Review
Extracellular signal-regulated kinases (ERK) 1 and 2 as a key molecule in pain research
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Abstract: Pain can be divided into nociceptive, inflammatory, and neuropathic pain. It is important to understanding the molecular mechanism of pain signaling in the development of pain relief therapies. Twenty years ago, extracellular signal-regulated kinases (ERK) 1 and 2, which are members of the mitogen-activated protein kinase superfamily, were identified as molecules activated in neurons by the exposure of peripheral tissues to noxious stimuli. Further studies have revealed that peripheral nerve injury induces ERK activation in glial cells, sensory neurons, and second-order neurons, albeit at different time points. Moreover, inhibition of ERK suppresses pathological pain in animals with peripheral nerve injury. Therefore, ERK is currently recognized as an important molecule in pain signaling and a potential novel target for pain treatment. This review introduces recent advances in revealing the regulation of ERK in pain research.

Keywords: ERK phosphorylation, inflammation, nociception, pain, peripheral nerve injury, tissue damage

Introduction
Pain is characterized by an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage [1]. Based on the type of damage, pain is often classified into the following three main categories: nociceptive, inflammatory, and neuropathic pain (Fig. 1) [2]. Nociceptive pain is triggered by a noxious stimulus; it is the body’s alarm signal to avoid danger and is usually acute and transient. Conversely, inflammatory pain is induced by tissue damage and inflammation; it is spontaneous and often accompanied by hypersensitivity to pain signals. Neuropathic pain develops when peripheral nerve fibers are damaged; it is a pathological signal because it occurs spontaneously or even in response to nonnoxious stimuli and is persistent. The molecular mechanism and cellular basis underlying these different types of pain must be urgently elucidated to aid the development of appropriate pain relief therapies.

During the past century, studies have revealed pain pathways from the periphery to higher brain centers and have identified ion channels, receptors, neurotransmitters, neuromodulators, kinases, and transcription factors as pain-related factors in the nervous system [3-5].

Extracellular signal-regulated kinases (ERK) 1 and 2, which are components of the mitogen-activated protein (MAP) kinase family, regulate cell mitosis, proliferation, differentiation, and survival during mammalian development via the transmission of signals from various cell surface receptors to cytoplasmic and nuclear effector molecules [6,7]. Initial neuroscience studies have demonstrated that ERK signaling is essential for the neural plasticity underlying learning and memory [8].

In 1999, Ji et al. found that noxious stimulation of the rat hind paw resulted in rapid and transient activation of ERK only in a small population of neurons in the visceral dorsal horn (SDH) [9]. Subsequent studies have revealed that ERK is activated in glial cells (such as microglia and astrocytes) and neurons [10-12] and is thus involved in various types of pain in different manners after peripheral nerve injury. This review introduces the major roles of ERK, which have been elucidated through pain research over the past 20 years.

ERK and nociceptive pain—Phosphorylated ERK (pERK) as a marker for neurons activated by nociceptive stimuli
The axons in somatosensory neurons are generally categorized into the following three classes based on their conduction velocity: Aβ, Aδ, and C fibers [13,14]. Myelinated Aβ fibers are normally involved in the transmission of tactile information, whereas both myelinated Aδ and unmyelinated C fibers are involved in the transmission of nociceptive information in response to noxious mechanical, thermal, or chemical stimuli.

Subcutaneous injection of capsaicin (a transient receptor potential vanilloid 1 [TRPV1] agonist) into the rat hind paw induces ERK phosphorylation in small neurons with unmyelinated fibers in the dorsal root ganglion (DRG) [15]. Conversely, electrical stimulation of Aδ fibers induces ERK phosphorylation primarily in neurons comprising NF200, a marker for neurons with myelinated fibers [14]. Taken together, these results suggest that ERK activation in DRG neurons is associated with excitation of specific sensory fibers.

In addition, application of nociceptive stimuli to a rat hind paw transiently induces ERK activation not only in sensory and primary neurons but also in a subset of medial superficial dorsal horn (DH) neurons [10]. Some of these neurons in laminae I and III/IV of the DH exhibit neurokinin 1 receptor (NK1R) immunoreactivity [16]. NK1R-expressing cells are considered projection neurons because they are labeled after injection of a neural tracer into the caudal ventrolateral medulla [17]. Therefore, at least a portion of nocicepti-induced pERK-positive neurons in the DH are presumed to be projection neurons.

Interestingly, ERK activation is exclusively induced by noxious stimuli (heat, cold, and noxious mechanical stimuli) but not by innocuous stimuli (warmth, coolness, and gentle touch) [10]. Furthermore, ERK activation occurs in a stimulation intensity-dependent manner and is affected by the duration of noxious stimulation [10,18]. These results suggest the important role of ERK signaling in pain perception and the use of pERK as a biomarker for activated cells involved in pain signaling to the somatosensory cortex and nocifensive reflex (Fig. 1A).

Trigeminal ganglion neurons send their peripheral axons to innervate the facial skin and various organs in the introrrenal area and a central axon to synapse on second-order neurons in trigeminal nuclei [19]. Electrophysiological studies and anatomical experiments have used different retro- and anterograde tracing methods and demonstrated that the neurons in each trigeminal spinal subnucleus are arranged somatotopically [20,21]. The ventral part of the spinal trigeminal subnucleus caudalis (Vc) comprises neurons with ophthalmic receptive fields, the region between the dorsal and ventral parts comprises neurons with maxillary receptive fields, and the dorsal part mainly comprises neurons with mandibular receptive fields [20]. Conversely, in the rostrocaudal direction, the caudal part of the Vc receives cutaneous inputs from the lateral parts of the face and head, whereas the rostral part of the Vc receives afferent inputs from the rostral parts of the face, i.e. the nose and mouth [21].

Consistent with the findings mentioned above, injection of capsaicin into the upper molar dental pulp results in rapid and transient activation of ERK in the superficial laminae of the center parts (on the dorsoventral axis) of the Vc and upper cervical cord [22]. Noma et al. (2008) injected capsaicin into various parts of the maxillofacial (including the tongue, lower gum, and upper and lower lips) and subcutaneous mental regions and
analyzed the distribution pattern of pERK signals in the brain [23]. They demonstrated that ERK phosphorylation is induced in the neurons in the somatotopically appropriate part of the ipsilateral Vc depending on the site of capsaicin injection [23]. These findings indicate that analyses of pERK signals in the primary sensory ganglia, SDH, and medulla have contributed to the understanding of pain pathways.

**ERK and inflammatory pain—pERK as a molecule regulating gene expression**

Clinically, inflammatory pain is induced by tissue damage and inflammation, and tissue damage results in the activation of inflammatory cells, which then release several inflammatory mediators, such as prostaglandin E2, interleukin-1β, and tumor necrosis factor-α [24]. In experimental models, inflammatory irritants, complete Freund’s adjuvant, carrageenan, and formalin have been frequently used to induce inflammation in the peripheral tissues of rodents [5].

Injection of formalin into rat hind paws reportedly resulted in ERK phosphorylation in SDH neurons and subsequently upregulated the expression of pain-related molecules, such as prodynorphin and NK-1 [25]. Elevated levels of ERK phosphorylation are induced in these formalin-injected models than in capsaicin-injected models [25], suggesting the involvement of enhanced ERK activation in the maintenance of central sensitization in the nervous system and pain hypersensitivity via regulation of gene expression (Fig. 1B).

Tooth movement using orthodontic appliances induces transient inflammation in the periodontal ligaments; therefore, patients feel discomfort or pain [26,27]. Similar to the former inflammatory models using formalin, continuous mechanical pressure on the teeth induced by orthodontic elastics in rat models resulted in ERK activation in Vc neurons [28]. pERK signals were observed in the nuclei of neurons and were colocalized with Fos, which is a protooncogene protein product. The intrathecal administration of an MEK1/2 inhibitor in the medulla suppressed both ERK phosphorylation and Fos expression, suggesting the involvement of ERK activation in the regulation of Fos expression. Fos regulates the transcription of several genes, including some genes that encode pain-related molecules, by forming a heterodimer with the Jun protein [29]. Studies have reported that the downregulation of Fos expression using a c-fos antisense oligodeoxynucleotide significantly suppressed inflammatory irritant-induced pain behavior in rodents [30,31]. Orthodontic treatment-induced discomfort or pain is usually transient but can persist for more than 1 week [32]. These findings suggest that the ERK signaling pathway plays an important role in prolonged orthodontic treatment-induced pain via the transcriptional regulation of pain-related genes by Fos.

**ERK and neuropathic pain—pERK as a therapeutic target for neuropathic pain**

Peripheral nerve injury often leads to somatosensory impairment, such as neuropathic pain (spontaneous pain, hyperalgesia, and allodynia) and, in some cases, hypoalgesia [33]. Several pain researchers have developed multiple models to identify the factors mediating the development of each type of neuropathic pain [34].

It is known that partial sciatic nerve ligation induces ERK phosphorylation in spinal astrocytes 21 days postlesion [35]. After dorsal root transection, ERK activation has been observed in spinal microglia 1-2 days after nerve injury [36]. Zhuang et al. investigated the ERK activation pattern in the spinal cord and nocifensive behavior at different time points after spinal nerve ligation (SNL) [37]. They found that ERK activation was enhanced in OX-42-immunoreactive (IR) microglia and GFAP-IR astrocytes on days 2 and 21, respectively. In addition, the intrathecal administration of a noncompetitive MEK1 inhibitor—PD98059—attenuated SNL-induced mechanical allodynia [37]. These findings suggest the importance of the sequential activation of ERK in the microglia and astrocytes in the induction and maintenance of neuropathic pain, respectively (Fig. 1C).

Suzuki et al. (2013) developed an experimental model of neuropathic pain based on infraorbital nerve chronic constriction injury (ION-CCI) in the trigeminal nervous system in rats [38]. Following noxious mechanical stimulation at 7 days after injury, the number of ERK-IR cells was significantly increased in rats with ION-CCI compared with rats who had undergone sham operations. In this experimental model, ERK-IR was induced in neurons but not in astrocytes or microglia. Furthermore, hypersensitivity to mechanical and thermal nocifensive behavior was significantly enhanced in rats with ION-CCI. However, hypersensitivity was reduced following the intrathecal administration of PD98059, suggesting that enhanced ERK phosphorylation in Vc neurons contributes to the development of hyperalgesia.

Using an inferior alveolar nerve-transected model (IANX), Okada-Ogawa et al. demonstrated that nerve injury-dependent hyperreactivity of...
astrocytes in the Vc is essential for the induction of hyperalgesia in the orofacial region. The intrathalamic administration of the astroglial aconitase inhibitor—sodium fluoride (FA)—prevented astrocyte hyperreactivity and ERK activation in Vc neurons on postoperative day 7 in rats operated using IANX. In addition, FA increased the head withdrawal latency to noxious heat stimulation of the maxillary whisker pad skin in rats operated using IANX [39].

These findings suggest that nerve injury-induced ERK activation in neurons and/or glial cells and astrocyte hyperreactivity are essential for the induction of hyperalgesia. Based on these results, ERK is considered a target for the treatment of neuropathic pain.

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Conflict of interest
The authors have no conflict of interest to declare.

References
1. Woolf CJ (2004) Pain: moving from symptom control toward mechanism-specific pharmacologic management. Ann Intern Med 140, 441-451.
2. Ueda H (2008) Peripheral mechanisms of neuropathic pain—involved in lysophosphatidic acid receptor-mediated demyelination. Mol Pain 4,411.
3. Julius D, Basbaum AI (2001) Molecular mechanism of nociception. Nature 413, 203-210.
4. Cruz CD, Cruz F (2007) The ERK 1 and 2 pathway in the nervous system: from basic aspects to possible clinical applications in pain and visceral dysfunction. Curr Neuropharmacol 5, 244-252.
5. Jia RR, Gereau RW 4th, Malcangio M, Strichartz GR (2009) MAP kinase and pain. Brain Res 140, 441-451.
6. Shi M, Asano M, Kirigaya J, Ogiso B, Ren K, Oki H et al. (2006) Phosphorylation of extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) in astrocytes in the lumbar spinal dorsal horn and the gracile nucleus. Pain 99, 175-184.
7. Zhuang ZY, Gerner P, Woolf CJ, Ji RR (2005) ERK is sequentially activated in neurons, and its involvement in peripheral sensitization. J Neurosci 22, 7377-7385.
8. Noma N, Tsuoi Y, Kondo M, Matsumoto M, Sesile BJ, Kitagawa J et al. (2008) Orga-
9. Ji RR, Brenner GJ, Woolf CJ (2002) ERK MAP kinase activation in superficial spinal cord neurons induces prodynorphin and NK-1 upregulation and contributes to persistent inflammatory pain hypersensitivity. J Neurosci 22, 478-485.
10. Bergius M, Karlsen SJ, Berggren U (2000) Pain in orthodontics. A review and discussion of the literature. J Orofac Orthop 61, 125-148.
11. Chen G, Zhang YQ, Qadri YJ, Serhan CN, Ji RR (2018) Microglia in pain: Detrimental and protective roles in pathogenesis and resolution of pain. Neuropharmacology 129, 170-179.
12. Ji RR, Donnelly CR, Nedergaard M.R (2019) Astrocytes in chronic pain and itch. Nat Rev Neurosci 20, 667-685.
13. Peirs C, Seal RP (2016) Neural circuits for pain: recent advances and current views. Sci Prog 29, 170-179.
14. Woolf CJ, Saltner MW (2000) Neuronal plasticity: increasing the gain in pain. Science 288, 1765-1769.
15. Jaggi AS, Jain V, Singh N (2011) Animal models of neuropathic pain. Fundam Clin Pharmacol 25, 1-28.
16. Ma W, Quirion R (2005) The ERK/MAPK pathway, as a target for the treatment of neuro-
17. Noma N, Tsuboi Y, Kondo M, Matsumoto M, Sesile BJ, Kitagawa J et al. (2008) Orga-
18. Ji RR, Brenner GJ, Woolf CJ (2002) ERK MAP kinase activation in superficial spinal cord neurons induces prodynorphin and NK-1 upregulation and contributes to persistent inflammatory pain hypersensitivity. J Neurosci 22, 478-485.