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MINI-REVIEW ARTICLE

The Epigenetic Mechanisms Involved in Chronic Pain in Rodents: A Mini-Review

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Abstract: Chronic pain is a common distressing neurological disorder and about 30% of the global population suffers from it. In addition to being highly prevalent, chronic pain causes a heavy economic and social burden. Although substantial progress has been achieved to dissect the underlying mechanism of chronic pain in the past few decades, the incidence and treatment of this neurological illness is yet not properly managed in clinical practice. While nerve injury-, chemotherapy- or inflammation-induced functional regulation of gene expression in the dorsal root ganglion and spinal cord are extensively reported to be involved in the pathogenic process of chronic pain, the specific mechanism of these altered transcriptional profile still remains unclear. Recent studies have shown that epigenetic mechanisms, including DNA/RNA methylation, histone modification and circular RNAs regulation, are involved in the occurrence and development of chronic pain. In this review, we provide a description of research on the role of epigenetic mechanism in chronic pain, summarize the latest clinical and preclinical advance in this field, and propose the potential directions for further research to elucidate the molecular mechanism underlying the pathogenesis of chronic pain.

Keywords: Chronic pain, DNA methylation, RNA N6-methyladenosine modification, histone acetylation, circular RNA, dorsal root ganglion, spinal cord.

1. INTRODUCTION

Chronic pain is an extremely prevalent disease. Global population-based estimates of chronic pain are approximately 30% [1-3]. It can cause serious physical and psychological disorders and considerably increase the economic burden on patients and society [4]. The causes of chronic pain include nerve injury, chemotherapy, and inflammation, *etc* [5-7]. Although considerable research efforts have been expanded toward elucidating the neurobiological mechanisms of chronic pain, the current therapeutic options, such as opioids and non-steroidal anti-inflammatory drugs, are ineffective or have severe side effects [8-10]. It is well known that the maintenance of chronic pain depends on the expression of pain-related proteins, which are regulated by the expression of corresponding genes [11, 12]. In recent years, accumulating studies have found that epigenetic modification is involved in the regulation of gene expression and plays an essential role in the process of chronic pain [13-15]. Epigenetics refers to a physiological or pathological process involving the reversible and heritable gene modifications without changes in the DNA sequence. It mainly includes DNA/RNA methylation, histone modification and non-coding RNA-mediated regulation [16, 17]. Most notably, chronic pain is also characterized as a reversible process that was substantially shaped by the compounding interaction between the noxious stimuli and neuropsychological environment. Hence, it is of fundamental importance to dissect the functional involvement of epigenetic modifications in chronic pain, and subsequently establish the biomarker signature (such as epigenetic regulatory biomarkers) closely related to the occurrence and development of chronic pain. Ultimately, these preclinical and clinical measurements will deliver promising molecular targets for the treatment of chronic pain and potential insights on the development of new analgesic drugs, which have far-reaching scientific significance. In this review, we will provide a brief overview of epigenetic modifications, especially DNA/RNA methylation, histone acetylation, and circular RNAs (circRNAs) regulation in the spinal cord and DRG in rodents, under the condition of chronic pain.

2. DNA/RNA METHYLATION AND CHRONIC PAIN

2.1. DNA Methylation

DNA methylation is a molecular process in which a methyl group is added to the fifth carbon of the cytosine, mainly in the context of neighboring cytosine and guanine nucleotides in the genome (5-CG-3 dinucleotides or CpG

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sites) [18, 19]. The process of DNA methylation is mediated by DNA methyltransferases (DMNTs), including DNMT1, DNMT3a, and DNMT3b. Methylation interferes with gene transcription either by preventing the interaction between transcription factors and promoters [20, 21] or by attracting methylated DNA-binding proteins, such as MBDs and MeCP2, which recruit transcriptional co-repressors or co-activators to mediate downstream changes in gene expression [22-24]. Ten-eleven translocation methylcytosine dioxygenases (TETs), including TET1, TET2, and TET3, are the main enzymes that mediate DNA demethylation [21]. Although the function of DNA methylation has been reported in other pathological states, only a few studies have demonstrated the potential role of DNA methylation in chronic pain.

2.2. DNA Methylation and Chronic Pain

Owing to improved genome-scale mapping of methylation, researchers can now comprehensively evaluate the level and sites of DNA methylation under different conditions. Using genome-wide detection methods, such as reduced representation bisulfite sequencing (RRBS) and/or digital restriction enzyme analysis of methylation (DREAM), researchers found that nerve injury (spinal nerve ligation or chronic constriction injury) and the chemotherapeutic drug oxaliplatin could induce DNA methylation remodeling in the DRG and spinal cord during the acute and chronic phases of neuropathic pain. These chromatin remodeling occurred not only inside but also outside of CpG islands (spanning exons, introns, intergenic regions, and/or repetitive sequences) [25-28]. Moreover, the chronic pain-associated changes of methylation level in different tissue such as the DRG and spinal cord has been a topic attracting remarkable attention from researchers.

In addition, transcriptional changes in certain genes mediated by methylation-related enzymes have been shown to be involved in chronic pain. In a model of peripheral nerve injury caused by sciatic nerve chronic constriction injury (CCI), global DNA methylation was increased in the spinal cord, and inhibition of DNA methylation attenuated the pain hypersensitivities induced by CCI [28]. Furthermore, nerve injury upregulated the expression of DNMT1 and DNMT3a in the DRG and/or spinal cord [29-31], but downregulated the expression of DNMT3b, which caused demethylation of *GPR151* gene promoter in spinal nerve ligation (SNL) mice model, and consequently facilitated the expression of G-protein-coupled receptor 151 (GPR151) in spinal cord contribute to the pathogenesis of neuropathic pain [32]. In addition to nerve injury, our recent studies reported that the decreased expression of DNMT3b in the dorsal horn is involved in three different chemotherapeutics-induced chronic pain, and it upregulated the expression of TRPC6 by mediating the hypomethylation of *PAX6* gene [33]. Mao and colleagues also found that the increased DNMT3a in the DRG contributed to the paclitaxel-induced chronic pain by decreasing the expression of the two-pore domain background potassium 1.1 ($K_{2p}1.1$) [34]. In general, DNMTs that target pain-related genes play an important role in the models of nerve injury and chemotherapy-induced chronic pain.

The opposite process is DNA demethylation generates 5-hydroxymethylcytosine (5hmC) through the oxidation of 5 mC by TET1-3 [35]. Literature has shown that TET1-3 is expressed in DRG of both naïve and nerve injury modeling mice [36]. SNL downregulated the expression of the μ-opioid receptor (MOR) and voltage-gated potassium channel subunit Kv1.2 by increasing their DNA methylation through TET1 in the DRG. Overexpression of TET1 in the DRG through microinjection of herpes simplex virus significantly alleviated the SNL-induced chronic pain [37]. In the spinal cord of different rodent models, TET1 is consistently involved in chronic pain by regulating gene transcription (*SOX10* and *mGluR5*) through demethylation of the gene promoter [38-40].

Methylated DNA-binding proteins, such as MBDs and MeCP2, as the functional executive proteins, modulate the expression of target genes by recruiting co-repressors or transcriptional activators [22, 23]. There is evidence that the upregulation of MeCP2 is directly involved in methylation-mediated gene silencing and leads to chronic pain [41, 42]. However, another study showed that MeCP2 plays an analgesic role in both acute pain transduction and spared nerve injury (SNI)-induced chronic pain [43]. The conflicting results may be due to differences in animal species, tissues and cell types of different studies. This suggests that, in the face of the complexity of the nervous system, we need to be more careful and accurate in defining the research object (especially different cell types in the same tissue), so as to get more precise and definite conclusion. MBD1-3 in the DRG and spinal cord is also involved, to some extent, in chronic pain induced by nerve injury. Specifically, the expression of MBD1 in DRG of SNL-induced chronic pain model rats was increased, the expression of MBD2 in spinal cord of CCI-induced chronic pain model rats was decreased and the expression of MBD3 need to be further explored [30, 44, 45].

With the overview of the above content (Table **1**), we found that the upregulation of methyltransferase DNMT3a is widely involved in chronic pain induced by a variety of causes (CCI, SNI, SNL and paclitaxel), and it promotes the occurrence and development of chronic pain by inhibiting the transcription of analgesic-related genes (*Oprm1*, *Oprk1* and $K_{2p}1.1$) by increasing the methylation level of these genes. However, the downregulation of DNMT3b induced by SNL and three chemotherapeutics promotes the chronic pain process by disinhibiting the expression of pain-related genes through post-transcriptional regulation mechanisms. These findings suggest that DNMT3a inhibition and DNMT3b overexpression may serve as a promising therapy for chronic pain management. While demethylases TET1-3 is expressed in DRGs of both naïve and SNI-induced mouse model of chronic pain, the changes in its expression, as well as its functional involvement, in chronic pain remain unclear. Upregulated TET1 and MeCP2 in the spinal cord are involved in chronic pain induced by nerve injury or complete Freund's adjuvant (CFA). Although these recent studies have linked the modification of DNA methylation to pain hypersensitivity, the role of DNMT1, TET2-3 and MBD1-3-dependent DNA demethylation modification in the develop-

Table 1. Overview of critical evidence for the involvement of DNA/RNA methylation in rodent models of chronic pain.

Type of Modification	Level of Global Modification	Enzyme Involved in Modification	Type of Chronic Pain (Model, Species)	Enzyme Alteration and Tissue Localization in Chronic Pain Condition	Method of Interfering the Enzyme	Outcomes of Intervention on Chronic Pain	Refs.
DNA methylation	Increased	MeCP2	Nerve injury-CCI, Rats	Increased, Lumbar spinal cord	5-azacytidine (i.t.)	Attenuated the mechanical allodynia and thermal hyperalgesia	[28]
-	-	DNMT1,3a,3b	Nerve injury-SNI, Rats	Increased, L4-L5 DRGs	-	-	[29]
-	-	DNMT3a	Nerve injury-CCI, Mice	Increased, Lumbar spinal cord	RG108 (i.t.)	Attenuated thermal hyperalgesia	[31]
-	-	DNMT3a, MBD1	Nerve injury-SNL, Rats and mice	Increased, L4-L5 DRGs	AAV5-*Dnmt3a* shRNA for rats/AAV5-*Dnmt3a* (DRG microinjection), *Dnmt3a*$^{fl/fl}$ mice,	Improved opioid analgesic effects/Increased MOR-gated presynaptic neurotransmitter release, Improved opioid analgesic effects	[30]
-	-	DNMT3a	Chemotherapeutics-Paclitaxel, Mice	Increased, L3-4 DRGs	*Dnmt3a*$^{fl/fl}$ mice (crossed with sensory-specific Advillin-*Cre*$^{+/-}$ mice)/HSV-*DNMT3a* (DRG microinjection) ,	Abolished mechanical allodynia and heat hyperalgesia/Produced mechanical allodynia and heat hyperalgesia	[34]
-	-	DNMT3b	Nerve injury-SNL, Mice	Decreased, L4-5 spinal cord	*DNMT3b* siRNA/LV-*DNMT3b*	Induced/alleviated mechanical allodynia	[32]
-	-	DNMT3b	Three different chemotherapeutics-paclitaxel, oxaliplatin, bortezomib, Rats	Decreased, L4-6 DRGs	-	-	[33]
-	-	TET1-3	Nerve injury-SNI, Mice	TET1,2 remain unchanged and TET3 increased, Lumbar DRGs	-	-	[36]
-	-	TET1	Nerve injury-SNL, Rats	L5 DRGs	HSV-TET1 ((DRG microinjection)	Blocked mechanical allodynia and abolished thermal hyperalgesia	[37]
-	-	TET1	Chemotherapeutics-oxaliplatin/Nerve injury-SNL, Rats	Increased, L4-6 spinal dorsal horn	TET1 siRNA (i.t.)	Ameliorated mechanical allodynia	[38, 39]
-	-	TET1	Nerve injury-SNL and inflammation-CFA, Rats	L4-5 spinal dorsal horn	LV-TET1 (i.t.)/TET1 siRNA	Produced /ameliorated mechanical allodynia and thermal hyperalgesia	[40]
-	-	MeCP2	Inflammation-CFA, Rats	L4-6 spinal dorsal horn	-	-	[41]
-	-	MeCP2	Nerve injury-SNI, Mice	Increased, L4-5 DRGs	MeCP2 T158A mice	Reduced mechanical sensitivity	[42]
-	-	MeCP2	Nerve injury-SNI, Mice	Increased, L4-6 spinal cord	Transgenic mice overexpressing MeCP2	weaken both acute mechanical pain and thermal pain	[43]
-	-	MBD1	Nerve injury-SNL and inflammation-CFA, Mice	L3-4 DRGs	*Mbd1*$^{-/-}$ mice/AAV5-Mbd1 (DRG microinjection)	Ameliorated /led to mechanical allodynia, heat hyperalgesia, and cold allodynia	[44]
-	-	DNMT1,3a,3b, MeCP2, MBD1-3	Nerve injury-CCI, Rats	DNMT3a, DNMT3b and MeCP2increased, MBD2 decreased, DNMT1, MBD1 and MBD3 unchanged Lumbar spinal cord	-	-	[45]
RNA methylation-m6A	Increased	METTL3	Inflammation-CFA, Mice	Increased, L4-5 spinal cord	METTL3 shRNA/ LV-METTL3	Iinhibited and reversed/produced thermal hyperalgesia and mechanical hyperalgesia	[55]
-	Decreased	METTL3	Inflammation-CFA,	Decreased, Spinal cord	Knocked down or conditionally deleting/overexpressed	Produced/attenuated of pain hypersensitivity	[56]

(Table 1) contd….

Type of Modification	Level of Global Modification	Enzyme Involved in Modification	Type of Chronic Pain (Model, Species)	Enzyme Alteration and Tissue Localization in Chronic Pain Condition	Method of Interfering the Enzyme	Outcomes of Intervention on Chronic Pain	Refs.
-	-	FTO	Nerve injury-SNL and CCI, Rats and mice	Increased, L4-5 DRGs	FTO siRNA (DRG microinjection) and *Fto*$^{fl/fl}$ mice (AAV5-*Cre* DRG microinjection)/AAV5-*Fto* (DRG microinjection),	Ameliorated /produced mechanical allodynia, heat hyperalgesia and cold allodynia	[57]
-	-	FTO	Nerve injury-SNL, Mice	L4 spinal dorsal horn	-	-	[58]

Abbreviations: CCI-chronic constriction injury; SNI-spared nerve injury; SNL-spinal nerve ligation; DRG-dorsal root ganglion; AAV-adeno-associated virus; HSV-herpes simplex virus; LV-lentiviral vector; CFA-complete freund's adjuvant.

ment of chronic pain remains elusive, in which more efforts from the researchers in this field are needed to thoroughly elucidate the regulation and function of DNA (de)methylation in the setting of chronic pain. In addition, the causal relationship between the changes of enzymes related to DNA methylation modification and the occurrence of chronic pain also needs to be further explored.

2.3. RNA Methylation

As one of the main RNA methylation modifications, the methylation at the N6 position of adenosine (m6A) was first revealed in 2012 by a technique known as methylated RNA immunoprecipitation sequencing [46]. m6A modification is typically located in the consensus motif RRACH (R = A or G; H = A, U, or C) and is enriched particularly around the 3′UTR near the stop codons and the transcription start site [47, 48]. The enzymes methyltransferase-like 3 (METTL3), methyltransferase-like 14 (METTL14), and Wilms' tumor 1-associating protein (WTAP) can catalyze the transfer of a methyl group from S-adenosyl methionine to adenine nucleotides of mRNA substrates and are called "writers". The dynamic nature of chemical modifications is a quintessential feature through which they regulate physiological functions, and m6A modification is no exception. m6A demethylation is mediated by two specific demethylases: fat-mass and obesity-associated proteins (FTO) and AlkB homolog 5 (ALKBH5). The function of m6A is mainly performed by the binding of several specific proteins, called "readers". Readers, specifically, YTH N6-methyladenosine RNA binding proteins (YTHDFs, including YTHDF1-3), have been shown to be involved in a number of RNA biogenesis processes, including translation, splicing, and degradation [46, 49-53].

2.4. m6A Modification in Chronic Pain

The m6A modification on the RNA molecular is known to be involved in diverse biological processes. However, very little is known about its role in chronic pain. Initially, researchers found that SNL, which induced neuropathic pain, elevated the level of m6A-tagged transcripts encoded by many regeneration-associated genes in the adult mouse DRG, and loss of either the m6A methyltransferase METTL14 or the m6A-binding protein YTHDF1 attenuated injury-induced protein translation in the DRG and reduced functional axon regeneration in the peripheral nervous system *in vivo* [54]. These results implied the effect of m6A modification on chronic pain. Indeed, some recent articles have identified the involvement of m6A modification in the regulation of chronic pain. The general contents are described as follows: Zhang and colleagues have revealed that METTL3, *via* positive modulation of pri-miR-65-3p processing, mediates a significant increase of spinal m6A modification in a mouse model of CFA-induced chronic inflammatory pain. Knockdown of spinal METTL3 by transfecting its corresponding shRNA into the spinal region could prevent and reverse CFA-induced pain behaviors and spinal neuronal sensitization. In contrast, lentivirus-mediated overexpression of spinal METTL3 induced pain behaviors and neuronal sensitization in naive mice [55]. In contrast, Pan and colleagues revealed a novel mechanism that downregulated spinal cord METTL3 coordinating with YTHDF2 contributes to the modulation of CFA-induced inflammatory chronic pain through stabilizing the upregulation of TET1 in spinal cord [56]. The existing literature have opposing views on the role of METTL3 in CFA-induced inflammatory chronic pain, so its role in pain needs to be further explored. Furthermore, peripheral nerve injury (*e.g.*, SNL and CCI) increased the expression of the m6A demethylase FTO in injured DRGs by activating Runx1, a transcription factor of FTO, and blocking FTO through FTO siRNA ameliorated pain symptoms and reversed the loss of m6A sites in Ehmt2 mRNA [57]. SNL promoted the FTO binding to the Mmp24 mRNA, which subsequently facilitated the translation of MMP24 in the spinal cord, and ultimately contributed to neuropathic pain genesis [58].

In summary, in the pain research field (Table **1**), m6A modification is an emerging but quite important research area in view of its high modification abundance and its great influence on mRNA stability and translation efficiency of pain-related genes. Until now, the overall change of m6A modification is controversial, and the mechanism of all the m6A-related enzymes involved in chronic pain needs further in-depth exploration.

3. HISTONE MODIFICATION IN CHRONIC PAIN

3.1. Type of Histone Modification and Histone Acetylation Process

The basic unit of DNA in cells is the nucleosome, formed by an octamer containing the four core histone proteins (H2A, H2B, H3, H4) wrapped by 147-base-pairs of DNA. The N-terminal histone tail, which is densely populated with basic lysine and arginine residues, protrudes from the nucleosome and can be subjected to extensive covalent post-translational modifications (PTMs), including acetyla-

tion, methylation, phosphorylation, SUMOylation and ubiquitination. PTMs recruit the remodeling enzymes that utilize the energy derived from the hydrolysis of ATP to influence nucleosome positioning, and mediate the function of these PTMs *via* changing chromatin structure [59, 60]. Histone acetylation modifications mainly occur on conserved lysine residues at the H3 and H4 N-terminal tail, which are catalyzed by the opposing action of two families of enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs) [59, 61]. Conventionally, histone acetylation alters the condensed chromatin into a more relaxed structure, and consequently facilitates gene transcription. In contrast, histone deacetylation tightly condenses chromatin resulting in gene silencing [62].

3.2. Histone Acetylation and Deacetylation in Chronic Pain

Evidence from various studies in rodents has suggested that chronic pain is associated with the altered acetylation of histone proteins mediated by HATs or HDACs, which results in abnormal transcription of pain-related genes in DRG and spinal cord. Current evidence has shown that the expressions of HATs (P300/CBP) is significantly upregulated in rodent spinal cord in chronic pain. Furthermore, spinal P300/CBP enhances the level of histone acetylation and promotes the expression of pain-related genes, by which it participates in the process of chronic pain [63-65]. Our previous studies found that chronic pain induced by injection of the chemotherapy drugs paclitaxel and/or vincristine was associated with the increased acetylation of global H4 (but not H3) expression and the increased levels of acetylated H4 (acH4) in the promoter of some chemokines (Cxcl12 and Cx3cl1) in spinal cord [66, 67]. Chemotherapy drug bortezomib treatment induced chronic pain, accompanied by the increased acetylation of histone H3 and H4 in NLRP3 promoter region in the DRG [68]. Lumbar 5 ventral root transection (L5-VRT) increased the level of H4 acetylation in Nav1.6 promoter region in the DRG and induced neuropathic pain [69]. Conversely, some researchers' findings suggested that the downregulation of ac-H3 may be involved in the development of CFA or SNL-induced chronic pain [70, 71].

18 HDAC genes are grouped into four classes: class I (HDAC1, 2, 3 and 8), class II (HDAC4, 5, 6,7, 9, 10), class III (sirtuin1-7) and class IV (HDAC11) [72]. A strong link between HDACs and nociceptive hypersensitivity has been indicated in different pain models. In the context of nerve injury induced chronic pain, the expression pattern and mechanistic action of different members of HDACs family may vary. CCI induced the upregulation of HDAC2 expression and downregulation of SIRT1 expression [73, 74]. Partial sciatic nerve ligation (PSNL) increased the expression of HDAC5, and knockdown of spinal HDAC5 expression attenuated PSNL-induced nociceptive hypersensitivity. Conversely, overexpression of spinal HDAC5 in naive mice immediately induced pain-like behaviors [75]. CFA -induced inflammatory chronic pain epigenetically suppressed KCC2 expression through HDACs-mediated histone hypoacetylation, resulting in decreased inhibitory signaling efficacy and sensitized pain behaviors [76]. Krukowski and colleges reported that pharmacological inhibition of HDAC6 completely reversed all hallmarks of the established cisplatin-induced peripheral neuropathy (including mechanical allodynia) by normalization of mitochondrial function in dorsal root ganglia [77]. In addition, several groups reported that the HDAC inhibitors had an antinociceptive effect in chronic pain. For example, HDAC1 selective inhibitor, LG325, exhibited a dose-dependent amelioration of mechanical allodynia induced by SNI in mice [78]. Intrathecal application of inhibitors targeting HDAC class I and II (SAHA, TSA, LAQ824) significantly delayed and attenuated the thermal hyperalgesia induced by CFA injection in the hindpaw [79]. Mechanical and thermal hypersensitivity were significantly attenuated by class I HDAC inhibitors in the rats with spinal nerve transection (SNT) [80].

Based on the observations described in Table **2**, abundant evidence indicate that histone deacetylation is a critical step in a variety of pathological hallmarks induced chronic pain. The existing studies demonstrate inconsistent changes in total level of acetylated histones and HATs/HDACs expression in chronic pain. In addition, inhibitors of either HATs or HDACs could alleviate chronic pain. Nonetheless, how HATs and HDACs are involved in chronic pain also remains elusive, and the role of histone acetylation and deacetylation in neuropathic pain need to be further verified.

4. NON-CODING RNA REGULATING CHRONIC PAIN

4.1. A Brief Overview of Non-coding RNA

With the development of sequencing technology, most transcripts other than annotated mRNAs do not seem to encode proteins and are therefore commonly referred to as non-coding RNAs (ncRNAs) [81]. Studies have found that some ncRNAs can be translated into short peptides as templates, but there is still some debate [82]. Recently, ncRNA has been found to be mainly divided into miRNAs, lncRNA and circRNAs. Although their biological functions have not been elucidated, an increasing number of studies have demonstrated that these ncRNAs are widely involved in important physiological functions and biological processes during the mammalian lifespan, such as spermiogenesis, cellular differentiation and neurogenesis [83, 84]. In the pain research field, the function of long non-coding RNA (lncRNA) and micro RNA (miRNA) in regulating the occurrence and development of chronic pain, ranging from transcriptional and post-transcriptional levels, has been extensively studied [85-87]. The unique miRNA and lncRNA expression signatures in chronic pain and correlation with higher neuropathic pain scores indicated its potential as a biomarker and therapeutic target for neuropathic pain [88-90]. Therefore, in this section, we will focus on the role of circRNAs in chronic pain. circRNA is a special type of RNA generated by a back- splicing process that has recently attracted increasing attention. As a new endogenous noncoding RNA, highly-

Table 2. Overview of critical evidence for the involvement of histone acetylation in rodent models of chronic pain.

Type of Modification	Level of Global Modification	Enzyme Involved in Modification	Type of Chronic Pain (model, species)	Enzyme Alteration and Tissue Localization in Chronic Pain Condition	Method of Interfering the Enzyme	Outcomes of Intervention on Chronic Pain	Refs.
Histone acetylation	-	P300/CBP	Nerve injury-CCI, Rats	Increased, Lumbar spinal cord	*P300* shRNA/P300 inhibitor-C646 (i.t.)	Attenuated the mechanical allodynia and thermal hyperalgesia	[63-65]
-	Increased	P300	Chemotherapeutics-pacitaxel and vincristine Rats	L4-L6 spinal dorsal horn	-	-	[66, 67]
-	Increased	P300	Chemotherapeutics-bortezomib/Nerve injury-L5-VRT, Rats	Increased, L4-6 DRGs	-	-	[68, 69]
-	Decreased	-	Inflammation-CFA, Rats	L4-L5 spinal dorsal horn	-	-	[70]
-	-	HDAC1-2	Nerve injury-CCI, Rats	HDAC1 increased in L4-6 DRGs, HDAC2 increased in L4-6 spinal cord	HDAC inhibitor-suberoylanilide hydroxamic acid/*HDAC2* siRNA (i.t.)	Attenuated mechanical and thermal hypersensitivity	[73]
-	Increased	Sirt1	Nerve injury-CCI, Rats	Increased, Spinal cord	-	-	[74]
-	-	HDAC5	Nerve injury-PSNL, Mice	Increased, Lumbar dorsal horn	*HDAC5* siRNA/LV-*HDAC5* (i.t.)	Attenuated/Produced mechanical and thermal hypersensitivity	[75]
-	-	HDAC1-8,11	Inflammation-CFA, Rats	L4-5 spinal dorsal horn	HDAC inhibitor-TSA (i.t.)	Attenuated mechanical and thermal hypersensitivity	[76]
-	-	HDAC6	Chemotherapeutics-cisplatin, Rats	Lumbar DRGs	HDAC6 inhibitor-ACY-1083 and ACY-1215 (i.p.)	Prevented and completely reversed mechanical allodynia	[77]
-	-	HDAC1	Nerve injury-SNI, Mice	Spinal dorsal horn	HDAC1 inhibitor-LG325 (i.t.)	Ameliorated mechanical allodynia	[78]
-	-	HDAC1-3,8	Inflammation-CFA/Nerve injury-SNT, Mice/Rats	L4-6 spinal dorsal horn	HDACs inhibitor-SAHA, TSA, LAQ824 (i.t.)	Attenuated thermal hyperalgesia	[79, 80]

Abbreviations: CCI-chronic constriction injury; L5-RT- ; PSNL-partial sciatic nerve ligation; SNI-spared nerve injury; SNT-spinal nerve transection; DRG-dorsal root ganglion; LV-lentiviral vector; CFA-complete freund's adjuvant.

expressed circRNAs in the nervous system have three important characteristics, including tissue specificity, non-degradability, and high conservation [91, 92]. These characteristics match the phenotype of neuropathic pain, thereby suggesting a possible therapeutic strategy for patients suffering from this specific type of pain.

4.2. Circular RNAs in Chronic Pain

Accumulating evidence has shown that circRNAs play multiple functions, including acting as miRNA sponges, regulating gene transcription and translation. However, the study of circRNAs in chronic pain is still in its early stages. Researchers have detected extensive circRNAs expression in the spinal dorsal horn and DRG following nerve injury [93, 94], and the expression of circRNAs, such as circRS-7, was positively associated with the progression of neuropathic pain [95]. Blocking the expression of circRNAs, such as circAnks1a or circFilip1l, attenuates the mechanical allodynia [96, 97]. Additionally, as miRNA sponge, circRNAs have been verified to regulate target gene expression and contribute to neuropathic pain. For example, following nerve injury, both circRS-7 and circRNA-Filip1l regulated the function of miR-135a-5p and miRNA-1224, then affected the expression of different target proteins [95, 97]. Furthermore, our recent study showed that SNL-induced circAnks1a upregulation can increase the transcription of vascular endothelial growth factor B (VEGFB) by promoting the transcription factor YBX1 into the nucleus [96]. Our unpublished data identified an upregulated exon-intron circFhit in the nucleus of inhibitory neurons in spinal dorsal horn following SNI, and found that the upregulated circFhit promoted the transcription of parental gene *Fhit* in cis. Furthermore, the upregulation of FHIT *via* suppressing the inhibitory synaptic transmission from dorsal horn inhibitory neurons induced the increase of $NK1R^+$ neurons excitability and pain behavior following SNI.

In conclusion, although there are a few studies on the role of circRNAs in chronic pain, the current evidence summarized in this review suggests that circRNAs may participate in chronic pain through different mechanisms. Nevertheless, the current research is far from sufficient to reveal the role and mechanism underlying circRNAs-mediated neuropathic pain.

5. DISCUSSION

In this review, we have summarized the current concepts on four epigenetic modifications in chronic pain from different dimensions. In terms of expression abundance and stability of modification, circRNA, as a newly discovered molecule, is highly-expressed in the nervous system. circRNAs have three pivotal characteristics- tissue specificity, non-degradability and high conservation [91, 92], which correspond to the three characteristics-refractory, persistence and universality of chronic pain. These characteristics strongly imply that circRNAs play an extremely important role in the occurrence and formation of chronic pain and may serve as a promising therapeutic target for pain management. Until now, the studies on circRNAs have focused on the mixed tissue analysis in the spinal cord and DRG. However, this approach can be flawed, since it neglects to consider the shifting contribution of different cell types across experimental conditions. In future studies, improvements should be made to identify the same type of cells (neurons, microglia or astrocytes) by specific cell markers and then detect epigenetic and transcriptional changes.

The relationship between DNA methylation and gene expression has been well established. The methyl group is transferred to DNA by DNMTs and interferes with gene transcription by blocking the interaction between transcription factors and promoters through spatial structures. TETs-mediated hypomethylation promotes gene expression, and MBDS and MeCP2, as transcriptional regulators, also regulate gene transcription [20-24. Among the enzymes involved in methylation modification, both DNMT3a inhibition and DNMT3b overexpression can relieve pain hypersensitivity apparently [30-34]. So, modulation of DNMT3a and DNMT3b may serve as a promising therapy for chronic pain management. The role of TETs and MBDS in chronic pain remains to be further explored.

RNA methylation is an emerging field of pain research, and the related studies are still lacking. According to the existing research evidence, RNA methylation is a reversible modification, and RNA methyltransferase and demethylase can regulate the mRNA stability and translation efficiency of pro- or anti-nociceptive genes to participate in chronic pain [55-58]. The investigation into RNA methylation continues to show a promise and complex picture about epigenetic gene regulation in the nervous system and may open a new avenue for chronic pain management.

Histone acetylation alters the condensed chromatin into a more relaxed structure, and consequently facilitates gene transcription. In contrast, histone deacetylation tightly condenses chromatin resulting in gene silencing [62]. As the most widely and deeply studied epigenetic modification in the field of chronic pain, there are many controversies about the results of histone acetylation research. In particular, both HATs inhibitors and HDACs inhibitors could alleviate chronic pain [60, 63-65, 73, 76-80]. Nevertheless, HDAC inhibitors are currently being tested in cancer and neurologic disorders in preclinical and phase I clinical trials, with initial promising results [98]. The intervention of acetylation modification process is most likely to provide new drug targets for pain treatment.

To date, although a large number of studies have been conducted around the mechanisms of epigenetic modification in chronic pain, scientific knowledge about epigenetics is far from comprehensive, and many issues remain to be investigated. Almost all of the research in the field of chronic pain has been conducted on animals and has yet to be translated to the clinical setting. This brings us to an important point: more translational research on humans is needed to advance our knowledge, explore the real clinical potential, and eventually make technology and therapeutics more accessible to the labs and clinics. In addition, future translational research in the field of epigenetic modification of chronic pain should draw more lessons from the research results from other fields, like clinical study of HDAC inhibitors in the treatment of cancer [98]. Finally, research should also consider some unanswered issues. For example, epigenetic regulation is tissue and site-specific. Can the epigenetic modification changes in the nervous system (spinal cord or DRG) be reflected in easily accessible body fluids (blood or urine)? In order to reduce invasiveness in human studies, it is essential to identify the epigenetic modifications that can be reliably assessed in accessible sites (*e.g.*, blood or saliva).

CONCLUSION

In conclusion, currently the cellular and molecular machinery underlying the development of chronic pain is unclear. The study of epigenetic modifications-DNA/RNA methylation, histone acetylation and circRNAs may shed lights on the chronic pain management. In the future, more studies are still needed to reveal the pivotal role of epigenetic modification in the diagnosis and treatment of chronic pain, which represents an additional layer of new drugs development based on these mechanisms.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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