Targeting Astrocyte Signaling for Chronic Pain

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Summary: Clinical management of chronic pain after nerve injury (neuropathic pain) and tumor invasion (cancer pain) is a real challenge due to our limited understanding of the cellular mechanisms that initiate and maintain chronic pain. It has been increasingly recognized that glial cells, such as microglia and astrocytes in the CNS play an important role in the development and maintenance of chronic pain. Notably, astrocytes make very close contacts with synapses and astrocyte reaction after nerve injury, arthritis, and tumor growth is more persistent than microglial reaction, and displays a better correlation with chronic pain behaviors. Accumulating evidence indicates that activated astrocytes can release proinflammatory cytokines (e.g., interleukin [IL]-1β) and chemokines (e.g., monocyte chemoattractant protein-1 [MCP-1]/also called CCL2) in the spinal cord to enhance and prolong persistent pain states. IL-1β can powerfully modulate synaptic transmission in the spinal cord by enhancing excitatory synaptic transmission and suppressing inhibitory synaptic transmission. IL-1β activation (cleavage) in the spinal cord after nerve injury requires the matrix metalloprotease-2. In particular, nerve injury and inflammation activate the c-Jun N-terminal kinase in spinal astrocytes, leading to a substantial increase in the expression and release of MCP-1. The MCP-1 increases pain sensitivity via direct activation of NMDA receptors in dorsal horn neurons. Pharmacological inhibition of the IL-1β, c-Jun N-terminal kinase, MCP-1, or matrix metalloprotease-2 signaling via spinal administration has been shown to attenuate inflammatory, neuropathic, or cancer pain. Therefore, interventions in specific signaling pathways in astrocytes may offer new approaches for the management of chronic pain. **Key Words:** Neuropathic pain, nerve injury, spinal cord, cytokine, chemokine, MAP kinase, glia.

INTRODUCTION

Pain is an unpleasant sensory experience and normally plays a protective role by warning us of potential harm to our body and enabling us to quickly remove the body part from noxious stimuli, and further learn to avoid them in the long run. When noxious peripheral stimulation is sensed, pain information is mainly transmitted by thin myelinated Aδ fibers and unmyelinated C fibers to the dorsal horn in the spinal cord, where second order nociceptive neurons are activated by neurotransmitters, such as glutamate and neuropeptides (e.g., substance P and calcitonin gene-related peptide), which are released from the primary afferents.[1] The information is further relayed to the thalamus, and it finally reaches the parietal lobe of the cerebral cortex for pain perception.[2,3] This type of pain is transient and referred to as acute or physiological pain.

However, under injury conditions, pain can be dissociated from its normal physiological role. It can persist for months to years, even after the original injury or inflammation has largely been healed. This type of pain is called chronic or pathological pain, as the consequence of damage or dysfunction of the peripheral nervous system and CNS (neuropathic pain), peripheral tissue damage or inflammation (inflammatory pain), and tumor invasion (cancer pain).[4–6] Chronic pain does not convey any useful information. Under injury conditions, painful pressure and thermal stimuli are grossly amplified (hyperalgesia). Even light touch is perceived painful (allodynia). Chronic pain creates considerable suffering for people affected, and it is extremely costly for the individual and for the community, with the estimated cost in the United States alone as more than $100 billion every year.[7]

Chronic pain is a maladaptive pain, resulting from the development of neural plasticity in the peripheral sensitization and CNS (central sensitization).[8–10] It was gen-

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erally believed for a long time that only neurons and their neural circuits were responsible for the development and maintenance of chronic pain, which led to the development of current therapeutics that have been focusing on neuronal targets, including drugs such as NMDA receptor antagonists, selective serotonin/norepinephrine reuptake inhibitors, opioid analgesics, and sodium channel blockers. Although these drugs have shown some effects in some patients,[11] they often produce a brief pain relief via transient blockade of neurotransmission. Notably, the side effects of these drugs, often CNS-related, such as nausea, sedation, drowsiness, and dizziness, as well as development of analgesic tolerance and addiction after opioid treatment, have greatly limited their universal use.[11,12] Therefore, research on other means of chronic pain treatment is in urgent demand. As a consequence, studies on non-neuronal cells, especially glial cells in chronic pain conditions, have increased exponentially in the last decade.

Glial cells are 10 to 50 times as numerous as neurons and consist of three major groups: 1) astrocytes, 2) microglia, and 3) oligodendrocytes.[13] Microglia are the resident macrophage-like cells of the CNS. Oligodendrocytes, which are derived from neuroectoderm, produce myelin to ensheath neuronal axons. Astrocytes are the most abundant cells in term of their number and volume, and they constitute 40% to 50% of all glial cells.[14] In normal conditions, microglia and astrocytes are relatively resting or quiescent; however, see reference.[15] After injury or under disease conditions, they can be converted to reactive states and participate in the pathogenesis of neurological disorders.[16–18] Increasing evidence has shown that microglia and astrocytes play important roles in the development of chronic pain.[18–27] Unlike microglia and oligodendrocytes, astrocytes form networks with themselves and are closely associated with neurons and blood vessels. It is estimated that a single astrocyte enwraps 4 to 6 neuronal somata and contacts 300 to 600 neuronal dendrites.[28] A close contact with neurons and synapses makes it possible for astrocytes to support and nourish neurons, and regulate the external chemical environment of neurons during synaptic transmission. In this review, we will discuss recent progress on astrocyte control of pain.

ASTROCYTE ACTIVATION IN PERSISTENT PAIN CONDITIONS

Glia activation is emerging as a powerful concept for understanding cellular mechanisms underlying chronic pain. Unfortunately, the term "glia activation" is poorly defined. In the pain research field, astrocyte activation is often referred to glial fibrillary acidic protein (GFAP) upregulation and astrogliosis (hypertrophy of astrocytes, as manifested by enlarged cell bodies and thick processes). The active astrocytes with gliosis are also called reactive astrocytes. Thus, in the following discussion we refer to this activation state as the reactive state, so as to separate from other activation states. It is well known that after peripheral nerve injury or inflammation or tumor invasion, astrocytes in the CNS (especially the spinal cord) undergo various biochemical, translational, transcriptional, and morphological changes. Therefore, astrocytes could display various activation states after peripheral sensory stimuli and injury. Some activation states occur within minutes, such as increases in intracellular Ca^{2+} and phosphorylation of signaling molecules. Some activation states occur after tens of minutes (e.g., translational regulation) and hours (e.g., transcriptional regulation). Other activation states may occur after hours or even days, such as astrocyte hypertrophy or astrogliosis.

Astrocyte reaction (GFAP upregulation and hypertrophy) has been found in various injury conditions that are associated with enhanced pain states. These conditions include 1) peripheral nerve injury, such as chronic constriction injury,[29] spinal nerve ligation (SNL) (FIG. 1),[30,31] and infraorbital nerve ligation[32,33]; 2) tissue injury/inflammation produced by intraplantar injection of complete Freund's adjuvant,[34] formalin,[35] zymosan[35]; and 3) tumor growth in the skin[36–38] and bone marrow.[39–41] Although astrocyte reaction can occur at supraspinal areas, such as the rostral ventramedial medulla after chronic constriction injury of the rat infra-orbital nerve,[32,33] the forebrain after complete Freund's adjuvant (CFA) injection,[34] and the gracile nucleus after partial sciatic nerve ligation,[42] most studies focus on the spinal cord dorsal horn.[19]

Notably, astroglial reaction after nerve injury is more persistent than microglial reaction (e.g., upregulation of the microglial markers CD11b/OX-42 and Iba-1, and hypertrophy of mciroglia). Astroglial reaction can last more than 150 days after nerve injury.[43] In most cases, microglial reaction precedes astrocytic reaction[34,44,45] and likely leads to astrocyte reaction.[46] Interestingly, nerve injury induces an increase in interleukin (IL)-18 and IL-18 receptor in reactive microglia and astrocytes, respectively, in the dorsal horn, suggesting an interaction between microglia and astrocytes in neuropathic pain.[47] However, astrocyte reaction is not always preceded by microglial reaction. Hald et al [40] showed that bone cancer resulted in marked spinal astroglial reaction without microglial reaction.

It has been shown that GFAP expression after inflammation or nerve injury requires NMDA receptor [48,49] and neuronal activity.[32,49] GFAP expression is also critical for morphological changes of astrocytes (astrogliosis)[34,35] and is often correlated with enhanced pain states[29,30,50]; however, see Reference.[31] Although intrathecal GFAP anti-sense oligonucleotide treatment in

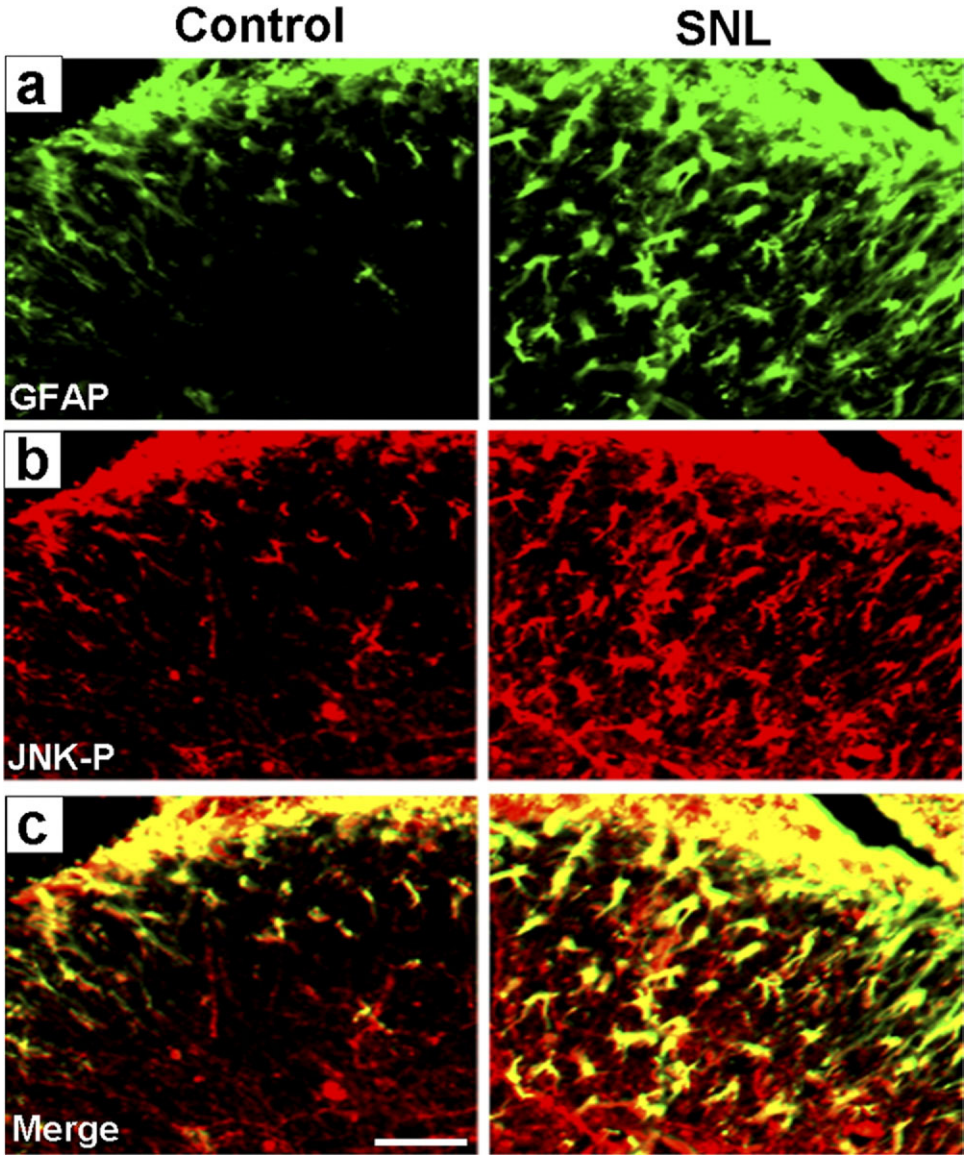

FIG. 1. Spinal nerve ligation induces a substantial increase in c-Jun N-terminal kinase (JNK) phosphorylation and GFAP expression in astrocytes in the spinal cord dorsal horn. Double staining reveals a co-localization of phosphorylated JNK with the astrocyte marker GFAP. Mice were sacrificed 10 days after nerve injury. Contralateral side of the spinal cord is used as the control. (Scale, 50 μm.)

nerve-injured animals was shown to reduce neuropathic pain behaviors,[51] this contribution of GFAP to chronic pain could be indirect via unknown mechanisms. It is generally believed that astrocytes control pain states by producing neuromodulators/pain mediators, such as cytokines, chemokines, and growth factors[22,32,33,52,53] (also see discussion as follows). The production and release of these mediators are not directly controlled by GFAP, rather by some key intracellular signaling pathways, such as the MAP kinase pathway. Remarkably, nerve injury and inflammation induce a persistent phosphorylation of c-Jun N-terminal kinase (JNK) in astrocytes, which may represent a different activation state of astrocytes that is not only correlated with pain hypersensitivity, but also with an underlying cause of this hypersensitivity (FIG. 1).[31,54–56]

ASTROCYTES CONTRIBUTE TO ENHANCED PAIN STATES

Several lines of evidence suggest that activated astrocytes are sufficient to produce chronic pain symptoms. Hofstetter et al.[57] reported that implantation of neural stem cells into the injured spinal cord causes allodynic-like hypersensitivity of the forepaws, which is mainly attributed to the conversion of the stem cells into astrocytes. Indeed, the allodynia is prevented when the neural cells are transfected with neurogenin-2 before transplan-

tation to suppress the generation of astrocytes.[57] Davies et al.[58] demonstrated that transplantation of glial-restricted precursor-derived astrocytes promotes the onset of mechanical allodynia. In particular, our recent data showed that intrathecal injection of tumor necrosis factor (TNF)-α-activated astrocytes is sufficient to induce the chronic pain hallmark, mechanical allodynia, in naive animals by releasing the chemokine CCL2.[59]

Further studies indicate that astrocytes are also required for the generation of persistent pain. Fluoroacetate and its metabolite fluorocitrate are general inhibitors for glial cells, especially astrocytes. Low doses of fluorocitrate specially disrupt astrocytic metabolism by blocking the glial-specific enzyme aconitase. Intrathecal injection of fluorocitrate or fluoroacetate has been shown to alleviate pain behaviors in animal models of inflammatory pain, neuropathic pain, and postoperative pain.[60–65] Of interest, fluorocitrate fails to inhibit muscle pain, which a pain condition that does not show obvious glial reaction.[66] L-alpha-aminoadipate is another relative specific cytotoxin for astrocytes.[67–69] Intrathecal injection of L-alpha-aminoadipate produces a dose-dependent attenuation of nerve injury-induced mechanical allodynia.[31,70]

There is an increasing list of signaling molecules in astrocytes that have been implicated in persistent pain (Table 1). The glial glutamate transporter-1 is abundantly expressed in astrocytes[71] and contributes to the clearance of glutamate from synaptic clefts and the extracellular space.[72,73] The altered expression and function of glutamate transporters could modulate glutamatergic transmission[74,75] and neuronal plasticity, such as long-term potentiation.[76,77] It has been demonstrated that nerve injury induces an initial increase,[78,79] followed by a persistent decrease of glial glutamate transporter-1 and glutamate-aspartate transporter in the spinal cord.[78–81] Inhibition of glutamate transporters causes an elevation in spinal extracellular glutamate concentrations and elicits spontaneous nociceptive behaviors and hypersensitivity to mechanical and thermal stimuli.[82,83] Gene transfer of glial glutamate transporter-1 into the spinal cord has no effect on acute mechanical and thermal nociceptive responses in naive animals, but it attenuates inflammatory and neuropathic pain.[84] These studies indicate a potential role of astroglial glutamate transporters in the recovery of chronic pain. However, the role of glutamate transporters in persistent inflammatory pain conditions could be different, because these transporters are not downregulated after inflammation. Trigeminal pain after tooth pulp inflammation is attenuated by intrathecal superfusion of methionine sulfoximine, an inhibitor of the astroglial enzyme glutamine synthetase, which is involved in the glutamate-glutamine shuttle.[85]

Astrocytes express proteases, such as tissue-type plasminogen activator (tPA) and matrix metalloproteases (MMP) that may be critical for the cleavage and release of signaling molecules from astrocytes. tPA is an extracellular serine protease and converts the plasminogen into the serine protease plasmin. Kozai et al.[86] showed that L4/5 root injury induces marked induction of tPA in activated astrocytes and a resultant increase of proteolytic enzymatic activity in the dorsal horn. Moreover,

Table 1. *Signaling Molecules in Astrocytes*

Signaling Molecule	Changes in Chronic Pain Conditions	Role in Chronic Pain	Reference No.
ALXR	Upregulation	Inhibition	55
bFGF	Upregulation	Facilitation	22, 102
CCL2/MCP-1	Upregulation	Facilitation	52
Connexin-43	Upregulation	Not tested	32, 96
Endothelin receptor-B	Upregulation	Not tested	101
ERK	Upregulation	Facilitation	100
GLAST	Downregulation	Not tested	80
GLT-1	Downregulation	Inhibition	78–80
IL-18 receptor	Upregulation	Facilitation	47
IL-1β	Upregulation	Facilitation	32, 33, 41
MMP-2	Upregulation	Facilitation	114
Neurokinin-2 receptor	Not tested	Facilitation	104
pJNK	Upregulation	Facilitation	31, 36, 52
pJNK1	Upregulation	Facilitation	56
p-c-jun	Upregulation	Not tested	31
TAK1	Upregulation	Facilitation	155
TNF-α	Upregulation	Facilitation	33
TPA	Upregulation	Facilitation	86

ALXR = lipoxin A4 receptor; bFGF = basic fibroblast factor; CCL2/MCP-1 = monocyte chemoattractant protein-1 (also called CCL2); ERK = extracellular signal-regulated kinase; GLAST = glutamate-aspartate transporter; GLT-1 = glutamate transporter-1; IL = interleukin; MMP-2 = matrix metalloproteinase-2; pJNK = phosphorylated c-Jun N-terminal kinase; TAK1 = transforming growth factor-activated kinase 1; TNF-α = tumor necrosis factor; TPA = tissue type plasminogen activator.

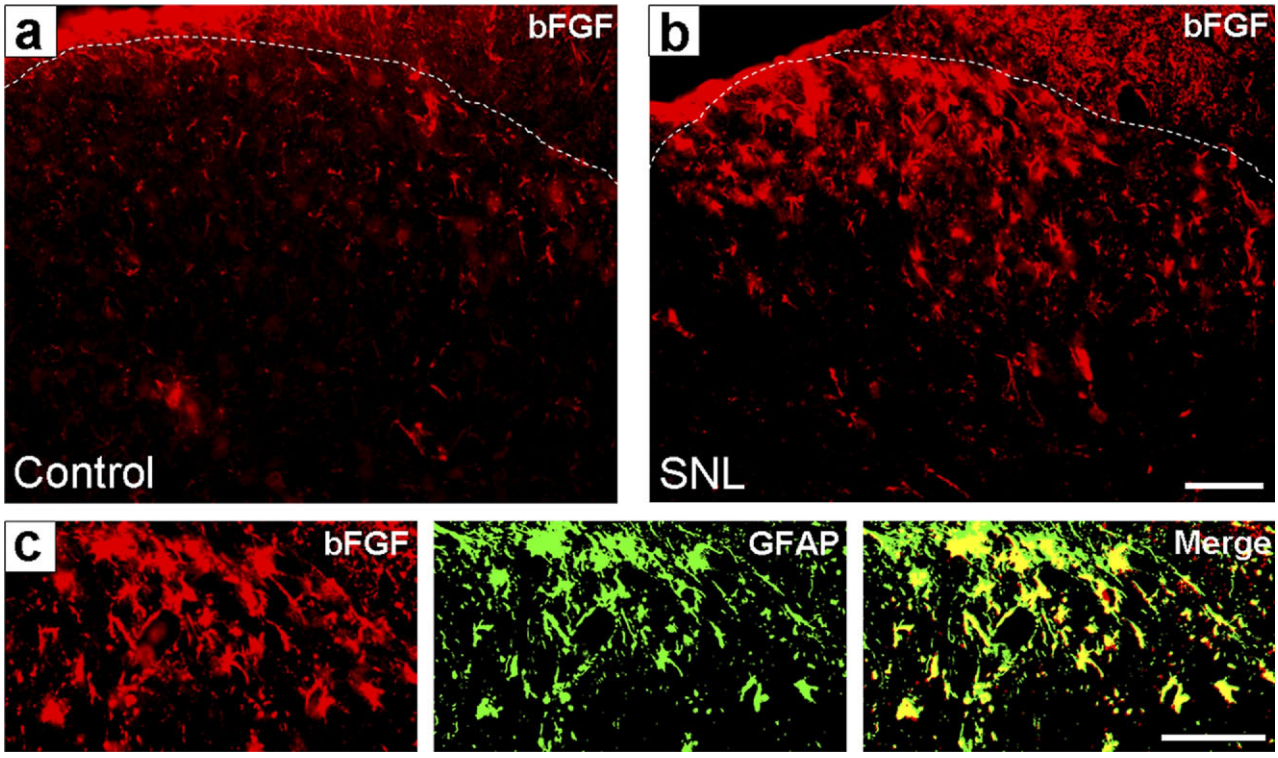

FIG. 2. Spinal nerve ligation (SNL) induces a marked basic fibroblast factor (bFGF) expression in spinal cord astrocytes. Double staining reveals a co-localization of bFGF with the astrocyte marker GFAP in the dorsal horn 3 weeks after nerve injury. (Scale, 50 μm.)

intrathecal administration of tPA inhibitor suppresses dorsal root ligation-induced mechanical allodynia. tPA-plasmin system may alter the excitability of dorsal horn neurons and pain transmission through the activation of growth factors[87,88] and modification of the NMDA receptors.[89,90]

Astrocytes are characterized by forming gap junction-coupled networks, which could transmit Ca^{2+} signaling in the form of oscillations through the networks.[91,92] The major structural components of gap junctions are connexins. In the mammalian nervous system, at least six connexins (Cx) (i.e., Cx26, Cx29, Cx30, Cx32, Cx36, and Cx43) have been identified. Among them, Cx30 and Cx43 are specifically expressed by astrocytes.[93,94] Interestingly, the expression of Cx43 increases markedly in response to facial nerve lesion,[95] spinal cord injury,[96] and CFA-induced inflammation,[32] indicating a role of Cx in chronic pain. Inhibition of gap junction function by carbenoxolone (i.e., a nonselective gap junction inhibitor) produces analgesia in different pain models.[97–99] Particularly, intrathecal injection of carbenoxolone reduces sciatic nerve inflammation-induced mechanical allodynia in the contralateral paw, suggesting a role of astrocytes network and gap junction in the spread of pain beyond the injury site.[98]

In addition, astrocytes also express phosphorylated JNK and JNK1 (FIG. 1),[31,56] phosphorylated extracellular signal-regulated kinase (ERK),[53,100] endothelin receptor-B,[101] TNF-α,[33] basic fibroblast factor (bFGF) (FIG. 2),[102,103] neurokinin-2 receptor,[104] IL-18 receptor,[47] IL-1β,[32,33,53,100] and monocyte chemoattractant protein-1 (MCP-1),[52,105] in response to nerve injury or inflammation. Importantly, pharmacological inhibition of these signaling molecules via spinal cord administration has been shown to reduce chronic pain symptoms (Table 1).

ASTROCYTES PRODUCE PRO-INFLAMMATORY CYTOKINES AND CHEMOKINES TO PROMOTE CHRONIC PAIN

IL-1β is a major pro-inflammatory cytokine and upregulated in the spinal cord under different chronic pain conditions.[35,62,106] Specifically, several studies have shown IL-1β upregulation in astrocytes after bone cancer,[41] nerve injury,[33] hind paw inflammation[53,107] and masseter inflammation.[32] IL-1β was also found in neurons in the spinal cord.[108,109] Several lines of evidence support an important role of IL-1β for pain sensitization. Inhibition of spinal IL-1β signaling with intrathecal IL-1 receptor antagonist or neutralizing antibody has been shown to alleviate inflammatory, neuropathic, and cancer pain.[32,33,62,106,107,110,111] Neuropathic pain is also markedly reduced in mouse strains with deletion of the IL-1 receptor type I or transgenic overexpression of IL-1 re-

ceptor antagonist.[112] Conversely, intrathecal injection of IL-1β is sufficient to elicit pain hypersensitivity.[113–117]

IL-1β released from astrocytes could directly modulate neuronal activity. Immunostaining shows that IL-1 receptor co-localizes with the NMDA receptor NR1 subunits in neurons of the spinal cord,[107] trigeminal nucleus,[32] and rostral ventromedial medulla.[33] In primary cultured neurons, IL-1β regulates the phosphorylation of the NMDA receptor NR2B and NR1 subunit.[32,118] IL-1β-mediated enhancement of NR1 subunit phosphorylation in the spinal cord may facilitate inflammatory pain and bone cancer pain.[107,119] In particular, our *ex vivo* electrophysiological study using patch clamp recordings in lamina II neurons demonstrated that bath application of IL-1β onto isolated spinal cord slices can markedly enhance NMDA-induced current.[106] Perfusion of spinal slices with IL-1β also increases the frequency and amplitude of spontaneous postsynaptic currents in dorsal horn neurons, indicating that IL-1β can directly enhance excitatory synaptic transmission.[106] Although the frequency increase of spontaneous postsynaptic currents result from increased glutamate release from presynaptic terminals, the amplitude increase is caused by enhanced signaling of glutamate receptor (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA]-subtype) in postsynaptic sites. IL-1β also increases the excitability of nociceptors via IL-1R, which is expressed in small-size primary sensory neurons,[120] leading to increased glutamate release in nociceptor central terminals in the spinal cord. Strikingly, IL-1β can further modulate inhibitory synaptic transmission in dorsal horn neurons. Bath application of IL-1β reduces the frequency and amplitude of spontaneous inhibitory postsynaptic currents and inhibits GABA- and glycine-induced currents in lamina II neurons,[106] which will contribute to disinhibition (loss of inhibition), an important mechanism that is increasingly appreciated for the generation of neuropathic pain.[121,122] Collectively, these studies suggest that IL-1β powerfully modulates synaptic transmission by: 1) enhancing excitatory synaptic transmission and 2) reducing inhibitory synaptic transmission. In addition, IL-1β also produces long-term neuronal plasticity in the pain circuit by inducing the phosphorylation of the transcription factor cAMP-response element binding protein[9,106] and expression of cyclooxygenase-2 in spinal cord neurons.[123]

IL-1β is synthesized as a precursor and requires a protease for its activation via cleavage to produce biological function. Notably, caspase-1 is not the only enzyme for IL-1β cleavage.[114] Metalloproteases have been implicated in the cleavage of extracellular matrix proteins, cytokines, and chemokines to control inflammation and tissue remodeling associated with various neurodegenerative diseases.[124–127] Several studies showed that MMP-9 and MMP-2 are involved in IL-1β cleavage.[114,125,128] Particularly, MMP-2 is persistently induced in astrocytes after spinal nerve ligation.[114] Treatment of MMP-2 siRNA in the late-phase of nerve injury blocks IL-1β cleavage in the spinal cord and reduces mechanical allodynia.[114] These data suggest that astrocyte-derived MMP-2 may maintain neuropathic pain by active cleavage of IL-1β. MCP-1 (also called CCL2) is the chemokine that is highly produced by astrocytes.

MCP-1 expression is increased in spinal cord astrocytes after spinal nerve ligation[52] and spinal cord contusion injuries.[105] Several studies demonstrate that activated astrocytes *in vitro* also produce MCP-1.[52,129–132] The MCP-1 was found in astrocytes in the brain after demyelinating lesions,[133,134] mechanical injury,[135] entorhinodentate axon transaction,[136] and focal cerebral ischemia.[137]

CCR2, the major receptor of MCP-1, is expressed in dorsal root ganglion neurons and increased in these neurons after nerve injury.[138] CCR2 is also constitutively expressed in spinal cord neurons,[52,139] which is upregulated after nerve injury.[52] Our recent study indicated a direct action of MCP-1 on spinal cord neurons. In isolated spinal cord slices, perfusion of MCP-1 immediately increases the frequencies of spontaneous postsynaptic currents and the amplitude in lamina II neurons of the dorsal horn.[52] MCP-1 also rapidly ($<$2 min) enhances NMDA- and AMPA-induced inward currents,[52] indicating a potentiation of glutamatergic synaptic transmission, which has been strongly implicated in central sensitization and hyperalgesia.[9,122] In addition, Gosselin et al.[139] demonstrated in neonatal cultures that MCP-1 inhibits GABA-induced currents in spinal neurons without affecting the electrical properties of these neurons. Thus, MCP-1 also modulates inhibitory synaptic transmission in spinal cord neurons.

In parallel with electrophysiological evidence, behavioral evidence shows that spinal injection of MCP-1 induces rapid heat hyperalgesia, starting at 15 min, peaking at 30 min, and recovering at 24 h.[52] Moreover, incubation of spinal cord slice with MCP-1 induces a rapid (within 5 min) phosphorylation of the extracellular signal-regulated kinase in superficial dorsal horn neurons,[52] which is regarded as a marker for spinal nociceptive neuron sensitization (central sensitization).[140] Thus, the rapid phosphorylation of ERK in dorsal horn neurons by MCP-1 supports a direct action of MCP-1 on spinal cord neurons and its involvement in central sensitization. In neuropathic pain models, MCP-1 neutralizing antibody reduces mechanical allodynia induced by SNL[52] or chronic constriction injury.[141] Nerve injury-evoked mechanical allodynia is also reduced by CCR2 antagonist or in mice lacking CCR2.[142–145] Taken together, these studies demonstrate an important role of MCP-1/CCR2 in chronic pain via astrocyte-neuron interaction (FIG. 3). In addition to a direct action of neurons, astrocyte-produced MCP-1 may also act on microglia to induce proliferation

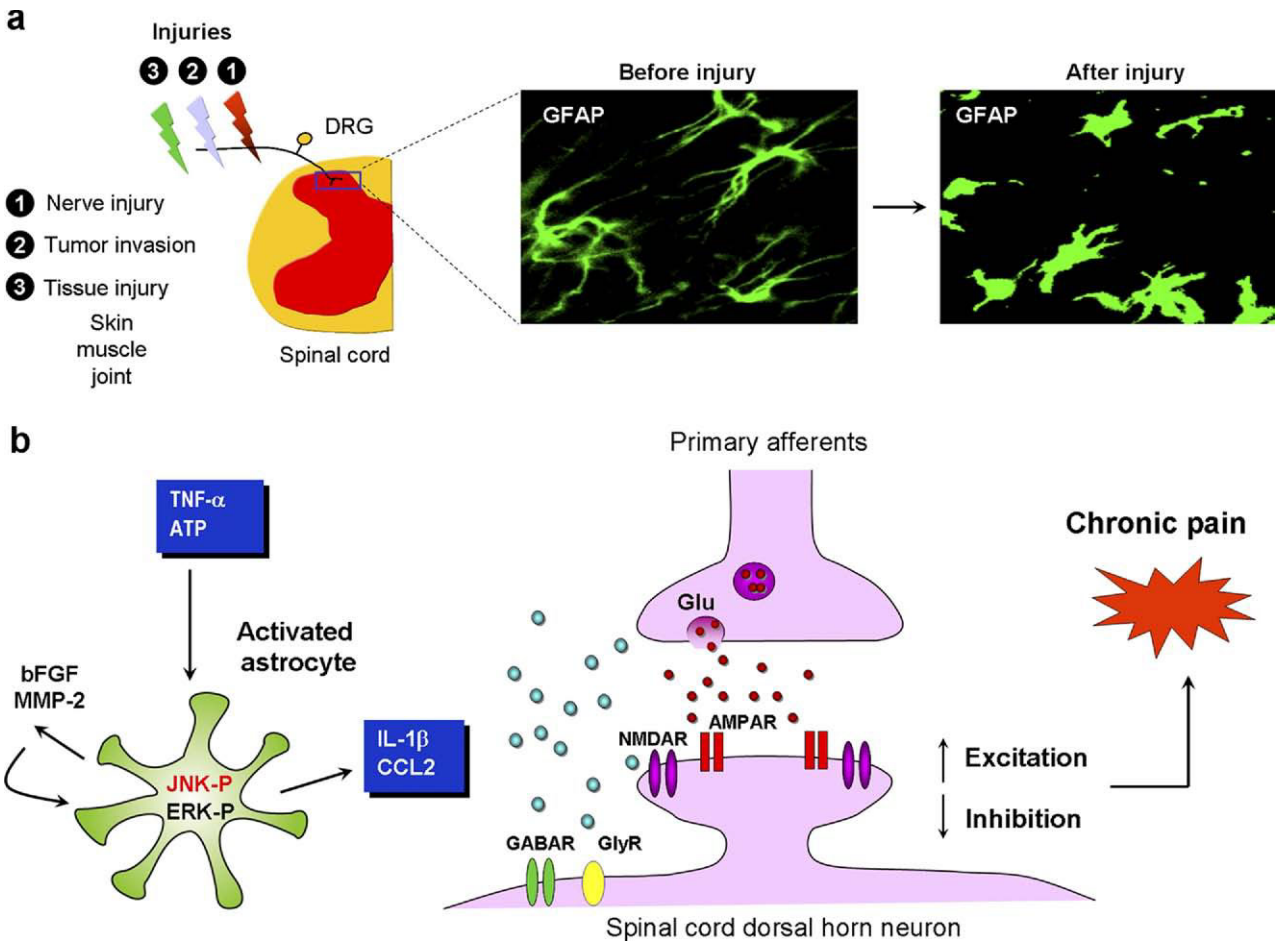

FIG. 3. Schematic showing how astrocytes in the spinal cord enhance synaptic transmission and promote chronic pain. (a) Peripheral injuries, such as nerve injury, tumor invasion (bone, nerve, and skin), and tissue injury (skin, muscle, joint) induce astrocyte reaction in the spinal cord. DRG = dorsal root ganglion. (b) Activated astrocytes show hyperphosphorylation of c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK) (only in the late phase), which leads to the production and release of interleukin (IL)-1β and monocyte chemoattractant protein-1 (MCP-1). IL-1β and MCP-1 act on presynaptic sites in the primary afferents to enhance glutamate release. They also act on postsynaptic sites in nociceptive dorsal horn neurons to enhance excitatory synaptic transmission via NMDA and AMPA receptors, and suppress inhibitory synaptic transmission via GABA and glycine receptors. While tumor necrosis factor (TNF)-α, mainly produced by microglia, induces transient activation of JNK, basic fibroblast factor, produced by astrocytes, elicits persistent activation of JNK. There is also a feedback loop between IL-1β, phosphorylation of the extracellular signal-regulated kinase, and metalloproteases (MMP)-2. IL-1β activates ERK to releases MMP-2, which in turn induces cleavage and activation of IL-1β. As a consequence of these signaling events in astrocytes, chronic pain is exaggerated and maintained. AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; bFGF = basic fibroblast factor; GABAR = gamma-aminobutyric acid receptor; Glu = glutamate; GlyR = glycine receptor; NMDAR = N-methyl-D-aspartate receptor.

and migration of microglia in the spinal cord, which can further enhance pain.[143]

MAP KINASE SIGNALING IN ASTROCYTES ENHANCES CHRONIC PAIN

Mounting evidence has demonstrated important roles of mitogen-activated protein kinases (MAPKs) (ERK, p38, and JNK) in chronic pain sensitization.[146] Of interest, these MAPKs are differentially activated in spinal cord glial cells after nerve injury. Whereas p38 is persistently activated in microglia at all the times examined,[31,147,148] ERK is only activated in microglia in the early phase (first several days) of nerve injury.[100] In the late phase (>3 weeks) of nerve injury, phosphorylation of ERK is induced in spinal astrocytes.[42,100] Spinal inhibition of this late-phase activation of ERK by intrathecal administration of an MAP kinase and ERK kinase inhibitor reverses mechanical allodynia, implicating a role of astrocytic ERK in the maintenance of neuropathic pain.[100] Intraplantar injection of CFA also induces phosphorylation of ERK in spinal cord astrocytes in the late-phase of this inflammatory pain condition.[149]

We will focus our discussion on JNK, also called stress-activated protein kinase, which is well known for its role in regulating apoptosis and neurodegeneration,[140] but JNK activation in spinal astrocytes after peripheral nerve injury is not associated with apoptosis in astro-

cytes.[31] Rather, JNK activation in astrocytes regulates the expression and release of chemokines.[52] SNL induces a persistent (>3 weeks) increase of phosphorylated JNK (pJNK) in the spinal cord, particularly in reactive astrocytes.[31] Increase in pJNK was also found in spinal astrocytes in other neuropathic conditions, such as partial sciatic nerve injury[42] and amyotrophic lateral sclerosis.[150] pJNK is further induced in spinal cord astrocytes in inflammatory pain conditions after intraplantar injection of carrageenan[55] and CFA.[56] In particular, CFA elicits a bilateral phosphorylation of JNK, starting at 6 hours and maintaining after 2 weeks.[56] Despite there are three isoforms of JNK (JNK1, JNK2, and JNK3), JNK1 is the isoform that is expressed in spinal astrocytes and hyperphosphorylated after SNL and CFA injection.[31,56] In parallel, inflammatory pain is reduced in mice lacking JNK1, but not JNK2.[56]

The role of astrocyte JNK in pain control has been also evaluated by intrathecal injection of the JNK inhibitor SP600125. Administration of SP600125, either before or after nerve injury, can both attenuate neuropathic pain after SNL.[31,151] SP600125 also suppresses neuropathic pain in a diabetes model of neuropathic pain.[152] The peptide inhibitor D-JNKI-1 is a more potent and selective inhibitor of JNK. A single bolus injection of D-JNKI-1 can block SNL-induced mechanical allodynia for more than 6 hours.[31]

How does JNK signaling in astrocytes control chronic pain? JNK activation in astrocytes results in the production of various inflammatory mediators. In cultured astrocytes there is a JNK-dependent expression of cyclooxygenase-2 and inducible nitric oxide synthase, as well as the release of nitric oxide, prostaglandin E2 and IL-6.[153] Notably, stimulation of astrocytes with TNF-α not only activates JNK, but also induces a marked upregulation of several chemokines, such as MCP-1, keratinocyte-derived chemokine, and IFN-γ-inducible protein 10.[52] Strikingly, TNF-α induces a substantial increase (>100-fold), both in the expression and release of MCP-1 in astrocyte cultures; this increase is completely blocked by JNK inhibition.[52] JNK activation in astrocytes also leads to the production of MCP-1 *in vivo*.[52] Thus, JNK activation in astrocytes can enhance pain via producing chemokines such as MCP-1, which is known to increase the sensitivity of dorsal horn neurons.[154]

JNK is activated by the transforming growth factor-activated kinase 1, a member of the MAPK kinase family. Interestingly, peripheral nerve injury induces transforming growth factor-activated kinase 1 upregulation in hyperactive astrocytes in the spinal cord.[155] Intrathecal administration of transforming growth factor-activated kinase 1 anti-sense oligodeoxynucleotides, either before and after nerve injury, can reduce nerve injury-induced mechanical allodynia.[155]

bFGF is a well-known activator of astrocytes and induces mitosis, growth, differentiation, and gliosis of astrocytes.[156,157] Spinal nerve ligation induces a substantial increase of bFGF in reactive astrocytes in the late phase (3 weeks after injury, FIG. 2). Intrathecal infusion of bFGF induces persistent JNK phosphorylation and GFAP expression in the spinal cord, which is associated with the development of mechanical allodynia.[22] Conversely, intrathecal injection of a bFGF neutralizing antibody can reverse nerve injury-induced mechanical allodynia.[102] Compared to a transient JNK activation by TNF-α, bFGF induces a sustained activation of JNK in astrocyte cultures.[22,52] Therefore, bFGF, produced in astrocytes in the late-phase of injury, may maintain chronic pain via sustained JNK activation in astrocytes.

CONCLUSIONS AND CLINICAL IMPLICATIONS

In summary, we have reviewed behavioral, histochemical, and electrophysiological evidence to support a rising role of astrocytes in chronic pain sensitization. We also demonstrate how astrocytes promote chronic pain via neuronal-glial interactions (FIG. 3). After peripheral nerve injury or tissue damage in the skin, muscle, or joint (e.g., arthritis), astrocytes are activated in the spinal cord in response to neurotransmitters/neuromodulators (e.g., ATP, glutamate, neuropeptides), and inflammatory mediators (e.g., TNF-α) released after injuries. Astrocyte activation may manifest as the activation of several intracellular signaling pathways, such as the JNK and ERK pathways or/and upregulation of GFAP and astrogliosis/hypertrophy (FIG. 3a). Activation of the JNK or/and ERK results in the production of pro-inflammatory cytokines and chemokines (e.g., IL-1β and MCP-1). These mediators can act at both presynaptic sites on primary afferents and postsynaptic sites on dorsal horn neurons to increase excitation and decrease inhibition of spinal cord nociceptive neurons, leading to enhanced pain states (FIG. 3b).

Given the important role of astrocytes in chronic pain facilitation, targeting astrocytes could reveal novel therapies for the management of chronic pain. However, caution must be taken when we consider strategies to target astrocytes, because astrocytes play an essential supportive and protective role in the CNS.[158] Inhibition of reactive astrocytes with the toxin fluorocitrate has been shown to retard neurovascular remodeling and recovery after focal cerebral ischemia.[159] Thus, it is important to target specific signaling events in astroctyes without disrupting the overall well being of astrocytes. As discussed in Table 1, all the signaling molecules that are induced in astrocytes under chronic pain conditions and that contribute to pain behaviors can be potentially targeted. In particular, JNK inhibitor not only exhibits

anti-allodynic action, but it also has a neuroprotecitve role.[160] JNK inhibitor further reduces tumor growth[36] and insulin resistance[161,162]; therefore, JNK inhibitor should be beneficial in pain conditions associated with cancer and diabetic neuropathy.

Finally, it is worth noting that all the evidence we present is from animal studies. Indeed, astrocytes from humans are quite different.[163] The human brain seems to contain subtypes of GFAP-positive astrocytes that are not represented in rodents. Strikingly, in the human cortex, astrocytes are >2-fold larger in diameter and extend 10-fold more GFAP-positive primary processes than their rodent counterparts. The domain of a single human astrocyte has been estimated to contain as many as 2 million synapses.[164] Hence, it is reasonable to postulate human astrocytes may play a more important role in chronic pain control than rodent astrocytes.

Acknowledgments: This work was supported by the National Institutes of Health R01 Grant No. NS54932, Grant No. NS67686, and Grant No. DE17794.

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