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Research Report

Spinal expression of Hippo signaling components YAP and TAZ following peripheral nerve injury in rats

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ABSTRACT

Previous studies have shown that the morphology and number of cells in the spinal cord dorsal horn could change following peripheral nerve injury and that the Hippo signaling pathway plays an important role in cell growth, proliferation, apoptosis, and dendritic remolding. In the present study, we examined whether the expression of YAP and TAZ, two critical components regulated by Hippo signaling, in the spinal cord dorsal horn would be altered by chronic constriction sciatic nerve injury (CCI). We found that (1) YAP was mainly expressed on CGRP- and IB4-immunoreactive primary afferent nerve terminals without noticeable expression on glial cells, whereas TAZ was mainly expressed on spinal cord second order neurons as well as microglia; (2) upregulation of YAP and TAZ expression followed two distinct temporal patterns after CCI, such that the highest expression of YAP and TAZ was on day 14 and day 1 after CCI, respectively; (3) there were also unique topographic patterns of YAP and TAZ distribution in the spinal cord dorsal horn consistent with their distinctive association with primary afferents and second order neurons; (4) changes in the YAP expression were selectively induced by CCI but not CFA-induced hindpaw inflammation; and (5) the number of nuclear profiles of TAZ expression was significantly increased after CCI, indicating translocation of TAZ from the cytoplasma to nucleus. These findings indicate that peripheral nerve injury induced time-dependent and region-specific changes in the spinal YAP and TAZ expression. A role for Hippo signaling in synaptic and structural plasticity is discussed in relation to the cellular mechanism of neuropathic pain.

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1. Introduction

Neuropathic pain often results from complicated neurological disorders such as neuropathy, spinal cord injury, multiple sclerosis and stroke (Cruccu et al., 2010). The current mainstay of neuropathic pain treatment relies heavily on medications that provide only symptomatic management. An alternative approach to improving neuropathic pain treatment is to better understand disease processes causing neuropathic pain in order to shift the strategy from symptomatic management to targeting the underlying mechanism of neuropathic pain (Finnerup et al., 2010).

To date, a number of mechanisms of neuropathic pain have been proposed, including ectopic discharges, sensitization of nociceptors, phenotypic switching, disinhibition, and neuroinflammation (Berger et al., 2011; Caterina et al., 2000; Devor, 2009; Eijkelkamp et al., 2010; Hughes et al., 2007; Wei et al., 2010). However, it is well known that peripheral nerve injury can change the shape, size and number of dendritic spines. Cell apoptosis has also been associated with neuropathic pain following nerve injury. For instance, although sciatic nerve lesion along failed to produce neuronal cell death in laminae I–III of the rat's spinal dorsal horn, a combination of sciatic nerve lesion and stimulation of myelinated fibers resulted in neuronal cell death in superficial layers of the spinal cord dorsal horn (Coggeshall et al., 2001). At least one of the consequences of neuronal cell death is the loss of GABAergic inhibitory interneurons following peripheral tissue injury (Mao et al., 2002; Scholz et al., 2005).

Recently, the Hippo signaling pathway has been shown to play an important role in neuronal development and diseases (Emoto, 2011). Activation of mammalian sterile 20-like 2 (MST2), a core component of Hippo signaling, induced neuronal cell death (Liu et al., 2012). On the other hand, Yes kinase-associated protein (YAP) is a downstream of Hippo signaling, which serves as a positive regulator of cell proliferation but a negative regulator of cell differentiation during mammalian neurogenesis (Zhang et al., 2012a), possibly by regulating sonic hedgehog homolog (shh) signaling (Fernandez et al., 2009; Lin et al., 2012). Loss of function of key Hippo signaling components leads to changes in cell proliferation, cell survival, tissue overgrowth, as well as cell shape and organ size (Boggiano and Fehon, 2012; Hipfner and Cohen, 2004; Justice et al., 1995; Udan et al., 2003; Zhang et al., 2012b). Furthermore, transcriptional coactivator with PDZ-binding motif (TAZ) is a transcriptional coactivator of YAP. The sequence of TAZ is similar to that of YAP despite their differences in the N-terminal proline-rich domain, second WW domain, and SH3 binding motif (Zhao et al., 2008). These findings suggest a similar functional role for YAP and TAZ because both are negatively regulated by the Hippo signaling pathway in mammals (Hao et al., 2008; Oka et al., 2008; Zhang et al., 2008; Zhao et al., 2007).

Therefore, it is possible that both YAP and TAZ as transcriptional coactivators play a role in cell growth and proliferation following peripheral nerve injury. In this study, we examined whether the expression of YAP and TAZ in the spinal cord dorsal horn would be altered following chronic constriction sciatic nerve injury (CCI) (Bennett and Xie). We found, for the first time, that (1) YAP and TAZ are differentially expressed in neuronal and glial cells with distinct topographic distribution patterns, (2) their expression was upregulated after CCI but at different time points, and (3) the YAP expression appeared to be selectively induced by nerve injury and was unchanged after CFA-induced inflammation.

2. Results

2.1. *Expression of YAP and TAZ in the spinal cord dorsal horn*

To identify the pattern of YAP and TAZ expression in the spinal dorsal horn in the absence of peripheral nerve injury, we first examined whether YAP and TAZ would be expressed on CGRP-immunoreactive and IB4-immunoreactive primary afferent nerve terminals as they represent peptide and non-peptide sensory neurons, respectively. We found that YAP (Fig. 1A and B) was extensively expressed on CGRP- as well as IB4-immunoreactive primary afferent nerve terminals. Specifically, YAP was co-expressed with CGRP-positive primary afferents in both lamina I and the outer layer of lamina II, as well as IB4-positive primary afferents in the inner layer of lamina III. In contrast, TAZ expression (Fig. 2A and B) was primarily associated with spinal cord second order neurons with little or no expression on primary afferent nerve terminals. The results indicate that YAP is expressed in both large and small primary sensory neurons, whereas TAZ is present primarily in spinal second order neurons.

2.2. *Expression of YAP and TAZ on glial cells*

We then examined whether YAP and TAZ would be expressed on glial cells in the spinal cord dorsal horn. At the L4–L5 level, both YAP and TAZ showed little co-expression with the astrocyte marker GFAP (Figs. 1 and 2C). However, TAZ was double-labeled with the microglial marker Iba1 (Figs. 1 and 2D), whereas no co-expression was detected between YAP and Iba1 (Fig. 2). Thus, only TAZ was expressed in microglial cells, and neither YAP nor TAZ in astrocytes, within the spinal cord dorsal horn.

2.3. *Topographic distribution patterns of YAP and TAZ expression after CCI*

Nociceptive threshold for both mechanical and thermal withdrawal on ipsilateral hindpaw was significantly decreased on post-CCI day1 and remained low up to at least post-CCI day14, as compared with sham-operated rats (Fig. 3A and B, $n=8$; $^{*}P<0.05$). There were no differences in withdrawal threshold on the contralateral hindpaw (Fig. 3A and B, $n=8$; $P>0.05$). After CCI, topographic distribution patterns of YAP and TAZ expression were noticeably different in the ipsilateral spinal cord dorsal horn (Fig. 4). Specifically, YAP was expressed only in lamina I–II, whereas TAZ expression was diffusely distributed across the spinal cord dorsal horn (Fig. 4). These distribution patterns are consistent with the basal expression of YAP (primary afferents) and TAZ (second order neurons) in the spinal cord dorsal horn, indicating that