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Spinal SHP2 Contributes to Exaggerated Incisional Pain in Adult Rats Subjected to Neonatal and Adult Incisions via PI3K

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Abstract—Neonatal injury-induced exaggeration of pain hypersensitivity after adult trauma is a significant clinical challenge. However, the underlying mechanisms remain poorly understood. Growing evidence shows that spinal Src homology-2 domain-containing protein tyrosine phosphatase-2 (SHP2) contributes to chronic pain in adult rodents. Here we demonstrated that the phosphorylation and expression of SHP2 in synaptosomal fraction of the spinal dorsal horn are elevated in adult rats subjected to neonatal and adult incisions (nIN-IN), and the upregulation of SHP2 is highly correlated with pain hypersensitivity. Intrathecal blockade of SHP2 phosphorylation using a SHP2 protein tyrosine phosphatase inhibitor NSC-87877, or knockdown of SHP2 by intrathecal delivery of small interfering RNA (siRNA), ameliorates mechanical allodynia and heat hyperalgesia in nIN-IN rats. Moreover, the expression of phosphatidylinositol 3-kinase (PI3K) in the spinal dorsal horn is significantly increased in nIN-IN rats. Intrathecal application of PI3K inhibitor, LY294002 or wortmannin, alleviates pain hypersensitivity in nIN-IN rats. Additionally, intrathecal administration of NSC-87877 or SHP2 siRNA attenuates the upregulation of PI3K. Finally, no alternation of SHP2 phosphorylation in the dorsal root ganglion and dorsal root of nIN-IN rats as well as PI3K expression in the dorsal root of nIN-IN rats intrathecally treated with NSC-87877 or SHP2 siRNA is observed. These results suggest that the phosphorylation and expression of SHP2 in the spinal dorsal horn play vital roles in neonatal incision-induced exaggeration of adult incisional pain via PI3K. Thus, SHP2 and PI3K may serve as potential therapeutic targets for exaggerated incisional pain induced by neonatal and adult injuries.

Key words: SHP2, PI3K, incisional pain, spinal dorsal horn, neonate.

INTRODUCTION

Children experience numerous invasive procedures and/or surgery during hospitalization (Stevens et al., 2003; Carbajal et al., 2008), which cause exacerbated pain after adult injury or nociceptive stimulus (Taddio et al., 1997; Peters et al., 2005; Hohmeister et al., 2010; Valeri et al., 2016). Despite of peripheral injury, persistent alterations in adulthood contain centrally mediated changes in the spinal dorsal horn (Walker et al., 2015, 2016; Li and Baccei, 2016). Several signalings in the spinal cord, such as p38 mitogen-activated protein kinase (MAPK), interleukin-1β and brain-derived neurotrophic factor (BDNF) (Schwaller et al., 2015; Soens et al., 2015; Gong et al., 2016; Ding et al., 2018), are found to be involved in the exaggeration of adult incisional pain induced by neonatal incision, however, the mechanisms are still largely unknown.

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Abbreviations: AMPA receptor, α-amino-3-hydroxy-5-methy-4-isoxazole propionate receptor; ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; DRG, dorsal root ganglion; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; ELISA, enzyme-linked immunosorbent assay; ERK, extracellular-regulated kinases; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; nIN rats, rats with only neonatal incision; nIN-IN rats, rats with neonatal incision and adult incision; NMDA receptor, N-methyl-D-aspartate receptor; nSham rats, rats with only neonatal sham operation; nSham-IN rats, rats with neonatal sham operation and adult incision; P3, postnatal day 3; PI3K, phosphatidylinositol 3-kinase; pSHP2, phosphorylated SHP2; PWL, paw withdrawal latency; PWT, paw withdrawal threshold; SDS, sodium dodecyl sulfate; SFK, Src-family kinase; SHP2, Src homology-2 domain-containing protein tyrosine phosphatase-2; siRNA, small interfering RNA.

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Molecular, cellular and behavioral studies have demonstrated that Src homology-2 domain-containing protein tyrosine phosphatase-2 (SHP2) contributes to chronic pain in adults. Spinal SHP2 regulates several pain-related signalings (Hayano et al., 2016; Lai et al., 2016). Consistent with the results of the brain (Pagani et al., 2009; Isosaka and Yuasa, 2010; Zhang et al., 2016), SHP2 participates in synaptic plasticity (Ding et al., 2015), a well-accepted mechanism of pathological pain (Sandkuhler, 2009; Liu and Zhou, 2015). Additionally, it has been documented that SHP2 modulates pain behaviors in adult rats with peripheral nerve injury (Peng et al., 2012; Ding et al., 2015), rats with complete Freund adjuvant injection (Lai et al., 2016) and patients with LEOPARD syndrome (Spatola et al., 2015). These findings lead to the possibility that spinal SHP2 may take part in neonatal incision-induced exaggeration of adult incisional pain.

Numerous growth factors and hormones can activate the phosphatidylinositol 3-kinase (PI3K)-dependent pathway via SHP2 (Tajan et al., 2015). The activation of PI3K is positively or negatively controlled by SHP2 in a receptor and cell context-dependent manner to keep the signal specificity of upstream receptors (Wu et al., 2001; Zhang et al., 2002; Zhou and Agazie, 2009). Furthermore, the PI3K pathway in response to SHP2 is associated with a variety of cellular functions, such as cell survival (Wheadon et al., 2003) and proliferation (Lahlou et al., 2003). Consistently, activated PI3K downstream of SHP2 plays a vital role in cancers (Zhou and Agazie, 2009; Liu et al., 2017; Tang et al., 2018) and developmental diseases (Edouard et al., 2010; Tajan et al., 2015). Additionally, PI3K in the mature spinal cord takes part in several types of pain (Pezet et al., 2008; Pritchard et al., 2016; Yang et al., 2017), notably incisional pain (Xu et al., 2014). However, whether or not spinal SHP2 takes part in neonatal incision-induced exaggeration of incisional pain via PI3K is still unknown.

In this study, we investigated the role and the underlying mechanism of SHP2 in neonatal incision-induced exaggeration of incisional pain after adult incision. Our results showed that the phosphorylation and expression of SHP2 in the spinal dorsal horn are both elevated, which are necessary for subsequent PI3K upregulation in adult rats subjected to neonatal and adult incisions.

EXPERIMENTAL PROCEDURES

Animals

All animal experimental procedures were performed in accordance with the guidelines of the International Association for the Study of Pain (Zimmermann, 1983) and were approved by the Animal Ethics Committees of Capital Medical University. Female Sprague–Dawley rat pups on postnatal day 3 (P3) or adults were obtained from the Department of Experimental Animal Sciences, Capital Medical University. Maternal separation and handling to rat pups were kept to a minimum, and litters aged postnatal day 21 were weaned. All the rats were raised in a temperature- and humidity-controlled environment with a 12-h light–dark cycle and had free access to food and water.

In this study, all behavioral and enzyme-linked immunosorbent assay (ELISA) tests were carried out in adult rats. Rats were assigned to seven groups: (i) Naive: non-operated adult controls; (ii) nSham: rats with sham operation on P3 and follow-up for 10 weeks, sham operation includes equivalent anesthesia, maternal separation and handling to the nIN group; (iii) nIN: rats having only neonatal incision and follow-up in adulthood; (iv) Sham: control animals with equivalent anesthesia and handling to the IN group in adulthood; (v) IN: age-matched animals have only hindpaw incision in adulthood; (vi) nSham-IN: rats with sham operation on P3 and incision in adulthood; (vii) nIN-IN: neonatal incision on P3 and adult incision at week 10.

Chemicals and recombinant lentivirus

The SHP2 protein tyrosine phosphatase inhibitor NSC-87877 (Tocris Bioscience, Bristol, UK), the reversible competitive PI3K inhibitor LY294002 (Sigma–Aldrich, St. Louis, MO, USA) and the irreversible PI3K inhibitor wortmannin (Sigma–Aldrich) were dissolved in dimethyl sulphoxide (DMSO, Sigma–Aldrich) as stock concentrations, aliquoted and stored at −20 °C. The stock solutions were diluted with sterile normal saline (NS) to final concentrations freshly before application. The final concentration of DMSO was ≤10%.

Recombinant lentivirus-short hairpin SHP2 (LV-shSHP2) was produced by Genechem (Shanghai, China) used in previous study (Ding et al., 2015). Plasmids expressed short hairpin SHP2 were used to construct lentivirus. Sense small interfering RNA (siRNA) sequence targeting SHP2 (GeneBank number NM-001177593.1): small interfering SHP2 TTAGGAACGTC-GATGTCAC was used to generate lentivirus short hairpin RNA. The negative control LV-Control had a scrambled sequence. BLAST homology search based on sense and antisense sequences was systematically carried out and confirmed that only a single mRNA sequence was targeted. The short hairpin RNAs were cloned into lentivirus vectors.

Hind paw incision

Rat pups were anesthetized with 2–3% isoflurane (Sigma–Aldrich). A small incision through the skin and fascia was made and the underlying plantaris muscle was elevated and incised longitudinally (Brennan et al., 1996). The same relative length of incision was carried out in rat pups and adult rats, extending from the midpoint of the heel to the first footpad (Schwaller et al., 2015), which can result in a longer incision in adults compared with neonatal rats. The skin was immediately closed with 5-0 silk in pups and 4-0 silk in adults.

Implantation of intrathecal catheter

Under anesthesia, intrathecal cannula implantation was carried out (Ding et al., 2015; Fang et al., 2015). A PE-10 polyethylene catheter was implanted between lumbar

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