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Protein phosphatase 2A (PP2A) activation promotes axonal growth and recovery in the CNS

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ABSTRACT

Current treatments to restore neurological deficits caused by axonal disconnection following central nervous system (CNS) injury are extremely limited. Protein phosphatase 2A (PP2A), one of the main serine-threonine phosphatases in mammalian cells, dephosphorylates collapsin response mediator protein-2 (CRMP2) in the developing CNS. In our study, we found that the major CNS inhibiting substrates, including chondroitin sulfate proteoglycans (CSPGs) and myelin associated glycoproteins (MAG), activated epidermal growth factor receptor (EGFR), but inactivated PP2A and downstream CRMP2. Both EGFR inactivation and PP2A activation promoted axon elongation in vitro in the presence of inhibitory substrates. EGFR blockage by AG1478 selectively attenuated the inactive form of PP2A in pY307 phosphorylation, thus increasing PP2A activity. EGFR activation by EGF attenuated PP2A activity, whereas mutation of Y307 to phenylalanine abolished the effect. Furthermore, PP2A activity was down-regulated immediately after spinal cord injury (SCI) in rats. Chronic application of D-erythro-sphingosine (DES), the PP2A agonist, to spinal cord-lesioned rats enhanced the activity of this phosphatase and dephosphorylated CRMP2 around the lesion. PP2A activation induced significant axon sprouting in the lesioned spinal cord and promoted function recovery after SCI. These findings suggest that PP2A works downstream of EGFR and dephosphorylates CRMP2 after CNS injury. Therefore, therapies targeting PP2A may be effective following SCI.

1. Introduction

Diffuse axonal injury is one of the key pathological features of human traumatic brain injury, neurodegenerative diseases, and locomotor neuronal dysfunction [1,2]. In addition to direct axonal damage, secondary damage results in cumulative neuronal injury. However, the molecular mechanisms underlying axonal stunting following secondary damage are not well understood. Glycogen synthase kinase-3 (GSK-3) inactivation promotes neurite and axon growth in vitro and in vivo [3]. Inactivation of the Rho-ROCK signaling pathway promotes CNS axonal regeneration and neurologic recovery after spinal cord injuries (SCI) in rats [4].

Protein phosphatase 2A (PP2A) is a principal Ser/Thr phosphatase that regulates cell cycle and differentiation [5]. The predominant form of PP2A is a heterotrimer, consisting of catalytic (PP2Ac), scaffolding (A), and variable regulatory subunits derived from three gene families (B, B′, B″) [6]. PP2Ac carboxymethylation at the L309 site hampers the formation of the heterotrimer [7,8]. Otherwise, phosphorylation of the PP2Ac at the Y307 site by receptor and non-receptor protein tyrosine kinases inactivates PP2A [9–11]. In neurodegenerative diseases, a key function of PP2A is to dephosphorylate the hyperphosphorylated tau protein [12,13]. In the developing CNS, PP2A initially occurs in all neurites of the unpolarized neurons. It is preferentially enriched in the distal segments of the axons and facilitates axon genesis by targeting collapsin response mediator protein-2 (CRMP2) [14]. CRMP2 is a chemorepellent in axon guidance and induces neuronal growth cone collapse. It binds tubulin heterodimers and modulates microtubule assembly [15,16]. Studies also revealed that treatments of increased CRMP2 activity positively promoted functional recovery after SCI [17].

Epidermal growth factor receptor (EGFR), a type of protein tyrosine kinase, was reported to be up-regulated after SCI. Its attenuation promotes axon growth and functional recovery after CNS injury [18,19]. The mechanisms underlying the role of EGFR in promoting axon elongation have not been well studied. However, as a tyrosine kinase, EGFR potentially phosphorylates PP2A at the tyrosine site Y307. Therefore, we hypothesized that PP2A signaling may promote axon outgrowth after CNS injury.

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In the present study, we focused on the role of PP2A activation on neurite and axonal growth in neurons in vitro and in vivo. We demonstrated that CNS inhibiting substrates, including chondroitin sulfate proteoglycans (CSPGs) and myelin inhibitors, significantly increased EGFR but attenuated PP2A activity. Treatment enhanced PP2A activity remarkably increased axon outgrowth and facilitated functional recovery after SCI by dephosphorylating CRMP2. Given the critical function of PP2A in regulating axon genesis and elongation, modulation of PP2A activity may be an important and effective therapeutic strategy targeting CNS axonal injuries.

2. Materials and methods

2.1. Materials and reagents

Neurobasal medium and B27 supplement were obtained from Invitrogen (San Diego, CA, USA). D-erythro-S (DES, 10 nM for cell culture and 1 μM in vivo) was purchased from Calbiochem (San Diego, CA, USA). CSPGs including neurocan, versican, phosphacan, and aggrecan were provided by Millipore (Billerica, MA, USA). Myelin associated glycoprotein and Epidermal growth factor (EGF 50 ng/ml) were purchased from Sigma (St. Louis, MO, USA). EGFR inhibitor AG-1478 (10 μM) was purchased from Merck (KGaA, Darmstadt, Germany). Biotinylated dextran amine (BDA) was ordered from Life Technologies.

Antibody against βIII-tubulin (1:200) was purchased from Santa Cruz (California, CA, USA), GFAP (1:200) for immunofluorescence; EGFR (1:500), pT1173-EGFR(1:500), Y307-PP2Ac (1:1000 for WB and 1:200 for immunofluorescence) and total PP2Ac (1:1000) were obtained from Abcam (Cambridge, UK); CRMP2(1:1000) PY514- CRMP2 (1:1000 for Western blot and 1:200 for immunofluorescence) were provided by Cell Signaling Technology (Camarillo, CA, USA); Actin (1:1000) was purchased from Sigma(St. Louis, MO, USA); and demethyl-PP2Ac 309 (1:1000) was from Millipore (Billerica, MA, USA).

2.2. Spinal cord injury model

Briefly, adult Sprague–Dawley (SD) male rats weighing approximately 240 to 260 g were supplied by the Experimental Animal Center

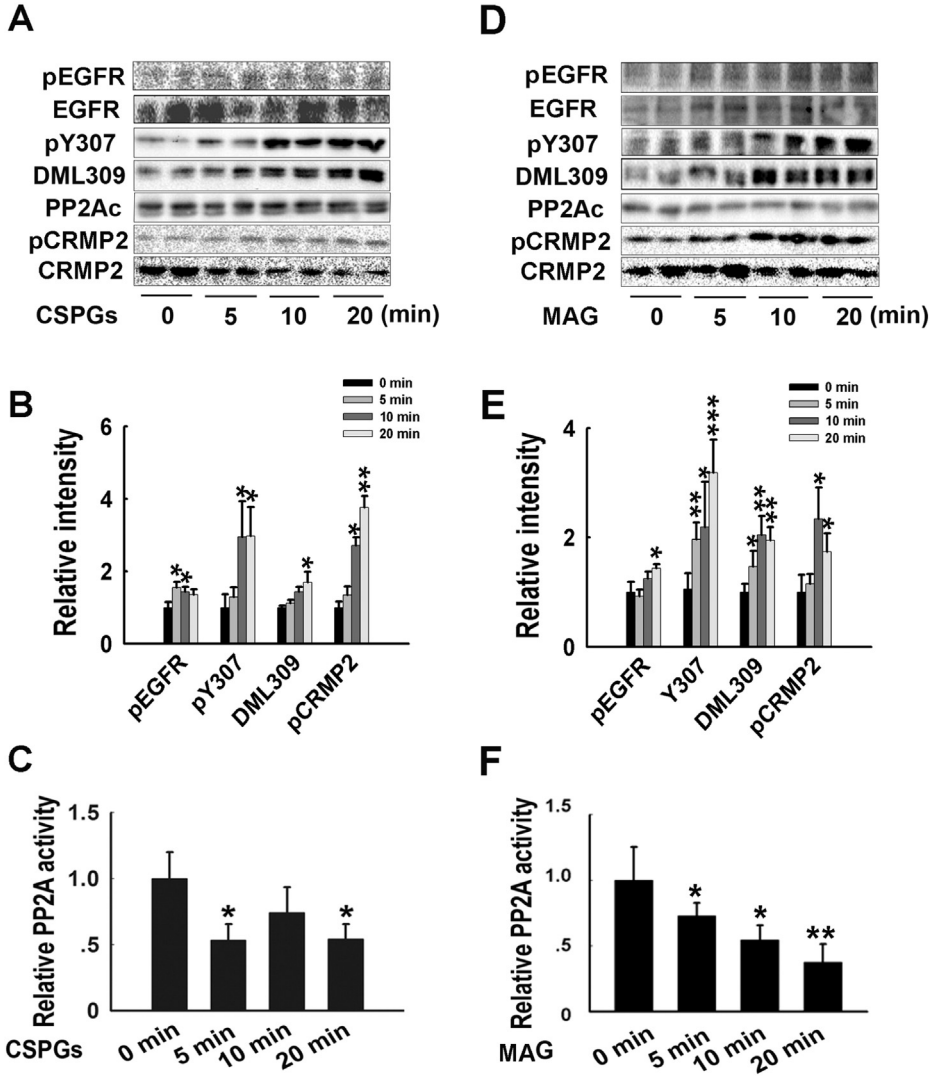

Fig. 1. Axonal growth inhibitors activate EGFR but inactivate PP2A and pCRMP2 in neuronal cultures. (A)Western blots indicated 24 h after CGN plating; the application of CSPGs (1.5 μg/ml) significantly affected the levels of pEGFR, pY307, DML 309 (inactive form), and pCRMP2, and total level of each was also measured in the supernatants of CGNs 24 h after cultures 5–20 min after exposure. (B) Quantitative analysis of the results in A. *, $p < 0.05$. (C) PP2A activity was examined in cell supernatants of cultured CGNs after CSPGs (1.5 μg/ml) application 5–20 min after exposures. *, $p < 0.05$ versus 0 min control neurons. (D)Western blots indicated 24 h after CGN plating; the application of myelin inhibitor MAG(570 ng/ml) significantly affected the levels of pEGFR, pY307, DML309 and pCRMP2, and the total level of each was also measured in the supernatants of CGNs 24 h after cultures 5–20 min after exposure. (E) Quantitative analysis of the results in C, *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ versus 0 min control neurons. (F) PP2A activity was examined in cell supernatants of cultured CGNs after MAG (570 ng/ml) application 5–20 min after exposure. *, $p < 0.05$, **, $p < 0.01$ versus 0 min control neurons.