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Noncoding RNAs: New players in chronic pain

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Abstract

Chronic pain is a common clinical symptom. Current treatments for this disorder are often inadequate or ineffective due to our deficient understanding of molecular mechanisms that trigger the initiation and maintenance of chronic pain. The changes in gene expression in primary sensory neurons of the dorsal root ganglion (DRG) following peripheral inflammation and nerve injury may contribute to chronic pain genesis. Newly identified noncoding RNAs play a critical role in the mechanism for gene regulation. Recent studies have shown that peripheral noxious stimuli drive expressional changes in noncoding RNAs and that these changes are associated with pain hypersensitivity under chronic pain conditions. This review first presents current evidence for the peripheral inflammation/nerve injury-induced change in expression of two types of noncoding RNAs, miRNAs and Kcna2 antisense RNA, in pain-related regions, particularly in the DRG, after peripheral inflammation and nerve injury. We then discuss how peripheral noxious stimuli induce such changes. We finally discuss potential mechanisms of how expressional changes in DRG miRNAs and Kcna2 AS RNA contribute to the development and maintenance of chronic pain. Understanding of these mechanisms may allow the development of novel therapeutic strategies for preventing and/or treating chronic pain.

Keywords

Noncoding RNA; miRNAs; Kcna2 antisense RNA; Dorsal root ganglion; Inflammatory pain; Neuropathic pain; Chronic pain

Chronic 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

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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 non-coding (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, which take all the credit for the structural and functional roles in cells. However, it has become increasingly clear that mammalian genomes encode not only protein-coding RNAs but also a vast number of ncRNA transcripts⁷. Since 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); 18–200 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 pain-related regions¹⁰. 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.

Here, we first review current evidence for the changes of two types of ncRNAs, miRNAs and *Kcna2* AS RNA, in pain-related regions, particularly in DRG, following peripheral inflammation and nerve injury. We then discuss how peripheral noxious stimuli induce such changes. We finally discuss potential mechanisms of how expressional changes in DRG miRNAs and *Kcna2* AS RNA contribute to the development and maintenance of chronic pain.

1. miRNAs in chronic pain

1a. 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^{17–19}. These miRNAs are coded by specific genes. Generally, a miRNA molecule is synthesized from a long RNA primary transcript known as the pri-miRNA (Fig. 1). In the cellular nucleus, the pri-miRNA is cleaved by Drosha, an RNAPIII endonuclease, to produce a characteristic stem-loop structure of about 60–70 nucleotides in length, known as a pre-miRNA (Fig. 1). After the pre-miRNA

is exported from the nucleus into the cytoplasm, it is cleaved by Dicer, another RNase III 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)²⁰. The single strands completely or incompletely bind to specific mRNA sequences, resulting in degradation or translational repression of the target mRNAs²¹.

1b. Expressional changes of miRNAs after noxious stimulation

The expression changes of miRNAs in response to noxious stimulation have been reported. Dr. Bai et al¹³ first stated that complete Freund's adjuvant (CFA)-induced peripheral inflammation in the rat masseter muscle significantly, but differentially, downregulated expression of mature miR-10a, -29a, -98, -99a, -124a, -134, and -183 in the ipsilateral mandibular division of the trigeminal ganglion (TG) within 4 h after CFA injection. Such downregulated miRNAs recovered differentially to a normal level or higher than normal level¹³. Expression and downregulation of miRNAs occurred in all sizes of TG neurons (but not in glial cells and other nonneuronal cells) that innervate the inflamed muscle, although the miRNA signals varied among neurons¹³. Injection of CFA into a hindpaw also reduced expression levels of miR-1, -16, -206, and -143 in the ipsilateral DRG neurons^{22;23}, but increased miR-1, -16, and -206 in the ipsilateral spinal dorsal horn neurons²². Interestingly, peripheral injection of formalin led to significant downregulation of miRNA-124a expression in the neurons of dorsal horn ipsilateral to injection²⁴. These studies provide promising evidence of miRNA changes in pain-related regions under inflammatory pain conditions.

In addition to peripheral inflammation, expressional changes of miRNAs were observed following 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^{14;22;25;26} and in the expression of miR-200b and -429 in the nucleus accumbens²⁷. L5 SNL also downregulated the expression of 59 miRNAs in the uninjured L4 DRG²⁸. Consistently, in the sciatic nerve transection or chronic constriction injury (CCI) model of neuropathic pain, the injured DRG showed reduced expression of several miRNAs, including miR-10a, -30b, -99a, -100, -143, -582-3p, and -720^{23;29}. In contrast, miR-21 in the injured DRG was upregulated following L5 SNL^{14;15}. The evidence indicates that the expression of miRNAs is differentially and spatially regulated in pain-related regions after peripheral inflammation and nerve injury.

Furthermore, expressional changes of miRNAs have also been observed in patients with painful diseases. In the biopsies of bladder pain syndrome, 31 miRNAs were differentially expressed³⁰. An inverse relationship was observed in which neurokinin1 mRNA/protein was downregulated and four miRNAs (miR-449b, -500, -328, and -320) were upregulated³⁰. Differential expression of 18 miRNAs was reported in blood from patients with complex regional pain syndrome³¹. In addition, miR-146a, -199a, and -558 may be linked to pain-related pathophysiology of osteoarthritis through regulation of the expression of cyclooxygenase-2^{32–34}. It appears that miRNA profiles have the potential to serve as biomarkers of pain.

1c. miRNAs regulated by inflammatory mediators in chronic pain

How peripheral noxious stimulation causes the alternations of miRNA expression in pain-related regions is unclear, 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 inflammation³⁵, suggesting at least partial involvement of inflammatory mediators in inflammation-induced changes in miRNA expression. A recent study revealed that stimulation with interleukin-1 β (IL-1 β), an inflammatory mediator, produced a significant reduction in miR-558 in normal and osteoarthritis chondrocytes possibly through IL-1 β -induced activation of MAP kinase and nuclear factor- κ B³⁴. IL-1 β also increased the expression of miR-21 in DRG neurons¹⁵, MING cells³⁶, and human pancreatic islets³⁶. AP-1, a transcription factor, may participate in this effect of IL-1 β as the promoter region of miR-21 contains the binding site of AP-1³⁷, and IL-1 β triggers AP-1 activation in DRG neurons¹⁵. Given that peripheral inflammation and nerve injury increase DRG IL-1 β expression, IL-1 β may be responsible for inflammation-induced downregulation of miR-558 and nerve injury-induced upregulation of miR-21 in the injured DRG (Fig. 2)^{15;34}.

1d. Potential mechanisms of miRNAs' effects in 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)²¹. Studies on inflammatory pain suggest that miRNAs specifically target pain-related genes (Fig. 2). When a miRNA-124a inhibitor was intravenously administered following formalin injection, the downregulation of miR-124a in the spinal cord was enhanced. This resulted in exaggerated formalin-induced nociceptive behaviors associated with an upregulation of the pain-relevant miRNA-124a target MeCP2 and proinflammatory marker genes in the spinal cord²⁴. In contrast, blocking formalin-induced downregulation of spinal cord miRNA-124a through pre-miRNA-124a administration counteracted these effects and reduced nociception by downregulating these target genes²⁴. miRNA-181a possesses multiple complementary binding sites for the GABA_A receptor subunit GABA_{A α -1} gene, suggesting a possible target for this miRNA. A neonatally zymosan-induced increase in miR-181a resulted in downregulation of the GABA_{A α -1} mRNA and protein in the spinal cord³⁸. This effect may contribute to neonatal cystitis-induced chronic visceral pain³⁸. 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 eliminated²¹. Conditional knockout of Dicer in DRG Nav1.8 neurons not only resulted in the loss of all mature miRNAs but also reduced pain-related transcripts including Nav1.7, Nav1.8, and CaMKII α in the primary sensory neurons³⁹. 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 cord³⁹. These mice also exhibited significant inhibition of inflammatory pain following formalin, CFA, and carrageenan injection³⁹. In contrast,

Dicer null mice displayed intact acute nociceptive behavior in response to electrical, mechanical, and thermal stimuli³⁹, 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 hypersensitivity³⁹, 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^{40–42}. Moreover, nerve injury-induced increases in abnormal ectopic discharges were found primarily in injured myelinated afferents and the corresponding large and medium DRG neurons^{43;44}. 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 pain¹⁴. Blocking this decrease through miR-7a overexpression in the injured DRG suppressed upregulation of the $\beta 2$ 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 pain¹⁴. Furthermore, mimicking nerve injury-induced downregulation of DRG miR-7a through intrathecal administration of a specific miR-7a inhibitor increased $\beta 2$ subunit protein levels in the DRG and led to pain-related behaviors in intact rats¹⁴. Another miRNA, miR-21, is persistently upregulated in the injured DRG neurons during the late phase of neuropathic pain¹⁵. Intrathecal miR-21 inhibitor alleviated nerve injury-induced mechanical and thermal hyperalgesia¹⁵. miR-21 may participate in neuropathic pain conditions by downregulating multiple targets including negative regulators of matrix metalloproteinases (which exhibit increased activity following nerve injury)⁴⁵, an endogenous inhibitor of phosphatidylinositol 3-kinase (that is decreased after nerve injury)⁴⁶, and negative regulators of extracellular signal-regulated kinase⁴⁷. 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).

2. Native Kcna2 AS RNA in chronic neuropathic pain

2a. Identification of native Kcna2 AS RNA and its expression in DRG

Long ncRNAs include AS RNA, double-stranded RNA, and long RNA species. Unlike 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^{7;48;49}, most long ncRNAs remain uncharacterized and their biological significance underestimated^{7;49;50}. We recently

identified a new native RNA that is 2.52kb in size and contains no apparent open reading frame¹⁶, 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 appears 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 specimens¹⁶. 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 large¹⁶. 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 non-peptidergic neurons, or for calcitonin gene-related peptide, a marker for small DRG peptidergic neurons. Compared to Kcna2 AS RNA, Kcna2 mRNA and protein are highly expressed in DRG. Approximately 70% of the DRG neurons were positive for Kcna2 protein^{16;51}. Most of these positive neurons were large in size^{16;51}. Double labeling of Kcna2 AS RNA with Kcna2 protein showed a tiny overlap between them¹⁶. It appears that Kcna2 AS RNA and Kcna2 protein have opposing expression and distinct subpopulation distribution in normal DRG neurons.

2b. MZF1-mediated increase of Kcna2 AS RNA after nerve injury

The data from our laboratory¹⁶ and those of others^{51–56} revealed that peripheral nerve injury time-dependently downregulated Kcna2 mRNA and protein in the injured DRG. In contrast, the level of Kcna2 AS RNA was time-dependently increased in the injured DRG following peripheral nerve injury (Fig. 3)¹⁶. 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 RT-PCR, we demonstrated that the ratios of Kcna2 mRNA to Kcna2 AS RNA were decreased, particularly in individual medium and large DRG neurons after SNL (Fig. 3)¹⁶. These results indicate that expression of Kcna2 AS RNA, like that of miRNAs, can be induced in the injured DRG following peripheral nerve injury.

Nerve injury-induced upregulation of Kcna2 AS RNA is triggered by myeloid zinc finger gene 1 (MZF1), a transcription factor belonging to the family of zinc finger proteins. The Kcna2 AS gene promoter contains the consensus MZF1-binding motif. Once bound to this motif, MZF1 promotes transcription of target genes^{57;58}. We found that MZF1 binds to this motif on the Kcna2 AS gene promoter in the DRG¹⁶, and SNL time-dependently increases MZF1 expression and its binding activity in the injured DRG¹⁶. Moreover, MZF1 directly promotes Kcna2 AS gene transcription and is co-expressed with Kcna2 AS RNA in DRG neurons¹⁶. It is very likely that nerve injury-induced upregulation of DRG Kcna2 AS RNA occurs specifically in response to the increased MZF1. It is worth noting that the increase in Kcna2 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.

2c. Kcna2 RNA specifically and selectively targeted by Kcna2 AS RNA

Nerve injury-induced opposing changes in expression of Kcna2 AS RNA and Kcna2 mRNA/protein in individual DRG neurons suggest that the increased Kcna2 AS RNA may be responsible for the decreased Kcna2 mRNA and protein under neuropathic pain conditions (Fig. 3). Consistent with this speculation, overexpression of full-length Kcna2 AS RNA in cultured HEK-293T cells or in cultured DRG neurons markedly knocked down Kcna2 mRNA, but not Kcna1 mRNA, Kcna4 mRNA, Scn10a, and their proteins¹⁶. In *in vivo* experiments, Kcna2 AS RNA overexpression time-dependently reduced Kcna2 mRNA in the DRG¹⁶. No changes were observed in the expression of Kcna1, Kcna4 and Scn10a at the levels of mRNA and protein in the DRGs injected with AAV-Kcna2 AS RNA¹⁶. These results suggest that nerve injury-induced DRG Kcna2 downregulation is likely caused by a nerve injury-induced increase in DRG Kcna2 AS RNA (Fig. 3). Kcna2 AS RNA functions as a biologically active regulator of Kcna2 mRNA and specifically and selectively targets Kcna2 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.

2d. DRG Kcna2 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 bodies¹⁻³. Voltage-dependent potassium channels (Kv) govern cell excitability. Application of Kv antagonists to sensory axons and to sites of ectopic afferent discharge facilitates ectopic firing⁵⁹⁻⁶². Injection of these antagonists into nerve-end neuromas provokes intense pain in humans⁶³. We found that selective reduction of Kcna2 expression in DRG by Kcna2 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 symptoms¹⁶. Rescuing nerve injury-induced downregulation of DRG Kcna2 by blocking nerve injury-induced upregulation of DRG Kcna2 AS RNA attenuated induction and maintenance of nerve injury-induced mechanical, cold, and thermal pain hypersensitivities¹⁶.

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^{64;65}, it is conceivable that peripheral nerve injury upregulates the expression of native Kcna2 AS RNA through activation of the MZF1 transcription factor in the injured DRG. This up-regulation silences the expression of DRG Kcna2 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, Kcna2 AS RNA may be an endogenous trigger in

neuropathic pain development and maintenance. Kcna2 AS RNA may be a potential target for the prevention and/or treatment of neuropathic pain.

3. Conclusion

The lines of evidence described above indicate that both miRNAs and Kcna2 AS RNA are endogenous instigators of chronic pain in peripheral and central nervous systems. miRNAs have been extensively studied recently and may be used as 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. Kcna2 AS RNA identified recently is the only long ncRNA to be involved in chronic pain¹⁶. As peripheral inflammation and nerve injury alters the expression of many other genes in addition to Kcna2 in pain-related regions^{1–3}, it is unknown whether they, like Kcna2, are regulated by AS RNAs or whether broad upregulation of AS transcription can be a general cellular response to peripheral inflammation and nerve injury. Given that Kcna2 AS RNA, unlike miRNAs, specifically and selectively targets the corresponding Kcna2, it is conceivable that the significance of long ncRNAs including AS RNAs in chronic pain will become even more apparent in the coming years.

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Summary Statement: This review highlights recent findings regarding the changes in noncoding RNAs in pain-related regions after persistent inflammation and nerve injury, and discusses how noncoding RNAs participate in the development and maintenance of chronic pain.

Fig 1.

Formation of mature miRNA. microRNA (miRNA) is transcribed from the genome (DNA) via RNA polymerase II (Pol II). The resulting pri-miRNA transcript is then cleaved via the endonuclease Drosha to create a 60–70 nucleotide long pre-miRNA. This transcript is then removed from the nucleus via exportin-5 to the cytoplasm where it is cleaved by Dicer, another endonuclease. The resulting double-stranded mature miRNA is unwound by a helicase or cleaved by Ago2. The single-stranded mature miRNA then acts as the core of RISC (RNA-induced silencing complex). This complex guides the miRNA to its target sequence located within the 3' untranslated region (3'UTR) of the target messenger RNA (mRNA). Incomplete or complete base-pairing results in degradation of the mRNA or inhibition of translation.

Fig 2.

Proposed model for the mechanism by which miRNAs contributes to chronic inflammatory and neuropathic pain. After peripheral inflammation or nerve injury, the increase in inflammatory mediators such as interleukin-1 β (IL-1 β) causes a change in the expression of microRNAs (miRNAs) in dorsal root ganglion (DRG) neurons. This change includes upregulation of some miRNAs (e.g., miR-7a) and downregulation 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).

Fig 3.

Kcna2 antisense (AS) RNA upregulation specifically and selectively targets Kcna2 expression in neuropathic pain. **A:** Under normal conditions, due to highly low expression of Kcna2 AS RNA, Kcna2 messenger RNA (mRNA) that is transcribed from the genome is translated into protein, resulting in expression of the Kcna2 channel at the cell membrane. **B:** Under neuropathic pain conditions, peripheral nerve injury promotes the expression of Kcna2 AS RNA that is transcribed from the opposing strand of the Kcna2 gene. Increased expression of Kcna2 AS RNA specifically and selectively inhibits expression of Kcna2 mRNA via extensive overlap of their complementary regions, including the transcription and translation inhibition sites, leading to reduced expression levels of the membrane Kcna2 channel only, not other Kcna family members (e.g. Kcna1).

Fig 4.

Proposed model for the mechanism of how Kcna2 AS RNA is involved in neuropathic pain. Nerve injury leads to an increase in MZF1, a transcription factor that enhances the transcription of Kcna2 AS RNA, in dorsal root ganglion (DRG). The Kcna2 AS RNA silences expression of the Kcna2 messenger RNA (mRNA) and protein. The reduced Kcna2 protein expression at DRG neuronal membrane results in reduced K^+ current (Kv), increases number of action potentials (AP) and neuronal excitability in DRG neurons, and produces spinal cord central sensitization and neuropathic pain symptoms (hyperalgesia and allodynia).

microRNAs associated with peripheral inflammation

Table 1

<i>Inflammatory models</i>	miRNAs	Change in Expression	Tissue	Target Gene	Reference
CFA into Masseter muscle	miR-29a, -98, -99a, -124a, -134, -183.	↓	Rat ipsilateral trigeminal ganglion	Unknown	G. Bai et al., 2007 ¹³
CFA into Hindpaw	miR-1, -16, 206	↓	Rat ipsilateral DRG neurons	Unknown	R. Kusuda et al., 2011 ²⁵
	miR-1, -16, 206	↑	Rat ipsilateral spinal dorsal horn neurons	Unknown	
	miR-143	↓	Murine ipsilateral DRG neurons	Unknown	S. Tam Tam, et al., 2011 ²⁶
Formalin Injection	miR-124a	↓	Murine ipsilateral dorsal horn neurons	MeCP2; Proinflammatory marker genes	KL Kynast, et al., 2013 ¹⁸
Bladder pain syndrome	miR-449b, -500, -328, -320; 31 miRNAs	↑ ↑/↓	Human Bladder Biopsies	Neurokinin 1 Unknown	F. Sanchez, et al., 2010 ³³
Complex Regional Pain Syndrome	18 different miRNAs	↓/↑	Human Blood	Unknown	IA.Orlova, et al., 2011 ³⁴
Osteoarthritis	miR-199a, -558	↓	Human chondrocytes	Cyclooxygenase-2	N. Akhtar, et al., 2012 ³⁵ ; SJ. Park, et al., 2013 ³⁷
	miR-146a	↑	Human Synoviocytes		X. Li, et al., 2011 ³⁶
Peritonitis model of self-limiting acute inflammation	miR-21, -146b, 208a	↑		Unknown	
	miR-302d	↓		Unknown	
	miR-142-3p	↓		IL-6, IL-23A, TGFBRI	
	miR-142-5p	↓	Murine exudates	c-Fos, ATF2, SRF, CREB	A. Recchiuti, et al., 2011 ³⁸
	miR-203	↑		STAT5, SOCS3, JAK1	
	miR-219	↓		TNF-α, TNF-αR, IL-1, IL-1R accessory protein	

Abbreviations: ATF2: Activating Transcription Factor 2; CCI: Chronic Constriction Injury; CFA: Complete Freund's Adjuvant; CREB: cAMP Response-Element Binding Protein; DRG: Dorsal Root Ganglia.; 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; TGFBRI: transforming growth factor Beta Receptor 1; TNF: Tumor Necrosis Factor.

Table 2

Noncoding RNAs associated with Peripheral Nerve Injury

Neuropathic pain models	ncRNAs	Change in Expression	Tissue	Target Gene	Reference
L5 Spinal Nerve Ligation	miR-1,-7a,-96,-103,-182,-183,-206	↓	injured DRG of Mice	For miR-7a, β2 subunit of voltage-gated sodium channels is a potential target	A. Sakai et al., 2013 ¹⁴ ; R. Kusuda et al., 2011 ²⁵ ; A. Favereaux et al., 2011 ²⁸ ; B.T. Aldrich et al., 2009 ²⁹ .
	miR-200b,-429	↓	nucleus accumbens of Mice	Unknown	S. Imai et al., 2011 ³⁰
	miR-21	↑	injured DRG of mice	Matrix metalloproteinases; endogenous inhibitors of phosphatidylinositol 3-kinase; negative regulators of extracellular signal-regulated kinase	A. Sakai et al., Brain 2013 ¹⁴ ; A. Sakai and H. Suzuki, 2013 ¹⁵ .
	59 miRNAs	↓	uninjured L4 DRG in Mice	Unknown	S.D. Von et al., 2011 ³¹ .
	Kcna2 AS RNA	↑	injured DRG of mice	Kcna2	X. Zhao et al., 2013 ¹⁶ .
Sciatic Nerve Transection/CCI	miR-10a,-30b,-99a,-100,-143,-582-3p,-720	↓	injured DRG of Rat	Unknown	T.S. Tam et al., 2011 ²⁶ ; T. Brandenburger et al., 2012 ³² .

Abbreviations: CCI: Chronic Constriction Injury; DRG: Dorsal Root Ganglion; miR: microRNA; ncRNAs: noncoding RNAs.