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Epigenetic Mechanisms of Neural Plasticity in Chronic Neuropathic Pain

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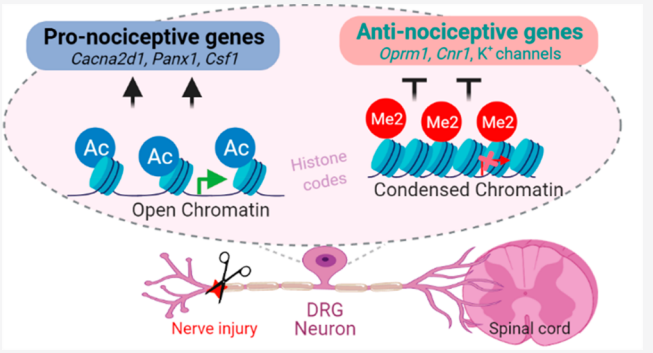
ABSTRACT: Neuropathic pain is a challenging clinical problem and remains difficult to treat. Altered gene expression in peripheral sensory nerves and neurons due to nerve injury is well documented and contributes critically to the synaptic plasticity in the spinal cord and the initiation and maintenance of chronic pain. However, our understanding of the epigenetic mechanisms regulating the transcription of pro-nociceptive (e.g., NMDA receptors and $\alpha 2\delta$-1) and antinociceptive (e.g., potassium channels and opioid and cannabinoid receptors) genes are still limited. In this review, we summarize recent studies determining the roles of histone modifications (including methylation, acetylation, and ubiquitination), DNA methylation, and noncoding RNAs in neuropathic pain development. We review the epigenetic writer, reader, and eraser proteins that participate in the transcriptional control of the expression of key ion channels and neurotransmitter receptors in the dorsal root ganglion after traumatic nerve injury, which is commonly used as a preclinical model of neuropathic pain. A better understanding of epigenetic reprogramming involved in the transition from acute to chronic pain could lead to the development of new treatments for neuropathic pain.



KEYWORDS: *Chromatin, DRG neuron, epigenetics, G9a, NMDA receptor, neuron-restrictive silencer factor, nociceptor, potassium channel, spinal cord*

1. INTRODUCTION

Neuropathic pain, caused by injury or damage to the somatic nervous system, often lasts for many months or even years and remains a major therapeutic challenge. Common symptoms include increased pain perception to a generally innocuous stimulus (allodynia), enhanced pain perception to noxious stimuli (hyperalgesia), and spontaneous pain. The common causes of neuropathic pain include diabetic neuropathy, spinal cord injury, viral infection (e.g., postherpetic neuralgia), and chemotherapy. This debilitating condition is highly prevalent in patients after nerve injury associated with trauma, limb amputation, mastectomy, and thoracotomy.[1,2] Similarly, neuropathic pain is a major adverse effect of several cancer chemotherapeutic agents, including paclitaxel.[3,4] The persistent pain caused by chemotherapy often leads to dose reduction or discontinuation of what is otherwise life-saving treatment. Because neuropathic pain responds poorly to conventional analgesics, identifying its molecular determinants is essential for developing new mechanism-based therapies.

The neuropathic pain state persists long after the initial injury is resolved. Thus, it has long been suspected that epigenetic mechanisms leading to altered gene expression must be involved. The reversible and dynamic changes in epigenetic modifications are pivotal in embryonic neurogenesis, cognition, synaptic plasticity, and neurodevelopmental and neurodegenerative diseases.[5,6] Nucleosomes are the basic units of chromatin where histones and DNA closely interact. Chromatin remodeling is crucial for regulating the physical access of transcription factors to DNA wrapped around the histone proteins.[7] Histone tails are positively charged domains that bind to negatively charged DNA. Histone modifications, including methylation, acetylation, ubiquitination, and phosphorylation, affect the chromatin structure and often occur in many combinations, altering gene transcription in a coordinated fashion. Neuropathic pain is associated with increased expression of pro-nociceptive genes and decreased expression

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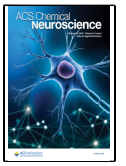

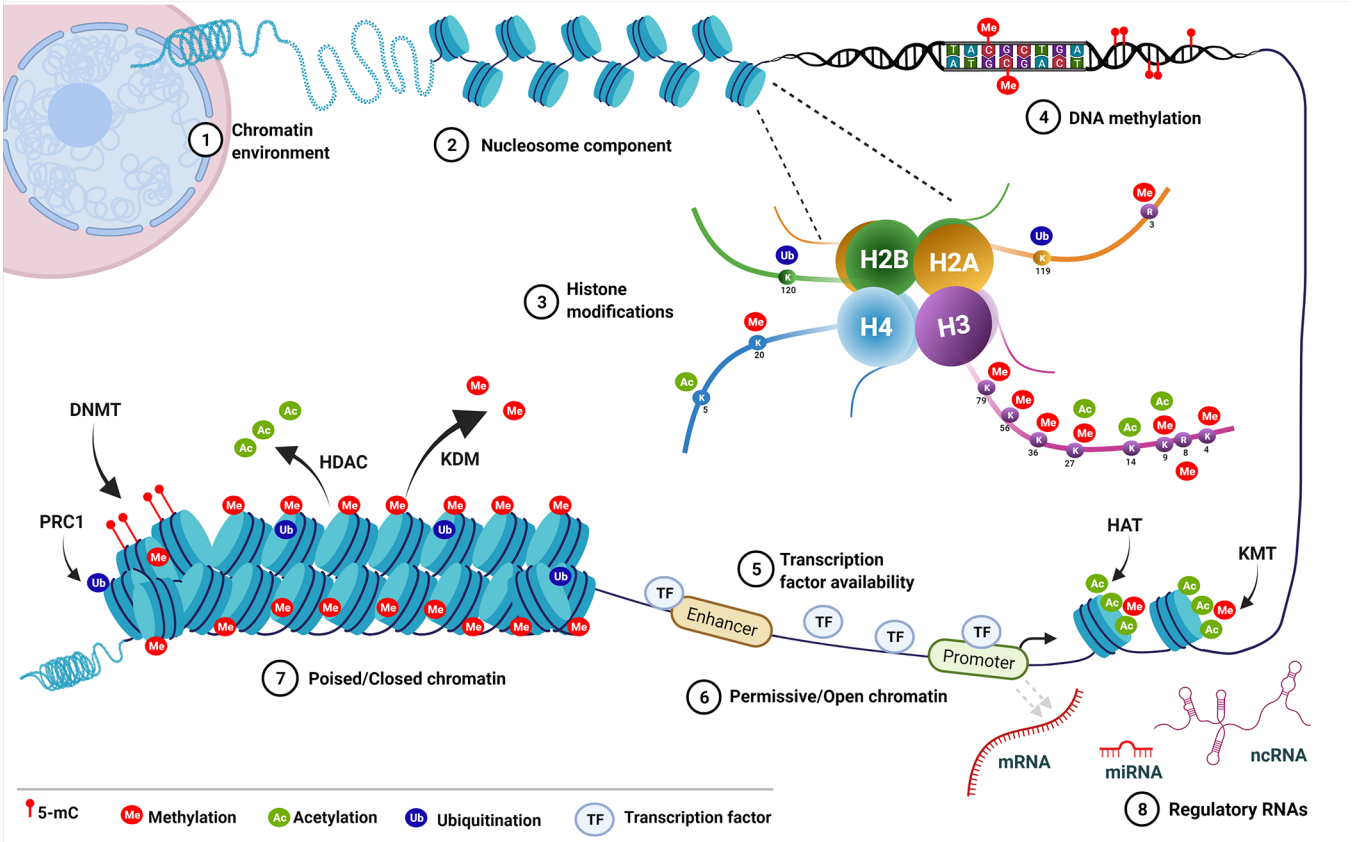

Figure 1. Schematic representation of epigenetic modifications. (1−3) Several amino acids on the N-terminal tails of core histone proteins undergo methylation, acetylation, and ubiquitination within the nucleus. K and R indicate the lysine and arginine residues, respectively. (4) The cytosine (C) base of the CpG dinucleotides of DNA undergoes methylation. (5, 6) The characteristic activating histone marks catalyzed by the histone acetyltransferase (HAT) and lysine methyltransferase (KMT) create a locally permissive open chromatin at the regulatory regions, allowing transcription factors to actively transcribe these genes. (7) Removal of acetylation marks by histone deacetylases (HDACs) or certain methylation marks by lysine demethylases (KDMs), together with the addition of ubiquitination marks on histone by polycomb repressive complex 1 (PRC1) or 5-mC methylation on DNA by DNA methyltransferases (DNMTs), results in condensed or closed chromatin that usually represses gene expression. (8) The noncoding RNAs (ncRNAs) and microRNA (miRNA) constitute a regulatory transcriptome network that determines the fate of the mRNA.

of antinociceptive genes, which play a critical role in sustained excitation of nociceptive neurons. Because most preclinical studies use traumatic injury of peripheral nerves as neuropathic pain models, the dorsal root ganglion (DRG), which contains the soma of primary sensory neurons, is used in the vast majority of epigenetic studies in the field. In this review, we mainly summarize recent studies on the epigenetic changes in the DRG in the development of neuropathic pain.

2. OVERVIEW OF EPIGENETIC COMPONENTS AND REGULATORS

Epigenetics, defined as changes in gene expression without a change in DNA sequence, plays a pivotal role in tissue- and cell type-specific gene expression via diverse molecular modifications to both DNA and chromatin. Epigenetic control of gene transcription predominantly includes alterations in DNA methylation and post-translational histone modifications, which are essential for long-lasting changes in gene expression even in mature neurons (Figure 1). In eukaryotes, DNA is greatly compressed and assembled into nucleosomes with histones. Nucleosomes are composed of 146 base pairs (bp) wrapped 1.7 times around an octamer of four core histones: H2A, H2B, H3, and H4. The protruding N-terminal tail of histones contains a high proportion of basic amino acids lysine and arginine, and their post-translational modifications are associated with chromatin remodeling and transcriptional control.[8,9] Various chromatin accessing proteins, including transcription factors and chromatin remodelers (polycomb and trithorax proteins), crosstalk with epigenetic modifiers to regulate gene transcription within neurons and glial cells in the nervous system.[10−12]

The lysine methyltransferases (KMTs) and protein arginine methyltransferases (PRMTs) are responsible for adding mono- (me1), di- (me2), or trimethylation (me3) marks on lysine and mono- and dimethylation marks on arginine residues, respectively, while the lysine demethylases (KDMs) remove them.[13−15] The histone and lysine acetyltransferases (HATs or KATs) add an acetyl group to the epsilon amino group of lysine residues (Figure 1). Unlike methylation, which does not alter the positive charge of the lysine side chain, acetylation neutralizes it. It causes steric bulkiness, leading to loosening of the histone−histone and DNA−histone interactions and subsequent DNA unpacking to allow chromatin access by transcription factors.[7,8] Acetylation also introduces "reader" proteins with bromodomains, such as the bromo and extra terminal (BET) proteins.[16] The acetylation marks are removed by histone or lysine deacetylases (HDACs or KDACs) that

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restore the positive charge of lysine residues, causing chromatin condensation and gene repression.[17,18]

In general, DNA methylation is a stable epigenetic mark, and DNA methylation patterns can be retained as a form of epigenetic memory.[19] Methylation of cytosine bases on DNA (particularly at CpG dinucleotides) is catalyzed by DNA methyltransferases (DNMTs). DNA cytosine methylation is actively reversed by ten-eleven translocase (TET) methylcytosine dioxygenases that sequentially remove the CpG 5-mC mark, resulting in demethylation and restoration to the unmodified cytosines.[20] In addition, noncoding RNAs (ncRNAs) participate in epigenetic regulation of gene expression.[21] Many writers, readers, and erasers of epigenetic modulations (Figure 1) are essential in faithful maintenance of the genetic information encoded in the DNA sequence for cell division, differentiation, development, and phenotypic identity.

3. ROLE OF HISTONE MODIFICATIONS IN NEUROPATHIC PAIN

3.1. Histone Methylation. *3.1.1. H3K9 Methylation.* Histone lysine methylation can cause repression or activation of gene transcription. For example, methylation at H3K4, H3K36, and H3K79 often leads to transcriptional activation, whereas H3K9, H3K27, and H4K20 methylation are repressive histone marks.[8,13] The heteromeric complexes of G9a (KMT1C or EHMT2) and GLP (G9a-like protein, KMT1D or EHMT1) methyltransferases catalyze the formation of H3K9me1 and H3K9me2 typically at the euchromatin region involved in transcriptional silencing.[22,23] In contrast, SUV39H1 (KMT1A) and SUV39H2 (KMT1B) catalyze H3K9me2 and H3K9me3 formation at the constitutive heterochromatin regions.[14]

A sustained increase in the excitability of primary afferent nerves is a hallmark of chronic neuropathic pain. A prominent feature of nerve injury-induced neuropathic pain is the sustained reduction in the expression of potassium channels in primary sensory neurons. Peripheral nerve injury induces long-lasting downregulation of many voltage-gated potassium (K_V) channels,[24−26] including K_V1.1 (encoded by *Kcna1*), K_V1.2 (*Kcna2*), K_V1.4 (*Kcna4*), K_V4.2 (*Kcnd2*), and K_V7.2 (*Kcnq2*). K_V channel activation inhibits action potential generation; their downregulation by nerve injury plays a role in the hyperexcitability of DRG neurons and increased pain sensitivity.[27−29] Nerve injury produced by spinal nerve ligation (SNL) causes a sustained increase in G9a expression and the catalytic product, H3K9me2, in the DRG.[25] Chromatin immunoprecipitation (ChIP) analysis indicates that nerve injury increases the enrichment of H3K9me2 at the promoters of at least four K^+ channel genes, *Kcna4*, *Kcnd2*, *Kcnq2*, and *Kcnma1*, resulting in their downregulation and reduced activity in DRG neurons. Moreover, intrathecal administration of UNC0638, a selective G9a inhibitor, or a G9a-specific siRNA restores the expression of all four K^+ channels in the DRG downregulated by nerve injury. Similarly, conditional knockout of G9a in DRG neurons rescues the expression of four K^+ channel genes in the injured DRG. Strikingly, mice with G9a ablated from DRG neurons do not develop chronic pain after nerve injury,[25] indicating a pivotal role of G9a in neuropathic pain development. In addition, RNA sequencing analysis of DRG tissues reveals that about 40 K^+ channel genes that are downregulated by nerve injury are normalized by treatment with UNC0638. These findings have been independently confirmed in G9a-floxed mice treated with AAV5-Cre, which showed that abrogation of G9a rescues the expression of *Kcna2* and *Kcna4* in the injured DRG.[30] These studies clearly indicate that G9a plays a major role in epigenetic silencing of a large number of K^+ channel genes, which contributes to the increased excitability of DRG neurons and development of chronic neuropathic pain after nerve injury. It will be interesting to determine whether early perioperative treatment with G9a inhibitors can prevent the development of chronic pain after tissue and nerve injury.

Opioid drugs and cannabinoids are often less effective for patients with neuropathic pain. The μ-opioid receptor (MOR, *Oprm1*) expressed in DRG neurons is essential for the analgesic effect of MOR agonists.[31,32] Traumatic nerve injury causes a profound and sustained reduction in the expression of MORs in the DRG, which explains the diminished efficacy of MOR agonists in neuropathic pain. Nerve injury increases the H3K9me2 enrichment at the *Oprm1* promoter, suggesting a potential role of G9a in repressing *Oprm1* transcription.[33] Indeed, conditional knockout of G9a in DRG neurons restores the expression level of MOR and the analgesic effect of morphine diminished by nerve injury.[33] In addition, nerve injury causes a prolonged reduction in the expression of the type 1 cannabinoid receptor (CB1, *Cnr1*) and the enrichment of H3K9me2 at the *Cnr1* promoter in the injured DRG.[34] Inhibition of G9a with UNC0838 or conditional knockout of G9a in DRG neurons restores the CB1 expression and potentiates the analgesic effect of the CB1 agonist diminished by nerve injury.[34] By restoring MOR and CB1 expression in DRG neurons and their central terminals, the G9a inhibitor potentiates the inhibitory effect of MOR and CB1 agonists on nerve injury-induced glutamatergic input to spinal dorsal horn neurons.[33,34] Thus, G9a inhibitors may be combined with MOR or CB1 agonists to increase their analgesic efficacy in neuropathic pain.

G9a can form a complex with other epigenetic regulators involved in chromatin remodeling, including HDAC1/2, the lysine-specific histone demethylase 1A (KDM1A or LSD1), Brahma-related gene-1, RE-1 silencing factor (REST, also known as neuron-restrictive silencer factor, NRSF), and REST co-repressor 1 (CoREST1).[12,35] G9a forms heteromeric complexes with REST, which recognizes the unique RE-1 site at the gene promoters. SNL increases the expression of REST in the DRG, and intrathecal injection of a REST-specific siRNA reduces pain hypersensitivity induced by SNL.[36] Furthermore, ablating REST in DRG neurons attenuates the development of neuropathic pain after nerve injury. The cholinergic receptor muscarinic 2 (mAChR2, *Chrm2*) is the predominant muscarinic receptor subtype expressed in the DRG and spinal superficial dorsal horn and is involved in the inhibition of nociception by endogenous acetylcholine and muscarinic receptor agonists.[37−39] Importantly, REST knockdown with siRNA or knockout in DRG neurons restores the expression level of mAChR2 in the injured DRG and the inhibitory effects of muscarinic receptor agonists on nociceptive transmission at the spinal cord level and nerve injury-induced allodynia and hyperalgesia.[36] It is still unclear whether REST and G9a form a repressive complex to silence the expression of antinociceptive genes in the injured DRG.

3.1.2. H3K27 Methylation. The repressive histone mark H3K27me3 is catalyzed by the polycomb repressive complex 2 (PRC2), which harbors the methyltransferase EZH2 (KMT6A) along with other core protein members.[14] Nerve injury also enhances EZH2 expression and enrichment of its

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catalytic product H3K27me3 at the promoter of *Kcna4*, *Kcnd2*, *Kcnq2*, and *Kcnma1* in the DRG.[25] Selective inhibition of EZH2 with GSK503 alone rescued the mRNA levels of some K^+ channel genes (but not *Kcna4*) in the injured DRG. Notably, H3K27me3 is also enriched at the *Oprm1* promoter in the injured DRG.[33] In addition, SNL seems to induce upregulation of KDM6B, a lysine demethylase, via demethylating H3K27me3 at the IL-6 gene promoter to increase the expression of IL-6 in the DRG and dorsal horn.[40] Although nerve injury increases EZH2 levels in the injured DRG, pharmacological inhibition of EZH2 with GSK503 has little effect on nerve injury-induced neuropathic pain.[25]

3.1.3. H3K4 Methylation. The members of the KMT2 family with their characteristic C-terminal SET (Su(var.)3−9 enhancer-of-zeste and trithorax) domains form multimeric protein complexes that sequentially add up to three methyl groups at H3K4 residues.[14] In contrast, the members of KDM1 and KDM5 families demethylate them.[9] The enrichment of H3K4me2 and H3K4me3 frequently peaks around the transcription start site of actively transcribed genes.[9,14] Nerve injury reduces H3K4me3 at the promoter of *Kcnd2*[25] and *Oprm1*[33] in the DRG. In contrast, H3K4me3 is enriched at the gene promoter of pannexin-1 (*Panx1*),[41] a large-pore membrane channel, resulting in transcriptional activation and increased *Panx1* expression in the injured DRG. Increased pannexin-1 activity in the DRG leads to the release of ATP and caspase-1 to cause pain hypersensitivity after nerve injury.[41] SNL also elevates KMT2A (MLL1, mixed-lineage leukemia 1) in the spinal dorsal horn and increases H3K4me3 enrichment at the promoter of mGluR5, resulting in its upregulation.[42] Increased mGluR5 expression and activity in DRG neurons and their central terminals in the spinal cord can augment nociceptive input via NMDA receptors to induce chronic neuropathic pain.[43,44]

In addition to primary sensory neurons, macrophages and glial cells (oligodendrocytes, astrocytes, and microglia cells) in the spinal dorsal horn and the satellite glial cells in the DRG can contribute to neuropathic pain. These cells may release pro-inflammatory cytokines, such as tumor necrosis factor-α, interleukin-1β (IL-1β), and IL-6, and chemokines, such as CXCL1 and CXCL8, in response to nerve injury or disease. Nerve injury rapidly increases the expression of colony-stimulating factor 1 in DRG neurons.[45] Increased trafficking of colony-stimulating factor 1 from the DRG to the spinal dorsal horn can activate microglial proliferation. H3K4me3 enrichment has been shown at the gene promoters of many cytokines and chemokines (e.g., CCL2 and CCL3) in the injured sciatic nerve.[46] However, the specific epigenetic regulators have not been clearly identified.

3.1.4. Arginine Methylation. In addition to the lysine methylation changes, nerve injury differentially affects the expression of PRMTs. Peripheral nerve injury increases PRMT4 (*Carm1*) expression but reduces the expression of PRMT8 and PRMT9 in the DRG in mice.[47,48] PRMT8 is unique among the PRMTs, because it is predominantly expressed in the nervous system and is selectively present at the synaptic site where it catalyzes arginine methylation of various synaptic proteins critical for dendritic spine maturation, synaptic plasticity, and cognitive functions.[49−51] Mice lacking *Prmt8* in the brain have reduced protein levels of the NMDA receptor subunit GluN2A and NMDA receptor currents largely via a post-translational mechanism.[49] However, it remains uncertain how decreased PRMT8 expression is causally associated with nerve injury-induced pain hypersensitivity.

On the other hand, nerve injury-induced overexpression of PRMT4 only occurs in the DRG. Interestingly, selective inhibition or knockdown of PRMT4 reduces nerve injury-induced pain hypersensitivities.[48] It is unclear what gene and protein targets are involved in PRMT4-induced arginine methylation of histone and non-histone proteins in neuropathic pain. In addition, PRMT7 interacts with $Na_V1.9$ (*Scn11a*) in the mouse DRG and methylates the arginine 519 (R519) residue in its hLoop1.[52] This increased methylation may facilitate the trafficking of $Na_V1.9$ to the cell membrane, augmenting neuronal excitability and nerve injury-induced neuropathic pain.

3.2. Role of Histone Acetylation in Neuropathic Pain.

In addition to histone methylation, acetylation is another common modification of histones and serves as a crucial epigenetic modulator for gene transcription.[53,54] The acetylation status of lysine residues in histone tails is dynamically regulated by the opposing activities of various HATs and HDACs. There are about 18 mammalian HDACs, and the functions of HDACs in the nervous system are highly heterogeneous.[55,56] HDAC proteins are grouped into four classes based on function and DNA sequence similarity. Classes I, II, and IV are "classical" HDACs, and class III is a family of NAD^+-dependent proteins known as sirtuins. Class I HDAC subtypes (HDAC1, -2, -3, and -8) are located primarily in the nucleus, whereas class II HDACs (HDAC4, -5, -6, -7, -9, and -10) shuttle between the nucleus and the cytoplasm. Among the nine HDACs detected in DRG tissue, HDAC2 is the most highly expressed.[25] Previous studies, largely using nonspecific HDAC inhibitors, provided only conflicting results about the functions of individual HDACs in pain regulation. For example, some reported that HDAC inhibitors partially reduce nerve injury-induced neuropathic pain,[57,58] whereas others showed that an HDAC inhibitor causes pain hypersensitivity in normal animals.[59] However, HDAC subtypes often have distinct and opposing functions in the nervous system.[60,61]

Histone lysine acetylation is mediated by HATs, read by BET proteins, and removed by HDACs. The acetylation marks on H3K9 and H3K27 are predominantly present at the transcription start site of active genes.[53,54] HDAC2, HDAC4, and HDAC5 are predominantly expressed in DRG neurons, whereas HDAC1 is primarily present in non-neuronal cells in the DRG.[25,62] SNL increases expression levels of HDAC1 and HDAC2 in whole DRG tissues.[25] Also, SNL increases the mRNA and protein levels of HDAC4, but not HDAC5, in the DRG.[25] Furthermore, *Hdac4* knockout mice show reduced thermal sensitivity compared with wild-type mice.[63] However, global changes of HDACs in DRG tissues cannot predict their abundance locally at the gene promoters. This critical difference reiterates the need to determine the enrichment of HDAC subtypes at individual gene promoters using genome-wide approaches so that the specific function of HDAC subtypes in neuropathic pain can be appropriately defined.

Repeated intrathecal injections of MS-275, a class I HDAC inhibitor, modestly reduce neuropathic pain but only if treatment starts before the induction of pain.[57] Treatment with MS-275 elevates the H3K9ac level in the spinal cord tissue[57] and reverses the expression of K2P Kv channels downregulated by oxaliplatin.[64] Similarly, intrathecal injection of suberoylanilide hydroxamic acid (SAHA), a class I and II

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inhibitor, slightly decreases SNL-induced pain hypersensitivity.[25] Sodium valproate, a nonselective HDAC inhibitor, upregulates GLT-1 and glutamate-aspartate transporter (GLAST) genes decreased by peripheral nerve injury.[65,66] A selective HDAC1 inhibitor, LG325, also reduces pain hypersensitivities induced by nerve injury in mice.[58] In addition, BET protein inhibition with JQ1 reduces nerve excitability and pain induced by nerve injury,[67] and the combination of HDAC (SAHA) and BET (i-BET762) inhibitors produces a greater reduction in nerve injury-induced pain hypersensitivity than each agent alone.[68]

Glutamate NMDA receptor-mediated synaptic plasticity at the spinal cord level is crucially involved in central sensitization and neuropathic pain development.[69−72] $\alpha2\delta$-1 (encoded by the *Cacna2d1* gene), previously known as a Ca^{2+} channel subunit, is expressed in neurons in the DRG, spinal dorsal horn, and various brain regions.[73,74] $\alpha2\delta$-1 is the binding site of gabapentinoids, the first-line treatment for neuropathic pain conditions. Both traumatic nerve injury and paclitaxel treatment cause substantial and prolonged upregulation of $\alpha2\delta$-1 in the DRG.[75,76] Overexpression of $\alpha2\delta$-1 alone at the spinal cord level causes a long-lasting neuropathic pain-like phenotype.[77,78] Conversely, genetic knockout of *Cacna2d1* profoundly attenuates neuropathic pain development after nerve injury or paclitaxel treatment. Recent studies revealed that upregulated $\alpha2\delta$-1 can form a protein complex with NMDA receptors to increase synaptic NMDA receptor trafficking in the spinal dorsal horn and that $\alpha2\delta$-1-bound NMDA receptors, not voltage-activated Ca^{2+} channels, mediate the analgesic effect of gabapentinoids in various animal models of neuropathic pain.[76,78−80] Interestingly, pain hypersensitivity induced by JNJ-26481585, an HDAC inhibitor, in naive animals is reversed by gabapentin.[59] This suggests that HDAC inhibition may induce $\alpha2\delta$-1 upregulation. Nevertheless, the role of individual HDAC subtypes in the regulation of $\alpha2\delta$-1 expression in neuropathic pain remains largely unknown.

SNL increases the enrichment of H3K9ac at the promoter of *Panx1*, resulting in upregulation of *Panx1* in the DRG.[41] Nerve injury also causes histone hypoacetylation at gene promoters, silencing sodium channel $Na_V1.8$ (*Scn10A*), potassium channels (*Kcnd2*, *Kcnd3*, *Kcnq2*, and *Kcnma1*), and *Oprm1*,[25,62,81,82] which may contribute to their downregulation in the injured DRG. In addition, the transcription coactivator protein p300 and CREB-binding protein (CBP) have HAT activity as well as bromodomains to read acetylated lysines. p300-CBP has been shown to upregulate the expression of cyclooxygenase-2 and brain-derived neurotrophic factor in the spinal cord of rats after nerve injury.[83] Upregulation of cyclooxygenase-2 early after nerve injury is critically involved in the initiation of neuropathic pain.[84] Further studies are required to identify the HAT subtypes involved in neuropathic pain development.

3.3. Role of Histone Ubiquitination in Neuropathic Pain. The protein ubiquitination system comprises ubiquitin, ubiquitin ligases, and deubiquitinating enzymes, which are primarily involved in protein degradation. The monoubiquitination at H2A-K118/K119 and H2B-K120 residues is catalyzed by E3 ubiquitin ligases of PRC1 and RING-finger protein 20 (RNF20), respectively.[85] Although the H2-K119Ub mark is associated with PRC-mediated heterochromatinization,[85] the H2B-K120Ub mark facilitates transcriptional elongation by recruitment of phosphorylated (active) RNA polymerase II.[86] SNL induces RNF20-mediated formation of H2B-K120Ub at the mGluR5 gene promoter that leads to recruitment of activated RNA polymerase II and increased mGluR5 transcription in the spinal dorsal horn.[87]

4. ROLE OF DNA METHYLATION IN NEUROPATHIC PAIN

DNA methylation and hydroxymethylation are closely associated with neural development.[19,88] DNA methylation is catalyzed by DNA methyltransferases (DNMTs). There are two types of DNMTs, *de novo* DNMT (DNMT3A and DNMT3B) and maintenance DNMT (DNMT1), which introduce a methyl group to the fifth carbon member of the cytosine ring forming 5-methylcytosine (5-mC). DNA methylation is removed by TET demethylases, resulting in the formation of 5-hydroxymethylcytosine (5-hmC). Interestingly, in mammals, the CpG rich regions of about 70% of gene promoters remain unmethylated and are associated with H3K4me3 in the active gene promoters or with the H3K27me3 and H2A-K119Ub at the repressive gene promoters.[89]

Nerve injury-induced DNA methylation changes in the nervous system have been systemically determined using highly quantitative digital restriction enzyme analysis of methylation (DREAM) and reduced representation bisulfite sequencing (RRBS) assays. This unbiased, genome-wide DNA methylation analysis shows that nerve injury induces dynamic and differential DNA methylation changes in the nervous system.[90] In this regard, SNL causes an initial increase in the methylation of CpG sites in the DRG 3 days after surgery, followed by DNA methylation changes at 8% of CpG sites with prevailing hypomethylation 3 weeks after injury. These hypomethylated CpG sites are primarily located outside the CpG islands, in introns, intergenic regions, and repetitive sequences.[90] A separate study using RRBS analysis shows a similar change in DNA methylation patterns in the DRG 3 days after SNL.[91] In contrast, SNL causes more gains of methylation in the spinal cord and prefrontal cortex. Notably, mimicking the nerve injury-induced DNA hypomethylation via a diet deficient in methyl donors or intrathecal treatment with RG108, a cell-permeable DNMT inhibitor, induces tactile allodynia and mechanical hyperalgesia in the naive rats.[90] SNL-induced CpG methylation reprogramming of the genes associated with axonal and ion channels is downregulated in SNL, whereas genes related to morphogenesis and development are upregulated. Although methylation reprogramming is correlated with increased gene expression variability, the pronociceptive or antinociceptive gene targets in the DRG directly affected by DNA hypomethylation are unclear.

5. ROLE OF NONCODING RNA IN NEUROPATHIC PAIN

The noncoding RNAs (ncRNAs) include long ncRNAs (lncRNAs), circular RNAs (circRNA), microRNAs (miRNAs), and naturally generated antisense RNAs (AS-RNAs), which regulate transcription, translation, and mRNA stability.[21] The ncRNAs are not translated into proteins but can participate in transcriptional and translational regulation. Nerve injury may silence *Kcna2* by increasing *Kcna2* antisense RNA (a lncRNA) via the transcription factor MZF1 in neuropathic pain.[92] A reduced Ds-lncRNA level after nerve injury facilitated enhanced binding of the RNA-binding transcriptional cofactor

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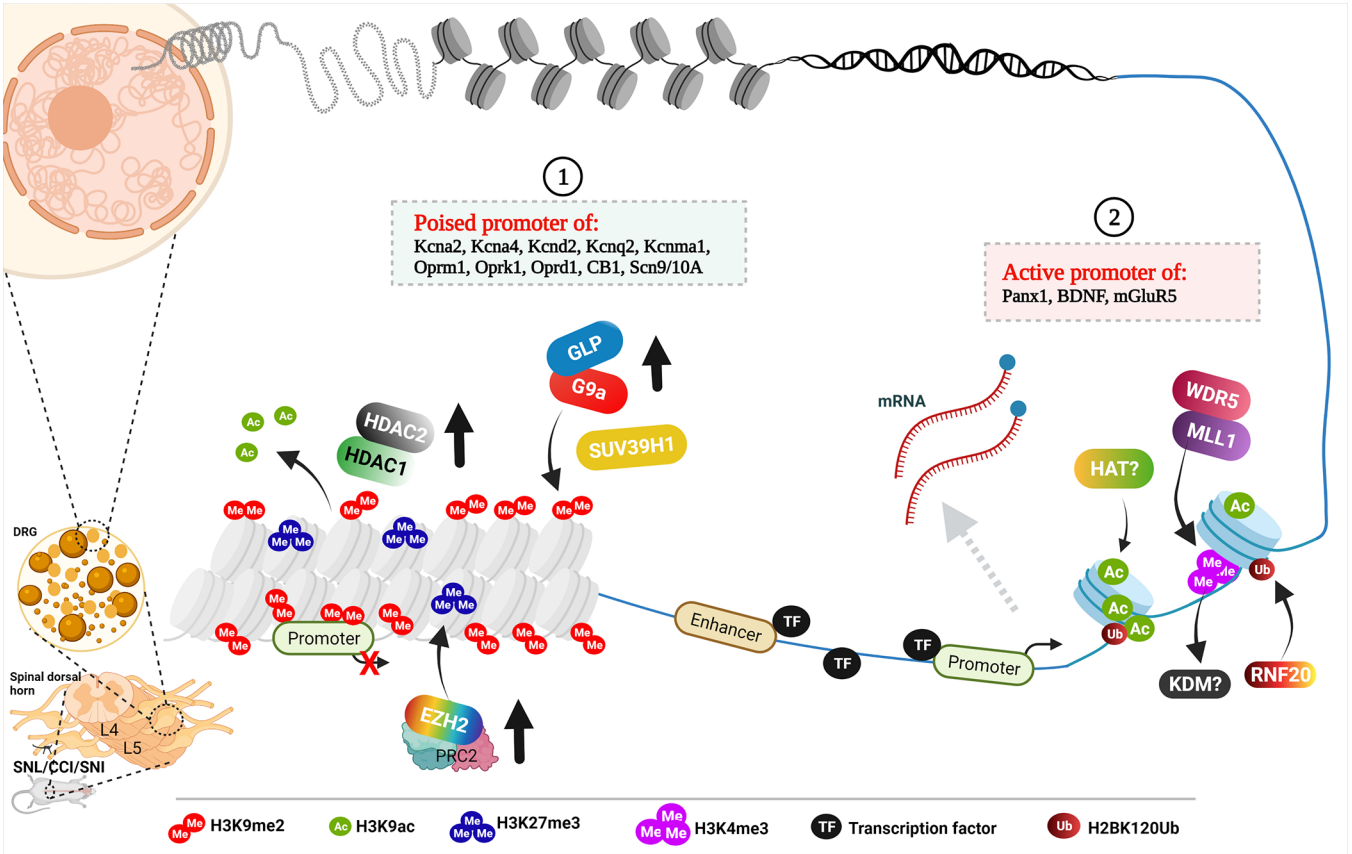

Figure 2. Nerve injury-induced epigenetic modifications and transcriptional control of genes associated with pain hypersensitivity in the DRG. (1) Nerve injury-induced enrichment of H3K9me2 and H3K27me3 repressive marks catalyzed by upregulated G9a/GLP complex and EZH2−PRC2, respectively. SUV39H1 catalyzes the formation of H3K9me3. Increased histone deacetylase (HDAC) activity removes the activating H3K9ac mark from the promoters of antinociceptive genes, repressing their expression in the DRG. (2) Increased level of active H3K4me3, H3K9ac or H2B-120Ub at the promoter of pronociceptive genes, augmenting their transcription. Although the specific histone acetyltransferase (HAT) is yet unknown, the MLL1-WDR5 complex may catalyze the formation of H3K4me3 after nerve injury. The ubiquitination of H2B-K120 is catalyzed by the RING finger protein RNF20.

RALY to RNA polymerase II, which further increased G9a transcription in injured DRG.[93]

In addition, some miRNAs may target nociception-related genes involved in neuropathic pain. For instance, nerve injury-induced $Na_V1.7$ upregulation coincides with decreased miR-182 levels.[94] Also, nerve injury-induced mechanical allodynia is associated with increased expression levels of miR-132-3p in the DRG and dorsal spinal cord.[95] Furthermore, overexpression of miR-18a, miR-19a, miR-19b, and miR-92a cluster members elicits mechanical allodynia, whereas blocking these miRNAs reduces mechanical allodynia caused by nerve injury.[96] The miR-17−92 cluster seems to cause pain mainly by downregulation of multiple K_V channels in the DRG.[96]

6. CONCLUSIONS AND PERSPECTIVES

Recent studies have provided substantial evidence showing that altered transcription of pronociceptive and antinociceptive genes in the DRG, via histone post-translational modifications, is important for the transition from acute to chronic pain after nerve injury. Current studies indicate that nerve injury mainly represses the transcription of many antinociceptive genes through G9a-mediated H3K9me2 in the DRG (Figure 2). Histone post-translational modifications are cooperative as the same or different lysine residues can be subjected to methylation, acetylation, or ubiquitination.[97] Thus, it is not surprising that the combined use of G9a and HDAC inhibitors synergistically attenuates pain hypersensitivity caused by nerve injury.[25] Various chromatin remodeling proteins can dynamically influence the expression of pronociceptive or antinociceptive genes that can generate sustained nociceptive signals from the periphery to the central nervous system after nerve injury (Figure 2). Although we have mainly discussed epigenetic changes that sustain chronic neuropathic pain, many changes in gene expression (e.g., *Oprm1* downregulation and *Cacna2d1* upregulation) occur within a few days after nerve injury.[33,75] Thus, the epigenetic regulation also applies to the acute phase of neuropathic pain.

Our understanding is still limited as to how the epigenome is reprogrammed in response to tissue and nerve injury and how various epigenetic regulators and transcription factors are coordinated to induce neural plasticity, leading to chronic neuropathic pain. Although we have learned a great deal about epigenetic regulators involved in downregulation of antinociceptive genes (e.g., K_V channel and opioid receptor genes), the epigenetic mechanisms responsible for persistent upregulation of many pronociceptive genes, such as cytokines and *Cacna2d1*, in neuropathic pain are generally unknown. It is essential to identify the unique gene targets regulated by individual epigenetic regulators, and effort should be made to differentiate genes involved in chronic pain from those required for nerve repair and regeneration. At present, the

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role of many epigenetic modifying enzymes in neuropathic pain has not been vigorously studied. For example, various HDAC subtypes are expressed in the DRG, and they likely have distinct functions in regulating gene transcription. Using specific genetic approaches targeting individual HDACs may help to address this issue. Caution should be made for the suggested roles of epigenetic regulators in neuropathic pain in the literature due to the use of potentially nonselective reagents (e.g., antibodies and inhibitors).

The vast majority of published studies use traumatic nerve injury as a neuropathic pain model to study epigenetic mechanisms. However, nerve injury-induced changes in epigenetic regulation may not be applicable to other neuropathic pain conditions, such as diabetic neuropathy and chemotherapy-induced neuropathy. In this regard, RNA sequencing analysis has revealed that altered levels of gene expression in the DRG are much smaller in paclitaxel-treated rats than in SNL rats.[25,90] Furthermore, only simple correlation analysis is performed about the relationship between neuropathic pain and epigenetic regulators or gene targets in many studies. Another major challenge in the study of epigenetic mechanisms of chronic pain is that tissues like the DRG and spinal cord are highly heterogeneous, consisting of different types of neurons and glial cells. Because the expression pattern of genes is tissue- and cell type-specific, it is important to define the function and changes of epigenetic regulators at gene promoter levels. Thus, single-cell sequencing and ChIP combined with gene sequencing (ChIP-seq) could be particularly helpful to define the epigenome map of nociception-related genes in chronic pain conditions in future studies. With a better and holistic understanding of the epigenomic landscape involved in the transition from acute to chronic pain, it is possible to identify specific epigenetic targets for treating refractory neuropathic pain. Because the epigenetic regulators are shared by many types of cells and tissues, using epigenetic interventions systemically for treating neuropathic pain is likely associated with various unavoidable adverse effects. One potential approach to minimize the adverse effects is to deliver epigenetic drugs locally to the DRG through intrathecal injection or transforaminal injection, which are commonly used in clinical practice.

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K.G. drafted the manuscript. H.-L.P. revised the manuscript.

Notes

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LIST OF ABBREVIATIONS:

DNMT, DNA methyltransferase; DRG, dorsal root ganglion; Ehmt2, euchromatic histone lysine methyltransferase 2; HAT, histone acetyltransferase; HDAC, histone deacetylase; KDM, lysine demethylase; KMT, lysine methyltransferase; miRNA, micro-RNA; ncRNA, noncoding RNA; PRMT, protein arginine methyltransferase; REST, RE-1 silencing factor; SNL, spinal nerve ligation

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