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C HRONIC pain usually caused by inflammation and tissue or nerve injury is a major public health problem worldwide. It is characterized by ongoing or intermittent burning pain, an enhanced response to noxious stimuli (hyperalgesia), and pain in response to normally innocuous stimuli (allodynia). Current treatment for this disorder has had restricted success due to our inadequate understanding of the mechanisms that lead to the initiation and maintenance of chronic pain. It is known that inflammation and nerve injury produce changes in the expression of receptors, enzymes, ion channels, neurotransmitters, neuromodulators, and structural proteins in primary sensory neurons of the dorsal root ganglion (DRG) at both transcriptional and translational levels.1–3 Such changes are considered to contribute to chronic pain development and maintenance.1–3 However, it is unclear how peripheral inflammation or nerve injury alters the expression of these genes and/or proteins in DRG. Understanding this mechanism may enable the development of new strategies to prevent and/or treat chronic pain.

Recent studies suggest that the mechanism for gene regulation involves widespread noncoding (nc) RNAs.4–6 RNA had long been thought to be a simple and intermediary component of gene expression, as it is transcribed from DNA and then translated into proteins in cells. However, it has become increasingly clear that mammalian genomes encode not only protein-coding RNAs but also a vast number of ncRNA transcripts.7 Because the function of each newly identified ncRNA has not been fully elucidated, the common practice is to group ncRNA transcripts based on transcript size: small/short ncRNAs (e.g., microRNAs [miRNAs]; 18 to 200 nucleotides) and long ncRNAs (e.g., native Kcna2 antisense [AS] RNA; >200 nucleotides).7–9 ncRNAs have been systematically identified in the mammalian nervous system, including in pain-related regions.10 They can be regulated and may govern the expression of both protein-coding and noncoding genes. An intriguing association between aberrant expression of ncRNAs and the development of diseases has been demonstrated recently.11,12 Previous studies have shown that peripheral inflammation and nerve injury drive changes in the expression of some miRNAs and Kcna2 AS RNA in DRG.13–16 These changes might be responsible for inflammation/nerve injury–induced alterations of some pain-associated genes, an increase in DRG neuronal excitability, and behavioral pain hypersensitivity.14,16 The evidence indicates that ncRNAs might be new key players in the mechanisms that underlie the development and maintenance of chronic pain.
miRNAs in Chronic Pain

Formation of miRNAs

Since the discovery of the first miRNA, lin-4 in *Caenorhabditis elegans*, hundreds of miRNAs have been identified in the nervous system. These miRNAs are coded by specific genes. Generally, a miRNA molecule is synthesized from a long RNA primary transcript known as a pri-miRNA (fig. 1). In the cellular nucleus, pri-miRNA is cleaved by Drosha, an RNAIII endonuclease, to produce a characteristic stem-loop structure of approximately 60 to 70 nucleotides in length, known as pre-miRNA (fig. 1). After pre-miRNA is exported from the nucleus into the cytoplasm, it is cleaved by Dicer, another RNAIII endonuclease, to produce double-stranded mature miRNA (fig. 1). The latter is either unwound via an unknown helicase or cleaved by the enzyme Ago2 to lead to a single-stranded miRNA (fig. 1). The single strands completely or incompletely bind to specific messenger RNA (mRNA) sequences, resulting in degradation or translational repression of target miRNAs. A recent link between miRNA-mediated poly(A)-tail length shortening and mRNA destabilization has been reported, suggesting another potential mechanism of ncRNA-mediated gene regulation.

Changes in the expression of miRNAs in response to noxious stimulation have been reported. Bai et al. reported a significant down-regulation of mature miR-10a, -29a, -98, -99a, -124a, -134, and -183 in the mandibular division of trigeminal ganglion ipsilateral to complete Freund’s adjuvant (CFA)–injected rat masseter muscle in a model of peripheral inflammation (table 1). Such down-regulated miRNAs were observed 4 h after injection and recovered differentially to a normal level or higher than normal level. Expression and down-regulation of miRNAs occurred in all sizes of trigeminal ganglion neurons (but not in glial cells and other non-neuronal cells) that innervate the inflamed muscle although the miRNA signals varied among neurons (table 1). Injection of CFA into a hind paw also reduced expression levels of miR-1, -16, -206, and -143 in the ipsilateral DRG neurons, but increased miR-1, -16, and -206 levels in the ipsilateral spinal dorsal horn neurons (table 1). These changes clearly correlate to CFA-induced peripheral inflammation. It should be noted that CFA-induced changes in miRNAs may be immune related as CFA also causes immune response. Interestingly, peripheral injection of formalin led to a significant down-regulation of miRNA-124a expression in the neurons of dorsal horn ipsilateral to injection (table 1). These studies provide promising evidence of miRNA changes in pain-related regions under inflammatory conditions.

In addition to peripheral inflammation, expression changes of miRNAs were observed after peripheral nerve injury. L5 spinal nerve ligation (SNL) induced a drastic decrease in the expression of miR-1, -7a, -96, -103, -182, -183, and -206 in the injured DRG and -582-3p, and -720 (table 2). In the sciatic nerve transection or chronic constriction injury model of neuropathic pain, the injured DRG showed reduced expression of several miRNAs, including miR-10a,-30b, -99a, -100, -143, -582-3p, and -720 (table 2). In contrast, miR-21 in the injured DRG was up-regulated after L5 SNL (table 2). Although these changes in expression may not be ruled out to be related to regeneration, the evidence indicates that the expression of miRNAs is differentially and spatially regulated in pain-related regions after peripheral nerve injury.

Furthermore, expression changes of miRNAs have also been observed in patients with painful diseases. In bladder...
biopsies from patients with bladder pain syndrome (also known as interstitial cystitis), 31 miRNAs were differentially expressed (table 1). An inverse relation was observed in which neurokinin-1 mRNA/protein was down-regulated and four miRNAs (miR-449b, -500, -328, and -320) were up-regulated. Differential expression of 18 miRNAs was reported in blood from patients with complex regional pain syndrome (table 1). In addition, miR-146a, -199a, and -558 may be linked to pain-related pathophysiology of osteoarthritis through regulation of the expression of cyclooxygenase-2 (table 1). It seems that miRNA profiles have the potential to serve as biomarkers of pain.

Table 1. miRNAs Associated with Peripheral Inflammation

<table>
<thead>
<tr>
<th>Inflammatory Models</th>
<th>miRNAs</th>
<th>Change in Expression</th>
<th>Tissue</th>
<th>Target Gene</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFA into masseter muscle</td>
<td>miR-29a, -98, -99a, -124a, -134, -183</td>
<td>↓</td>
<td>Rat ipsilateral trigeminal ganglion</td>
<td>Unknown</td>
<td>Bai et al.13</td>
</tr>
<tr>
<td>CFA into hind paw</td>
<td>miR-1, -16, -206</td>
<td>↓</td>
<td>Rat ipsilateral DRG neurons</td>
<td>Unknown</td>
<td>Kusuda et al.25</td>
</tr>
<tr>
<td></td>
<td>miR-1, -16, -206</td>
<td>↑</td>
<td>Rat ipsilateral spinal dorsal horn neurons</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Formalin injection</td>
<td>miR-143</td>
<td>↓</td>
<td>Murine ipsilateral DRG neurons</td>
<td>MeCP2; proinflammatory marker genes</td>
<td>Tam Tam et al.26</td>
</tr>
<tr>
<td></td>
<td>miR-124a</td>
<td>↓</td>
<td>Murine ipsilateral spinal dorsal horn neurons</td>
<td>Unknown</td>
<td>Kynast et al.18</td>
</tr>
<tr>
<td>Bladder pain syndrome</td>
<td>miR-449b, -500, -328, -320; 31 miRNAs</td>
<td>↑/↓</td>
<td>Human bladder biopsies</td>
<td>Neurokinin-1</td>
<td>Sanchez et al.33</td>
</tr>
<tr>
<td>Complex regional pain syndrome</td>
<td>18 different miRNAs</td>
<td>↑/↓</td>
<td>Human blood</td>
<td>Unknown</td>
<td>Orlova et al.34</td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td>miR-199a, -558</td>
<td>↓</td>
<td>Human chondrocytes</td>
<td>Cyclooxygenase-2</td>
<td>Akhtar et al.35; Park et al.37</td>
</tr>
<tr>
<td>Peritonitis model of self-limiting acute inflammation</td>
<td>miR-21, -146b, -208a</td>
<td>↑</td>
<td>Murine exudates</td>
<td>Unknown</td>
<td>Recchiuti et al.38</td>
</tr>
<tr>
<td></td>
<td>miR-302d</td>
<td>↓</td>
<td>Injured DRG of mice</td>
<td>For miR-7a, β2 subunit of voltage-gated sodium channels is a potential target</td>
<td>Sakai et al.14; Kusuda et al.25; Favereaux et al.28; Aldrich et al.29</td>
</tr>
<tr>
<td></td>
<td>miR-142-3p</td>
<td>↓</td>
<td>Nucleus accumbens of mice</td>
<td>Unknown</td>
<td>Imai et al.30</td>
</tr>
<tr>
<td></td>
<td>miR-142-5p</td>
<td>↓</td>
<td>Injured DRG of mice</td>
<td>Matrix metalloproteinases; endogenous inhibitors of phosphatidylinositol 3-kinase; negative regulators of extracellular signal–regulated kinase</td>
<td>Sakai et al.14; Sakai and Suzuki15</td>
</tr>
<tr>
<td></td>
<td>miR-203</td>
<td>↑</td>
<td>Injured DRG of mice</td>
<td>STAT5, SOCS3, JAK1</td>
<td>Sakai et al.14; Sakai and Suzuki15</td>
</tr>
<tr>
<td></td>
<td>miR-219</td>
<td>↑</td>
<td>Uninjured L4 DRG in mice</td>
<td>For miR-7a, j2 subunit of voltage-gated sodium channels is a potential target</td>
<td>Sakai et al.14; Kusuda et al.25; Favereaux et al.28; Aldrich et al.29</td>
</tr>
<tr>
<td>Sciatic nerve transection/CCI</td>
<td>miR-10a, -30b, -99a, -100, -143, -582-3p, -720</td>
<td>↓</td>
<td>Uninjured L4 DRG in mice</td>
<td>Unknown</td>
<td>von Schack et al.31</td>
</tr>
<tr>
<td></td>
<td>Kcna2 AS RNA</td>
<td>↑</td>
<td>Injured DRG of mouse</td>
<td>Kcna2</td>
<td>Zhao et al.16</td>
</tr>
<tr>
<td></td>
<td>miR-10a, -30b, -99a, -100, -143, -582-3p, -720</td>
<td>↓</td>
<td>Injured DRG of rat</td>
<td>Unknown</td>
<td>Tam Tam et al.26; Brandenburger et al.32</td>
</tr>
</tbody>
</table>

ATF2 = activating transcription factor 2; CCI = chronic constriction injury; CFA = complete freund’s adjuvant; CREB = cyclic adenosine monophosphate response-element binding protein; DRG = dorsal root ganglion; IL = interleukin; MeCP2 = methyl-CpG-binding protein 2; miR = microRNA; ncRNAs = noncoding RNAs; SOCS3 = suppressor of cytokine signaling-3; SRF = serum response factor; STAT5 = signal transducer and activator of transcription; TGFBR1 = transforming growth factor β receptor 1; TNF = tumor necrosis factor.

Table 2. Noncoding RNAs Associated with Peripheral Nerve Injury

<table>
<thead>
<tr>
<th>Neuropathic Pain Models</th>
<th>ncRNAs</th>
<th>Change in Expression</th>
<th>Tissue</th>
<th>Target Gene</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>L5 spinal nerve ligation</td>
<td>miR-1, -7a, -96, -103, -182, -183, -206</td>
<td>↓</td>
<td>Injured DRG of mice</td>
<td>For miR-7a, j2 subunit of voltage-gated sodium channels is a potential target</td>
<td>Sakai et al.14; Kusuda et al.25; Favereaux et al.28; Aldrich et al.29</td>
</tr>
<tr>
<td></td>
<td>miR-200b, -429</td>
<td>↓</td>
<td>Nucleus accumbens of mice</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>miR-21</td>
<td>↑</td>
<td>Injured DRG of mice</td>
<td>Matrix metalloproteinases; endogenous inhibitors of phosphatidylinositol 3-kinase; negative regulators of extracellular signal–regulated kinase</td>
<td>Sakai et al.14; Sakai and Suzuki15</td>
</tr>
<tr>
<td></td>
<td>59 miRNAs</td>
<td>↓</td>
<td>Uninjured L4 DRG in mice</td>
<td>For miR-7a, j2 subunit of voltage-gated sodium channels is a potential target</td>
<td>von Schack et al.31</td>
</tr>
<tr>
<td></td>
<td>Kcna2 AS RNA</td>
<td>↑</td>
<td>Injured DRG of mouse</td>
<td>Kcna2</td>
<td>Zhao et al.16</td>
</tr>
<tr>
<td></td>
<td>miR-10a, -30b, -99a, -100, -143, -582-3p, -720</td>
<td>↓</td>
<td>Injured DRG of rat</td>
<td>Unknown</td>
<td>Tam Tam et al.26; Brandenburger et al.32</td>
</tr>
</tbody>
</table>

CCI = chronic constriction injury; DRG = dorsal root ganglion; miR = microRNA; ncRNAs = noncoding RNAs.
miRNAs Regulated by Inflammatory Mediators in Chronic Pain

How peripheral noxious stimulation causes the alternations of miRNA expression in pain-related regions is incompletely understood, but it is very likely that miRNA expression may be controlled by inflammatory mediators (fig. 2). Administration of resolvin D1, an anti-inflammatory lipid mediator, counter-regulated the expression of miR-21, -142, -146b, -203, -208a, -219, and -302d in a murine peritonitis model of self-limiting acute inflammation (table 1). An inflammatory mediator, produced a significant reduction in miR-558 in normal and osteoarthritis chondrocytes possibly through IL-1β–induced activation of mitogen-activated protein kinase and nuclear factor-κB (table 1). IL-1β also increased the expression of miR-21 in DRG neurons, the insulinoma cell line MIN6, and human pancreatic islets. Activator protein 1 (AP-1), a transcription factor, may participate in this effect of IL-1β as the promoter region of miR-21 contains the binding site of AP-1, and IL-1β triggers AP-1 activation in DRG neurons. Given that peripheral inflammation and nerve injury increase DRG IL-1β expression, IL-1β may be responsible for inflammation-induced down-regulation of miR-558 and nerve injury-induced up-regulation of miR-21 in the injured DRG (fig. 2).

Potential Mechanisms of miRNAs' Effects on Chronic Pain

It has been demonstrated that miRNAs exert their functions through their complete or incomplete sequence homology to the 3’-untranslated region of target mRNAs, resulting in a block in translation or mRNA degradation (fig. 1). Studies on inflammatory pain suggest that miRNAs specifically target pain-related genes (fig. 2). When a miRNA-124a inhibitor was intravenously administered after formalin injection, the down-regulation of miR-124a in the spinal cord was enhanced. This resulted in exaggerated formalin-induced nociceptive behaviors associated with an up-regulation of the pain-relevant miRNA-124a target methyl CpG-binding protein 2 and proinflammatory marker genes in the spinal cord. In contrast, blocking formalin-induced down-regulation of spinal cord miR-124a through pre-miRNA-124a administration counteracted these effects and reduced nociception by down-regulating these target genes. miRNA-181a possesses multiple complementary binding sites for the γ-aminobutyric acid (GABA)A receptor subunit GABAAb1 gene, GABRA1, suggesting a possible target for this miRNA. A neonatally zymosan-induced increase in miR-181a resulted in down-regulation of the GABAAb1 mRNA and protein in the spinal cord. This effect may contribute to neonatal cystitis-induced chronic visceral pain. Identification of the target genes of miRNAs with specific changes in chronic pain may provide insight into the role of miRNAs in chronic pain development and maintenance.

The importance of miRNAs in pain is further validated in a study in which the activity of Dicer, a key enzyme in mature miRNA formation (fig. 1), is eliminated. Conditional knockout of Dicer in DRG Nav1.8 neurons resulted in not only the loss of all mature miRNAs but also the reduced pain-related transcripts including voltage sodium channel (Nav) 1.7, Nav1.8, and Ca2+/calmodulin-dependent protein kinase II in the primary sensory neurons. The conditional null mice failed to display inflammatory mediator-induced enhancement in excitability of Nav1.8 sensory neurons and formalin-induced c-FOS expression in spinal cord. These mice also exhibited significant inhibition of inflammatory pain after formalin, CFA, and carrageenan injection. In contrast, Dicer null mice displayed an intact acute nociceptive behavior in response to electrical, mechanical, and thermal stimuli, indicating that the loss of mature miRNAs in the nociceptors does not affect acute pain transmission to the spinal cord and brain. Therefore, miRNAs may be potential targets for the prevention and/or treatment of chronic inflammatory pain.

The functional role of miRNAs in neuropathic pain has also been observed (fig. 2). Although Dicer null mice exhibited intact SNL-induced pain hypersensitivity, the role of miRNAs in neuropathic pain cannot be ruled out as deletion of DRG Nav1.8 or most DRG nociceptors had no effect on neuropathic pain. Moreover, nerve injury–induced increases in abnormal ectopic discharges were found primarily in injured myelinated afferents and the corresponding large and medium DRG neurons. Thus, miRNAs expressed in large and medium DRG neurons may be involved in the production of abnormal spontaneous activity and neuropathic pain initiation. Indeed, miR-7a is expressed in small, medium, and large DRG neurons and robustly decreased in the injured DRG in the late phase of neuropathic pain. Blocking this decrease through

Fig. 2. Proposed model for the mechanism by which microRNAs (miRNAs) contribute to chronic inflammatory and neuropathic pain. After peripheral inflammation or nerve injury, the increase in inflammatory mediators such as interleukin (IL)-1β causes a change in the expression of miRNAs in dorsal root ganglion (DRG) neurons. This change includes up-regulation of some miRNAs (e.g., miR-7a) and down-regulation of other miRNAs (e.g., miR-21), resulting in an alteration in pain-related genes, such as an increase in β-subunit of voltage-gated sodium channels (Nav), in DRG. Such an alteration leads to an increase in DRG neuronal excitability, spinal central sensitization, and pain hypersensitivity (hyperalgesia and allodynia).
miR-7a overexpression in the injured DRG suppressed up-regulation of the β2 subunit protein of voltage-gated sodium channels in the DRG, normalized long-lasting hyperexcitability in nociceptive neurons, and attenuated established neuropathic pain without affecting acute pain and inflammatory pain. Furthermore, mimicking nerve injury–induced down-regulation of DRG miR-7a through intrathecal administration of a specific miR-7a inhibitor increased β2 subunit protein levels in the DRG and led to pain-related behaviors in intact rats. Another miRNA, miR-21, is persistently up-regulated in the injured DRG neurons during the late phase of neuropathic pain (table 2). The intrathecal administration of a miR-21 inhibitor (a single-stranded RNA with chemical modifications) alleviated nerve injury–induced mechanical and thermal hyperalgesia, may participate in neuropathic pain conditions by down-regulating multiple targets including negative regulators of matrix metalloproteinases (which exhibit increased activity after nerve injury), an endogenous inhibitor of phosphatidylinositol 3-kinase (that is decreased after nerve injury), and negative regulators of extracellular signal–regulated kinase. miRNAs may also be therapeutic targets for intractable chronic neuropathic pain.

Taken together, it is very likely that inflammatory mediators produced by peripheral inflammation or nerve injury act on peripheral nociceptors and then change the expression of DRG miRNAs. These changes may alter pain-related gene expression and lead to an increase in neuronal excitability in DRG, resulting in spinal cord central sensitization and pain hypersensitivity in response to peripheral stimulation (fig. 2).

Native Kcna2 AS RNA in Chronic Neuropathic Pain

Identification of Native Kcna2 AS RNA and Its Expression in DRG

Long ncRNAs include AS RNA, double-stranded RNA, and long RNA species. Unlike the study of miRNAs, the study of long ncRNAs is still in its infancy. Although long ncRNAs may be implicated in gene-regulatory roles such as chromosome dosage compensation, imprinting, epigenetic regulation, cell cycle control, nuclear and cytoplasmic trafficking, transcription, translation, splicing, and cell differentiation, most long ncRNAs remain uncharacterized and their biological significance underestimated. We recently identified a new native RNA that is 2.52 kb in size and contains no apparent open reading frame, indicating that it is a long ncRNA. We named it Kcna2 AS RNA because most of its sequence is complementary to the voltage-gated K+ channel Kcna2 RNA (also known as Kv1.2 RNA). This AS RNA seems to be transcribed from the opposing DNA strands of the Kcna2 RNA gene at the same genomic locus. Under normal conditions, Kcna2 AS RNA was expressed in pain-related areas, including DRG, from rats, although the signals were weak. It is also observed in DRGs from mouse, monkey, and human specimens. Using in situ hybridization histochemistry, we found that Kcna2 AS RNA was detected exclusively in DRG neurons. Approximately one fifth of neurons are labeled in the DRG of normal rats. Most are medium-sized although some are small and a few are large. Consistent with this subpopulation distribution pattern, the double-labeling observations showed that the majority of Kcna2 AS RNA–labeled neurons are positive for neurofilament-200 protein, a marker for myelinated A-fibers and large and medium DRG neurons. Some were positive for P2X3/isolectin B4, the markers for small DRG nonpeptidergic neurons, or for calcitonin gene–related peptide, a marker for small DRG peptidergic neurons. Compared with Kcna2 AS RNA, Kcna2 mRNA and protein are highly expressed in DRG. Approximately 70% of the DRG neurons were positive for Kcna2 protein. Most of these positive neurons were large in size. Double labeling of Kcna2 AS RNA with Kcna2 protein showed a tiny overlap between them. It seems that Kcna2 AS RNA and Kcna2 protein have opposing expression and distinct subpopulation distribution in normal DRG neurons.

Myeloid Zinc Finger Gene 1–Mediated Increase of Kcna2 AS RNA after Nerve Injury

The data from our laboratory and those of others revealed that peripheral nerve injury time-dependently down-regulated Kcna2 mRNA and protein in the injured DRG. In contrast, the level of Kcna2 AS RNA was time-dependently increased in the injured DRG after peripheral nerve injury (fig. 3). Such an increase occurred predominantly in large DRG neurons. No changes in the amount of Kcna2 AS RNA were observed in intact DRG, spinal cord, and other pain-related brain regions. Furthermore, using single-cell
quantitative reverse-transcription polymerase chain reaction, we demonstrated that the ratios of Kcn2 mRNA to Kcn2 AS RNA were decreased, particularly in individual medium and large DRG neurons after SNL (fig. 3).16 These results indicate that expression of Kcn2 AS RNA, like that of miRNAs, can be induced in the injured DRG after peripheral nerve injury (table 2).

Nerve injury–induced up-regulation of Kcn2 AS RNA is triggered by myeloid zinc finger gene 1 (MZF1), a transcription factor belonging to the family of zinc finger proteins. The Kcn2 AS gene promoter contains the consensus MZF1-binding motif. Once bound to this motif, MZF1 promotes transcription of target genes.61,62 We found that MZF1 binds to this motif on the Kcn2 AS gene promoter in the DRG,16 and SNL time-dependently increases MZF1 expression and its binding activity in the injured DRG.16 Moreover, MZF1 directly promotes Kcn2 AS gene transcription and is coexpressed with Kcn2 AS RNA in DRG neurons.16 It is very likely that nerve injury–induced up-regulation of DRG Kcn2 AS RNA occurs specifically in response to the increased MZF1. It is worth noting that the increase in Kcn2 AS RNA might be induced by other transcription factors and/or caused by increases in RNA stability and other epigenetic modifications. These possibilities will be addressed in future studies.

Kcn2 AS RNA Specifically and Selectively Targeted by Kcn2 AS RNA

Nerve injury–induced opposing changes in the expression of Kcn2 AS RNA and Kcn2 mRNA/protein in individual DRG neurons suggest that the increased Kcn2 AS RNA may be responsible for the decreased Kcn2 mRNA and protein under neuropathic pain conditions (fig. 3). Consistent with this speculation, overexpression of full-length Kcn2 AS RNA in cultured HEK-293T cells or in cultured DRG neurons markedly knocked down Kcn2 mRNA, but not Kcn1 mRNA, Kcn4 mRNA, Scn10a (Nav1.8), and their proteins.16 In vivo experiments, Kcn2 AS RNA overexpression time-dependently reduced Kcn2 mRNA in the DRG.16 No changes were observed in the expression of Kcn1, Kcn4, and Scn10a at the levels of mRNA and protein in the DRGs injected with AAV-Kcn2 AS RNA.16 These results suggest that nerve injury–induced DRG Kcn2 down-regulation is likely caused by a nerve injury–induced increase in DRG Kcn2 AS RNA (fig. 3). Kcn2 AS RNA functions as a biologically active regulator of Kcn2 mRNA and specifically and selectively targets Kcn2 in primary sensory neurons in neuropathic pain. This effect may be related to the extensive overlap of their complementary regions, including the transcription and translation initiation sites (fig. 3).16

DRG Kcn2 AS RNA as a Trigger in Neuropathic Pain Genesis

Although the detailed mechanisms by which nerve injury leads to neuropathic pain are still elusive, it is generally believed that neuropathic pain is induced by abnormal spontaneous activity that arises in neuromas and the medium and large DRG cell bodies.1,3 Voltage-dependent potassium channels (Kv) govern cell excitability. Application of Kv antagonists to sensory axons and to sites of ectopic afferent discharge facilitates ectopic firing.63-66 Injection of these antagonists into nerve-end neuromas provokes intense pain in humans.67 We found that selective reduction of Kcn2 expression in DRG by Kcn2 AS RNA decreased total Kv current, depolarized the resting membrane potential, decreased current threshold for activation of action potentials, increased the number of action potentials in large and medium DRG neurons, and produced neuropathic pain symptoms.16 Rescuing nerve injury–induced down-regulation of DRG Kcn2 by blocking nerve injury–induced up-regulation of DRG Kcn2 AS RNA attenuated induction and maintenance of nerve injury–induced mechanical, cold, and heat pain hypersensitivities.16

Given that nociceptive neurotransmitters and/or modulators (substance P and calcitonin gene–related peptide) in the injured myelinated fibers and in large and medium DRG neurons are dramatically increased at the early stage after nerve injury,68,69 it is conceivable that peripheral nerve injury up-regulates the expression of native Kcn2 AS RNA through activation of the MZF1 transcription factor in the injured DRG. This up-regulation silences the expression of DRG Kcn2 mRNA and protein, resulting in a decrease of total Kv current and an increase of ectopic discharge in large and medium DRG neurons. Ectopic discharge triggers the release of nociceptive transmitters and/or modulators in primary afferent terminals, leading to central sensitization in the dorsal horn and major symptoms of neuropathic pain (fig. 4). Thus, Kcn2 AS RNA may be an endogenous trigger in neuropathic pain development and maintenance. Kcn2 AS RNA may be a potential target for the prevention and/or treatment of neuropathic pain.

Conclusion

The lines of evidence described above indicate that ncRNAs including miRNAs and Kcn2 AS RNA in peripheral and central nervous systems are endogenous instigators of chronic pain. The lines of evidence described above indicate that ncRNAs including miRNAs and Kcn2 AS RNA in peripheral and central nervous systems are endogenous instigators of chronic pain.

Fig. 4. Proposed model for the mechanism of how Kcn2 AS RNA is involved in neuropathic pain. Nerve injury leads to an increase in myeloid zinc finger gene 1 (MZF1), a transcription factor that enhances the transcription of Kcn2 AS RNA, in dorsal root ganglion (DRG). The Kcan2 AS RNA silences expression of the Kcn2 messenger RNA (mRNA) and protein. The reduced Kcn2 protein expression at DRG neuronal membrane results in reduced Kv current (Kv), increases number of action potentials (AP) and neuronal excitability in DRG neurons, and produces spinal cord central sensitization and neuropathic pain symptoms (hyperalgasia and allodynia).
pain. miRNAs have been extensively studied in the past decade and may be used as prognostic and diagnostic biomarkers and potential new drug targets for chronic inflammatory pain and neuropathic pain; however, miRNAs have multiple and specific downstream targets due to their small size. This characterization may result in the limited use of miRNAs in chronic pain treatment because they might interfere with other physiological functions and produce potential side effects. Compared with the previous reviews on miRNAs in pain processing, this review updates current knowledge on miRNAs in chronic pain. More importantly, this review summarizes the latest finding on a long ncRNA Kcna2 AS RNA in chronic pain, which has not been discussed in previous reviews. Although the studies on long ncRNAs are still at the early stage, accumulating evidence indicates that they specifically and selectively target their corresponding gene’s expression. As peripheral inflammation and nerve injury alter the expression of many other genes in addition to Kcna2 in pain-related regions, it is very likely that those genes, like Kcna2, are regulated by a corresponding long ncRNAs. Significant regulations of long ncRNA transcription may be a general cellular response to peripheral inflammation and nerve injury and participate in the induction and maintenance of chronic pain. Given that long ncRNAs have the characterization of specifically and selectively targeting the corresponding genes, it is conceivable that the significance of long ncRNAs in chronic pain will become even more apparent in the coming years.

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Competing Interests
The authors declare no competing interests.

Correspondence
Address correspondence to Dr. Tao: Department of Anesthesiology, New Jersey Medical School, Rutgers, The State University of New Jersey, 185 South Orange Avenue, MSB, F-548, Newark, New Jersey 07103, yuanxiang.tao@njms.rutgers.edu. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. Anesthesiology’s articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

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Anesthesiology Reflections from the Wood Library-Museum

Paul Meyer Wood and His Collectibles: Packing and Moving

During World War I, the U.S. Army Ambulance Corps drilled (above) volunteers like young Paul Meyer Wood, through many lessons, including how to pack and move “valuables.” Those lessons would assist him later in life as Dr. Wood shifted anesthesia antiques in his Wood Library-Museum (WLM) from downtown New York City out to Foregger’s boat house in Long Island and upstate to Mrs. Wood’s “Meyer Family Home” in Highland Falls. Dr. Wood never lived to see his namesake museum open formally in Park Ridge, Illinois, on Busse Highway (1963) or to see the WLM’s move within the same town to North Northwest Highway (1992). So, what might the ever-patient Dr. Wood, the master of packing and moving anesthesia antiques, have commented about the most recent move of the WLM and its “mother ship” American Society of Anesthesiologists to Schaumburg, Illinois? Why, “Forward, march!” (Copyright © the American Society of Anesthesiologists, Inc.)

George S. Bause, M.D., M.P.H., Honorary Curator, ASA’s Wood Library-Museum of Anesthesiology, Schaumburg, Illinois, and Clinical Associate Professor, Case Western Reserve University, Cleveland, Ohio. UUYO@aol.com.