the down-regulation of *oct-3/4* and *nanog* mRNA expressions (Urano et al., 2006). In NGF-treated PC12 cells, PRP19α is phosphorylated by Akt kinase and triggers the formation of the PRP19α/14-3-3β/CDC5L core spliceosome complex in nuclei, by which NGF-induced neuronal differentiation of PC12 cells is promoted (Urano-Tashiro et al., 2010). A splicing variant of PRP19α, PRP19β, possesses an additional 19 amino acid residues in the N-terminal region. The constitutive expression of PRP19β suppresses neuronal differentiation through the inhibition of cyclophilin A, which acts as a transcriptional activator, and inversely promotes glial cell differentiation in RA-treated P19 cells as a neuron/glia switch molecule (Urano et al., 2006). However, the functional role of PRP19β in astrocyte differentiation remains unclear.

Extracellular stimuli as inducers of astrocyte differentiation are interleukin 6 (IL-6) family cytokines and bone morphogenetic protein (BMP) family cytokines (Bonni et al., 1997; Gross et al., 1996). The IL-6 family cytokines, IL-6, IL-11, leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), cardiotrophin (CT)-1, and oncostatin M (OSM) use the common receptor subunit gp130 for signal transduction and, as a consequence, elicit similar and overlapping physiological responses (Heinrich et al., 1998). In response to the activation of gp130, Janus kinase (JAK) phosphorylates the Tyr705 residue of signal transducer and activator of transcription 3 (STAT3) transcription factor, which then induces gene expression of the astrocyte markers glial fibrillary acidic protein (GFAP) and S100β by binding to their promoter region (Miller and Gauthier, 2007). The BMP family cytokines, BMP2 and BMP4, induce signals by first binding to their respective heterotrimeric serine/threonine kinase receptors, which then activate the downstream transcription factors, Smad1, 5, and 8 (Chen et al., 2004). LIF signaling induces astrocyte progenitor cells, whereas BMP signaling promotes astrocyte maturation (Bonaguidi et al., 2005). In precursor cells exposed to these two cytokines, Smad forms a transcriptional complex with activated STAT3 and p300/CBP, which then initiates astrocyte-specific gene expression (Nakashima et al., 1999a, 1999b).

Src homology domain 2 (SH2)-containing protein tyrosine phosphatase (SHP2) is a widely expressed growth factor-regulated protein tyrosine phosphatase that represses the gp130/JAK/STAT pathway (Fischer et al., 2004). SHP2 is required for the phospho-Tyr759 of activated gp130 and negatively regulates receptor and JAK activation (Lehmann et al., 2003; Ernst and Jenkins, 2004). SHP2 also enhances activation of the MEK/ERK pathway, which promotes neurogenesis (Miller and Gauthier, 2007). As a constitutively activated mutation of SHP2 causes aberrant neural development in Noonan Syndrome, SHP2 is essential for normal cortical cell-fate decisions (Gauthier et al., 2007). Protein tyrosine phosphatase 1B (PTP1B) serves as a protein inhibitor of activated STAT3 and suppressor of cytokine signal transduction (Valentino and Pierre, 2006). PTP1B inhibits the activation of JAK2 through dephosphorylation of Tyr1007 and reduces phospho-Tyr705 of activated STAT3 (Akasaki et al., 2006; Lund et al., 2005).

It has been reported that cAMP-elevating stimuli including a pituitary adenylate cyclase-activating polypeptide induce astrocytogenesis of rat cortical precursors, indicating that cAMP plays an important role in astrocyte differentiation

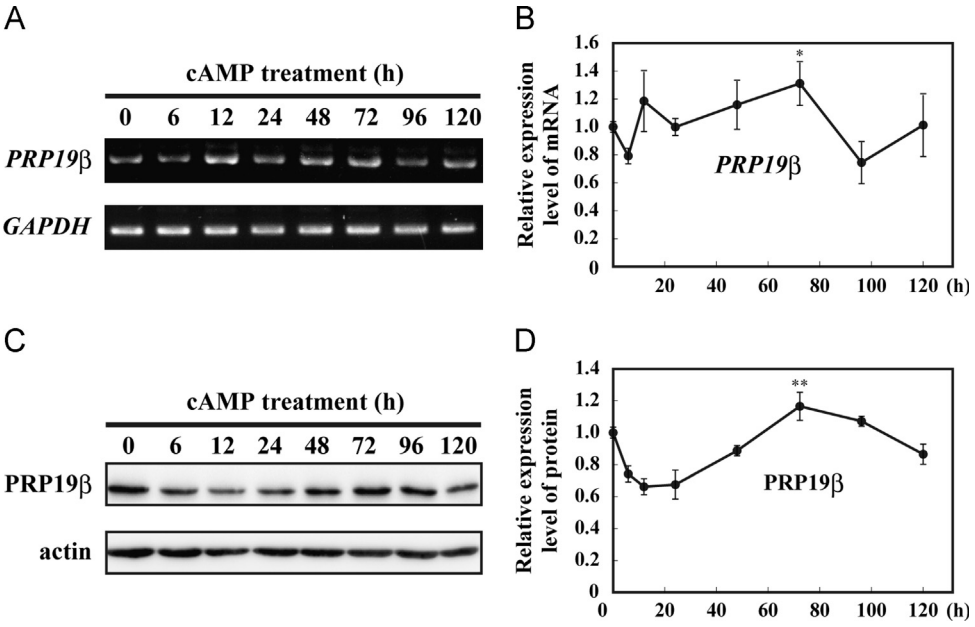

Fig. 1 – Induction of *PRP19β* gene expression during cAMP-triggered astrocyte differentiation of C6 cells. C6 cells were treated with 1 mM cAMP for various times, and total RNAs and cell lysates were prepared and analyzed by RT-PCR and WB with anti-PRP19α/β antibody, respectively. Glyceraldehyde 3′-phosphate dehydrogenase (*GAPDH*) mRNA and actin were used as internal controls. (A) Expression pattern of *PRP19β* mRNA after cAMP addition. (B) Quantification of the expression level of *PRP19β* mRNA indicated in (A). *$P<0.05$ compared with 0 h. $n=3$. (C) Expression pattern of PRP19β protein after cAMP addition. (D) Quantification of the expression level of PRP19β protein indicated in (C). **$P<0.03$ compared with 0 h. $n=3$.