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## Epigenetic regulation of chronic pain

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### Abstract

Chronic pain arising from peripheral inflammation and tissue or nerve injury is a common clinical symptom. Although intensive research on the neurobiological mechanisms of chronic pain has been carried out during previous decades, this disorder is still poorly managed by current drugs such as opioids and non-steroidal anti-inflammatory drugs. Inflammation-, tissue injury-, and/or nerve injury-induced changes in gene expression in sensory neurons of the dorsal root ganglion (DRG), spinal cord dorsal horn, and pain-associated brain regions are thought to participate in chronic pain genesis; however, how these changes occur is still elusive. Epigenetic modifications including DNA methylation and covalent histone modifications control gene expression. Recent studies have shown that peripheral noxious stimulation changes DNA methylation and histone modifications and that these changes may be related to the induction of pain hypersensitivity under chronic pain conditions. This review summarizes the current knowledge and progress in epigenetic research in chronic pain and discusses the potential role of epigenetic modifications as therapeutic antinociceptive targets in this disorder.

### Keywords

DNA methylation; Histone acetylation; Histone methylation; Dorsal root ganglion; Spinal cord; Inflammatory pain; Neuropathic pain; Chronic pain

### Introduction

Chronic pain is a major public health problem that affects approximately 30% of the general population in the USA. It is a cause of grave physiological and psychological distress in

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those affected, and it places significant pressures on the health care system. About 100 billion US dollars are spent on chronic pain related health care expenses, and many patients experience a loss of productivity [1]. Chronic pain usually arises from inflammation, or tissue and nerve injury. Although intensive research on the neurobiological mechanisms of chronic pain has been carried out during previous decades, this disorder is still poorly managed by current drugs such as opioids and non-steroidal anti-inflammatory drugs, which are ineffective and/or produce severe side effects [2]. Peripheral inflammation and nerve injury produce transcriptional and translational changes in the expression of receptors, enzymes, ion channels, neurotransmitters, neuromodulators, and structural proteins in primary sensory neurons of dorsal root ganglion (DRG), spinal cord, and other pain-related regions in the brain [2–4]. These changes contribute to the induction and maintenance of chronic pain; however, how these changes are regulated by peripheral noxious stimuli is still not fully understood.

Recent studies have suggested that the mechanism for gene regulation involves epigenetic modifications. Environmental toxins, medications, diet, and psychological stress alter epigenetic processes such as DNA methylation, covalent histone modification (e.g., acetylation and methylation), and non-coding RNA expression. Accumulating evidence demonstrates that these processes play an important role in synaptic plasticity during memory formation as epigenetic changes correlate with hippocampal activity [5–10]. Given that peripheral and central sensitization under chronic pain conditions share common mechanisms with the neuronal plasticity of memory formation, it is very likely that similar epigenetic mechanisms occur under both conditions. Indeed, peripheral inflammation and nerve injury drive changes in DNA methylation, histone modifications, and non-coding RNAs in pain-related regions [8;9;11–14]. These changes might be responsible for inflammation/nerve injury-induced alterations of some pain-associated genes in central neurons. The evidence suggests that modification of epigenetic processes participates in the mechanisms that underlie the induction and maintenance of chronic pain.

The role of non-coding RNAs including microRNAs and long non-coding RNAs in chronic pain has recently been discussed [8]. This article focuses on the evidence for the changes in DNA methylation and histone modification, mostly in DRG and spinal cord, under chronic pain conditions. We explore how these changes are induced by peripheral noxious stimuli and how these epigenetic processes regulate pain related genes. We finally deduce potential mechanisms of how the changes in DNA methylation and histone modification contribute to the development and maintenance of chronic pain.

## 1. Histone modification in chronic pain

### 1a. The Process of histone modification

The nucleosome is the basic unit of chromatin, composed of about 140 base pairs of DNA wrapped around a histone octamer. Histones are small, alkaline proteins categorized into five major families: H1/H5, H2A, H2B, H3 and H4. Histones H2A, H2B, H3 and H4 are known as the core histones, while histones H1 and H5 are known as linker histones. The N-terminal histone tail protrudes from the nucleosome and can be post-translationally modified, including acetylation, methylation, phosphorylation, citrullination, SUMOylation,

ubiquitination, and ADP-ribosylation [15]. These modifications result in changes in the three-dimensional chromatin structure and gene expression [15].

### 1b. Histone acetylation and deacetylation in chronic pain

Histone acetylation and deacetylation are the processes by which the histones on lysine residues within the N-terminal tail and on the surface of the nucleosome core are acetylated by histone acetyltransferase (HAT) or deacetylated by histone deacetylases (HDACs) [16]. Acetyl-Coenzyme A is the major source of the acetyl group in histone acetylation [16–19]. Conventionally, histone acetylation makes the condensed chromatin into a more relaxed structure, and consequently promotes gene transcription. In contrast, histone deacetylation tightly condenses chromatin resulting in gene silencing [16] (Figure 1).

**Effect of HDAC inhibitors on inflammatory pain**—Evidence from a pharmacological study has shown that HDAC inhibitors can relieve inflammatory pain [20–22]. A 5-day subcutaneous treatment with either of the two HDAC inhibitors, MS-275 and SAHA, substantially reduced nociceptive behaviors in the second phase of the formalin test and led to an increase in mGluR2 (but not mGluR1a, mGluR4 or mGluR5) in the dorsal root ganglion (DRG) [21](Table 1). This antinociception could be abrogated by an mGlu2/3 receptor antagonist. The induction of DRG mGlu2 receptors in response to SAHA was associated with increased acetylation of p65/RelA on lysine 310, a process that enhances the transcriptional activity of p65/RelA at nuclear factor-kappaB-regulated genes [21]. Given that transcription of the mGlu2 receptor gene is activated by p65/RelA in DRG neurons, HDAC inhibition may produce antinociception by up-regulating mGlu2 receptor expression in DRG. However, it is not clear whether formalin injection changes the histone acetylation conditions and HDAC expression and activity in the DRG.

It was reported that complete Freund's adjuvant (CFA)-induced peripheral inflammation increased the levels of class IIa HDAC members (HDAC4, 5, 7, 9), but not class I HDAC members (HDAC1, 2, 3), in the spinal dorsal horn [20]. Intrathecal administration of HDAC inhibitors targeting class II (SAHA, TSA, LAQ824) or IIa (VPA, 4-PB) significantly delayed the development of thermal hyperalgesia and attenuated existing thermal hyperalgesia in a CFA-induced inflammatory pain model [20] (Table 1). It appears that class I and II or IIa HDAC members function differentially in inflammatory pain models. A recent investigation from Zhang and colleagues revealed the epigenetic mechanism of inflammatory pain in central pain-modulating neurons. CFA induced the increase of global histone H3 and H4 acetylation in brainstem nucleus raphe magnus (NRM), a crucial supraspinal site for maintenance of pain hypersensitivity [22]. However, acetylated H3 was reduced in the Gad2 gene promoter region which epigenetically suppresses the transcription of Gad2 (encoding glutamic acid decarboxylase 65) and consequently causes impaired inhibitory function. Local injection of HDAC inhibitors TSA and SAHA into NRM reversed this effect and produced a similar analgesic effect on CFA-induced inflammatory pain using systemic administration [22](Table 1).

**Effect of HDAC inhibitors on visceral pain**—One study on visceral pain also provided evidence to support the involvement of central epigenetic mechanisms in pain [23].

Intracerebroventricular administration of TSA significantly attenuated water avoidance stress induced visceral hypersensitivity in rats [23] (Table 1). However, the targets of histone acetylation were not known in this study.

**Effects of HAT inhibitors and HDAC inhibitors on neuropathic pain**—Several groups reported that the HDAC inhibitors had an antinociceptive effect in neuropathic pain. Oral administration of Sodium butyrate, a HDAC inhibitor, not only attenuated chronic constriction injury (CCI)-induced pain hypersensitivity but also reduced the CCI-induced increase in TNF- $\alpha$  in the sciatic nerve [24] (Table 1). However, how HDAC inhibition affects TNF- $\alpha$  expression under CCI-induced neuropathic pain conditions is unknown. Intrathecal pre-treatment with class I HDAC inhibitors (MS-275 or MGDC0103) attenuated mechanical and thermal hypersensitivity in models of traumatic nerve injury and antiretroviral drug (stavudine)-induced peripheral neuropathy [25] (Table 1). This analgesic effect may be related to the increase in global H3K9ac in the spinal cord but not in DRG, suggesting that any potential mechanism could be found in the central nervous system [25]. Interestingly, the acetylation changes at the promoters of some pain-related genes, like mu opioid receptor, Kv4.3, Nav1.8, and brain-derived neurotrophic factor (BDNF), in DRG neurons have been reported in a neuropathic pain model [26–29] (Table 1). Nerve injury-induced reductions of histone H3 and H4 acetylation at the promoter regions of mu receptor, Nav1.8, and Kv4.3 silence their expression in DRG and may represent an underlying cause of common negative symptoms associated with neuropathic pain [27;28], whereas nerve injury-promoted increases in histone H3 and H4 acetylation at the promoter regions of BDNF in DRG and in histone H4 acetylation at the promoter regions of Cdk5 in spinal cord up-regulates the expression of BDNF and Cdk5, respectively, and may contribute to the induction or maintenance of neuropathic pain [29;30] (Table 1).

Interestingly, data from other groups showed that HAT inhibitors also had an antinociceptive effect in neuropathic pain. The HAT inhibitor anacardic acid relieved spinal nerve ligation (SNL)-induced neuropathic pain by suppressing the hyperacetylation of histone H3 in the promoter region of macrophage inflammatory protein 2 (MIP-2) and its receptor chemokine CC motif receptor 2 (CXCR2), resulting in the blockade of SNL-induced up-regulation of MIP-2 and CXCR2 in the injured sciatic nerve [31;32] (Table 1). In another report, CCI increased the expression of p300, a HAT E1A binding protein, in the lumbar spinal cord [33;34] (Table 1). Intrathecal administration of p300 shRNA or an inhibitor of p300 HAT reversed CCI-induced mechanical allodynia and thermal hyperalgesia and suppressed the expression of cyclooxygenase-2 (COX-2) in spinal cord [33;34](Table 1). Consistently, the intrathecal administration of resveratrol, an activator of Sirt1 (a classic III HDAC), attenuated CCI-induced mechanical allodynia and thermal hyperalgesia, reversed the CCI-induced decrease in spinal Sirt1, and blocked the CCI-induced increase in spinal histone H3 acetylation [35]. The analgesic effect of HAT inhibition was also reported in other persistent pain conditions. In an incision model, injection of the HAT inhibitor anacardic acid intraperitoneally reduced incision-induced pain hypersensitivity [36;37]. As expected, the HDAC inhibitor suberoylanilide hydroxamic acid exacerbated mechanical hypersensitivity after incision [36;37] (Table 1). Given that neuropathic pain and opioid tolerance/opioid-induced hyperalgesia share some common

intracellular pathways in their mechanisms [38], the evidence suggests that histone modification is also involved in the development and maintenance of opioid tolerance and opioid-induced hyperalgesia. Indeed, daily administration of the HAT inhibitor curcumin with morphine for 4 days reduced the development of morphine-induced mechanical allodynia, thermal hyperalgesia, tolerance, and physical dependence [39]. Conversely, the HDAC inhibitor SAHA enhanced these responses [39] (Table 1). The intrathecal injection of resveratrol suppressed the established morphine analgesic tolerance, reversed the morphine-induced decrease in spinal Sirt1, and attenuated the morphine-induced increase in spinal histone H3 acetylation [40]. Interestingly, baicalin, a flavonoid compound isolated from Huang Qin, ameliorated SNL-induced neuropathic pain by suppressing HDAC1 expression and preventing histone-H3 acetylation in the spinal cord dorsal horn [41] (Table 1).

Given that the degree of histone acetylation is controlled by the enzymes HATs and HDACs, conventionally, HAT inhibitors or HDAC activators should have opposite effects compared to HDAC inhibitors. Based on previous observations described above, how HATs and HDACs are involved in neuropathic pain is still elusive. The role of histone acetylation and deacetylation in neuropathic pain remains to be verified.

### 1c. Histone methylation and demethylation in chronic pain

Histone methylation is another process of histone modification by which methyl groups are transferred to amino acids of histone proteins in chromosomes (Figure 1). Histone methylation is catalyzed by S-adenosylmethionine- (SAM) dependent histone lysine methyltransferases (KMTs) and protein arginine methyltransferases (PRMTs), whereas histone demethylation is catalyzed by histone N-methylated lysine residue demethylases and the peptidyl arginine deiminases [15;42]. Histone methylation could repress or activate gene transcription depending on the sites and content being methylated. In general, methylation of histone H3 at Lys9 or Lys27 (H3K9 or H3K27) or histone H4 at Lys20 (H4K20) correlates with transcriptional repression, whereas methylation of H3K4, H3K36 and H3K79 correlates with enhanced transcription [15].

Although histone methylation has been reported to participate in the mechanism of formation of long-term memories and learning [7;43–45], the role of histone methylation in chronic pain is still unclear. Evidence indicates that histone methylation may be related to the expression of chemokine (C-C motif) ligands (CCLs), a class of small cytokines, in neuropathic pain [32;46]. The peripheral nerve injury-induced reduction in H3K27me3 in the promoter region of monocyte chemoattractant protein 3 (MCP-3, known as CCL7) might be responsible for the nerve injury-induced increase in the expression of MCP-3 in spinal cord [46]. Interleukin 6 may be involved in this response as the increased MCP-3 expression was almost abolished in interleukin 6 knockout mice with partial sciatic nerve ligation [46]. Peripheral nerve injury also increased the mRNA levels of CCL2, CCL3 and their receptors (CCR2 and CCR1/CCR5, respectively) in the injured sciatic nerve. These increases could be related to the increased H3K4me3 in the promoter regions of these cytokine genes [32]. An increase in global histone methylation was also observed in spinal cord after intrathecal injection of pertussis toxin, which induced significant thermal hyperalgesia [47]. However, whether these methylation sites are really required for these changes in gene expression is

unknown. Furthermore, whether nerve injury-induced changes in histone methylation contribute to neuropathic pain remains to be investigated. A recent study showed that an increase in the expression of MeCP2 in mouse central nucleus of the amygdala (CeA) was caused by both CFA-induced chronic inflammatory pain and repeated morphine exposure [48]. The increased MeCP2 bound to and repressed the transcriptional repressor histone dimethyltransferase G9a, resulting in a reduction in G9a-catalyzed repressive marker H3K9me2 and an increase in the expression of brain-derived neurotrophic factor in CeA [48]. Overexpression of CeA MeCP2 or knockdown of CeA G9a facilitated behavior of morphine reward, whereas knockdown of CeA MeCP2 inhibited behavior of morphine reward [48]. Whether such epigenetic cascade occurs in neuropathic pain needs to be confirmed.

## 2. DNA methylation and chronic pain

### 2a. Process of DNA methylation

In mammalian cells, DNA methylation is a biochemical process, in which a methyl group is added to the 5<sup>th</sup> carbon of cytosine residues situated adjacent to a guanine residue (CpG site). DNA sequences with a high concentration of CpG residues are referred to as CpG islands and are generally located at the start of the gene sequence within the promoter region (Figure 2). The process of DNA methylation is mediated by a group of DNA methyltransferases (DNMTs) that includes DNMT1, DNMT3a, and DNMT3b; this protein family also includes DNMT2, an inactivated isoform, and DNMT3L, which lacks the conserved catalytic domain [49–51]. DNMT1 maintains the methylation of DNA that is already established at the genome and is considered to be the primary maintenance DNMT [50;51]. Both DNMT3a and DNMT3b act as de novo methyltransferases and methylate unmethylated DNA [50;51]. Evidence has now expanded the role of DNMT1 to also include facilitation of de novo DNA methylation by DNMT3a and DNMT3b at gene promoters [6;50;52–54].

DNA methylation interferes with gene transcription by (1) physically interfering with the binding of transcription factors and (2) serving as docking sites for methyl-CpG-binding domain proteins (MBDs) [55;56] (Figure 2). MBDs contain a specific domain of ~70 residues, the methyl-CpG-binding domain, which directly binds to one or more methylated CpGs of a gene promoter. MBDs function as docking sites, in which they recruit other transcriptional co-repressors, such as histone deacetylases (HDAC), to the targeted gene for gene silencing, or co-activators, such as CREB1, for transcription activation [55–57]. The ability to recruit these proteins may be why DNA methylation has such a profound effect on gene expression.

### 2b. DNA methyltransferases and DNA methylation in chronic pain

**Effect of the DNMT inhibitors on inflammatory pain**—Although the function of DNA methylation has been reported in other pathological states, so far, only a few studies have demonstrated the potential role of DNA methylation and the activity and expressional levels of DNMTs in pain. Cystathionine- $\beta$ -synthase (Cbs) synthesizes hydrogen sulfide, an endogenous gas molecule, which is necessary and sufficient to elicit mechanical pain



hypersensitivity and increased excitability of DRG neurons. Peripheral inflammation induced by CFA leads to demethylation of the cystathionine- $\beta$ -synthase (*cbs*) gene in DRG [58]. This demethylation may be associated with the CFA-induced increase in expression of *Cbs* mRNA and protein in DRG and could influence the induction of inflammation-induced mechanical hypersensitivity [58]. Given the fact that peripheral inflammation did not decrease DNMT expression and activity in DRG [12], it is unclear how demethylation occurs in DRG under chronic inflammatory pain conditions. The level of DNA methylation is controlled by both DNMTs and demethylation enzymes (e.g., ten-eleven translocation dioxygenases). Whether peripheral inflammation changes the expression and activity of DNA demethylation enzymes in DRG remains to be determined. Interestingly, a recent study reported the CFA-induced hypermethylation of CpG islands in the miR-129 promoter in spinal cord neurons [59]. This methylation may regulate chronic inflammatory pain by targeting CaMKII $\gamma$  [59].

**Effect of the DNMT inhibitors on neuropathic pain**—In addition to peripheral inflammation, peripheral nerve injury caused by sciatic nerve chronic constriction injury (CCI) increased the level of global DNA methylation in the spinal cord [60]. Blocking spinal cord DNA methylation with intrathecal 5-azacytidine attenuated CCI-induced thermal and mechanical pain hypersensitivities [60]. CCI also increased the level of DNA methylation in the proximal promoter region of the  $\mu$  opioid receptor gene in DRG [61]. This increase may be related to the CCI-induced decrease in the analgesic effect of opioids [61]. In the spared nerve injury-induced neuropathic pain model, DNMT1 and DNMT3a (but not DNMT3b) transcripts were up-regulated in the injured DRGs [62]. Interestingly, the level of global DNA methylation was reduced in the prefrontal cortex and amygdala (but not the visual cortex and thalamus) following spared nerve injury [63]. This reduction strongly correlated with the severity of pain behaviors [63]. It appears that nerve injury-induced changes in DNA methylation are spatially different in the central nervous system and are implicated in distinct functions in spinal and supraspinal levels under neuropathic pain conditions. However, which type of cells in the nervous system express these changes is elusive. A recent study reported that DNMT1 was found in both neurons and satellite glial cells of DRG, DNMT3a in DRG satellite glial cells, and DNMT3b in DRG neurons [62], but the conclusion remains uncertain because no specific neuronal and glial markers were used [62]. Additionally, the specificity and selectivity of the antibodies used were not addressed [62]. These earlier studies raise several unanswered questions. For example, is the expression and/or activity of DNMTs and demethylation enzymes spatially and temporally changed following peripheral inflammation or nerve injury? If so, which type of DNMT? What are the downstream targeted genes of DNMTs under chronic pain conditions? Do DNMT inhibitors lead to side effects in addition to antinociception given that they are pharmacologically non-selective for specific DNMTs? Broader future investigations are required.

**DNA methylation in patients with chronic pain**—Alterations of DNA methylation have also been observed in patients with painful diseases. Women with fibromyalgia showed significant differences in DNA methylation patterns compared to aged-matched healthy controls, when genomic DNA isolated from whole blood was examined [64]. Fibromyalgia

associated genes with differential methylation include brain-derived neurotrophic factor, histone deacetylase 4, N-Acetyltransferase 15, protein kinase C alpha, and protein kinase G1 [64]. Increased methylation at the extracellular matrix protein SPARC (Secreted Protein, Acidic, Rich in Cysteine) gene promoter was reported in patients experiencing chronic low back pain associated with disc degeneration [65]. The endothelin B receptor gene promoter was heavily methylated in human oral squamous cell carcinoma lesions, which are highly painful, whereas this promoter was not methylated in human oral dysplasia lesions, which are typically not painful [66]. A regulatory DNA methylation region in the CpG-island shore of the TRPA1 promoter was reported to have a possible impact on TRPA1 gene expression and thermal sensitivity [67]. Joint resident synovial fibroblasts from patients with rheumatoid arthritis exhibited a global hypomethylation or both hypomethylation and hypermethylation patterns compared to patients with osteoarthritis or healthy controls [68–70]. This hypomethylation was identified in key genes relevant for rheumatoid arthritis, related to multiple pathways, and associated with increased gene expression [70]. A reduction of DNMT1 and an increase in the expression of *S*-adenosyl methionine decarboxylase, spermidine/spermine N1-acetyltransferase, and polyamine-modulated factor1-binding protein1 may be associated with hypomethylation in rheumatoid arthritis [68;71]. Additionally, promoter methylation states of the death receptor 3, interleukin (IL) 6, IL10, ILR2 and chemokine ligand 12 genes were altered in blood mononuclear cells and synovial fibroblasts in rheumatoid arthritis [42;72]. It appears that DNA methylation has the potential to serve as a biomarker for some types of painful disorders (e.g., autoimmune disorders or inflammation).

### 2c. methyl-CpG-binding domain proteins (MBDs) in chronic pain

As discussed above, DNA methylation-triggered gene transcriptional changes require a family of MBDs. The MBD family is composed of methyl-CpG-binding protein 2 (MeCP2) and MBD1–4. Each of these proteins, with the exception of MBD3, is capable of binding specifically to methylated DNA [73]. Accumulating evidence indicates that MeCP2 may be related to chronic pain. MeCP2 is associated closely with Rett syndrome, a neurodevelopmental disorder, which is primarily caused by mutations in the MeCP2 locus and patients display decreased pain sensitivity [74;75]. Preclinical studies showed that the expression of MeCP2 and the level of its phosphorylation were increased in the superficial dorsal horn under CFA-induced inflammatory pain conditions [76;77]. This phosphorylation is controlled by a descending serotonergic pathway as serotonergic depletion prevented CFA-induced MeCP2 phosphorylation [76]. It has been demonstrated that once MeCP2 is phosphorylated, it can be dissociated from the promoter regions of genes that have been repressed [78]. Therefore, the CFA-induced increase in MeCP2 phosphorylation may facilitate gene expression in the superficial dorsal horn under inflammatory pain conditions. However, the significance of how this increased phosphorylation is related to descending inhibitory serotonergic function in this model is unclear. The changes in MeCP2 expression following peripheral nerve injury are inconsistent. An increase in MeCP2 expression was observed in the spinal cord of CCI rats [60], whereas a decrease was detected in the superficial dorsal horn of rats after spared nerve injury [77]. The role of MeCP2 in neuropathic pain remains to be further clarified.



## Future perspectives

The evidence described above suggests that histone modifications and DNA methylation in DRG and spinal cord are involved in chronic pain. The conclusion depends on the behavioral observations following pharmacological inhibitor administration. These inhibitors have varying degrees of specificity and selectivity for the corresponding epigenetic enzymes. They may also exert their effects through non-epigenetic mechanisms, resulting in potential side effects. For example, HATs and HDACs are not histone-specific and can also acetylate and deacetylate, respectively, other targets in the cytoplasm. These factors call for careful interpretation of current findings claiming the role of a particular epigenetic enzyme in chronic pain. Therefore, the development of pharmacologic inhibitors for isoform- or subtype-specific epigenetic enzymes and/or the use of targeted genetic inhibition of isoform- or subtype-specific epigenetic enzymes will be required. Moreover, whether these epigenetic enzymes are activated by peripheral noxious insults and how their activation contributes to chronic pain remain to be investigated. Given that chronic pain remains a challenging condition to manage and that the contribution of epigenetic mechanisms underlying this disorder is becoming increasingly recognized, it is conceivable that the significance of histone modification and DNA methylation in chronic pain will become even more apparent in the coming years.

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### Exclusive summary

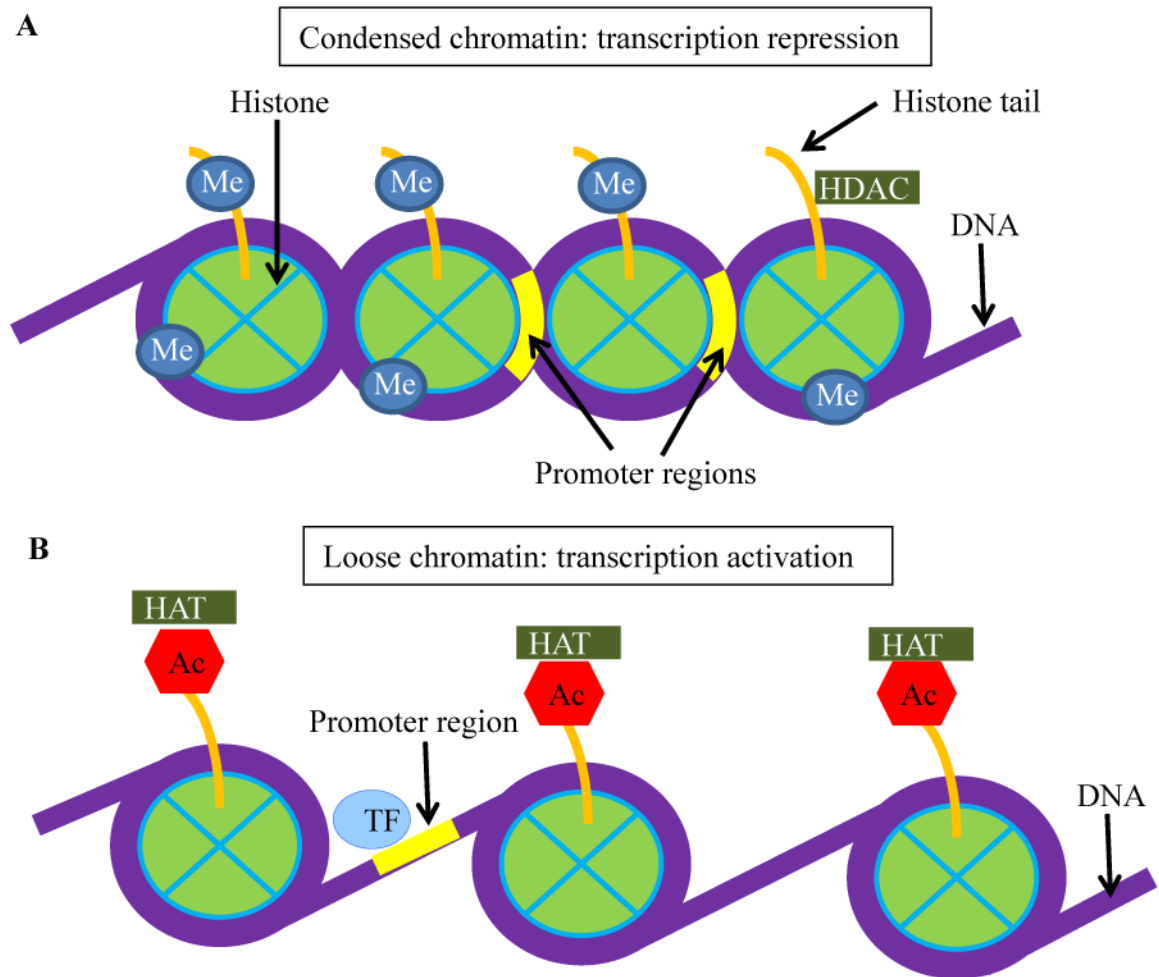
#### Histone modification in chronic pain

- HDAC inhibitors reduce inflammatory pain and visceral pain.
- Both HAT inhibitors and HDAC inhibitors show antinociceptive effects in neuropathic pain.
- Peripheral nerve injury or inflammation alters histone methylation in the promoter regions of some pain-related genes.

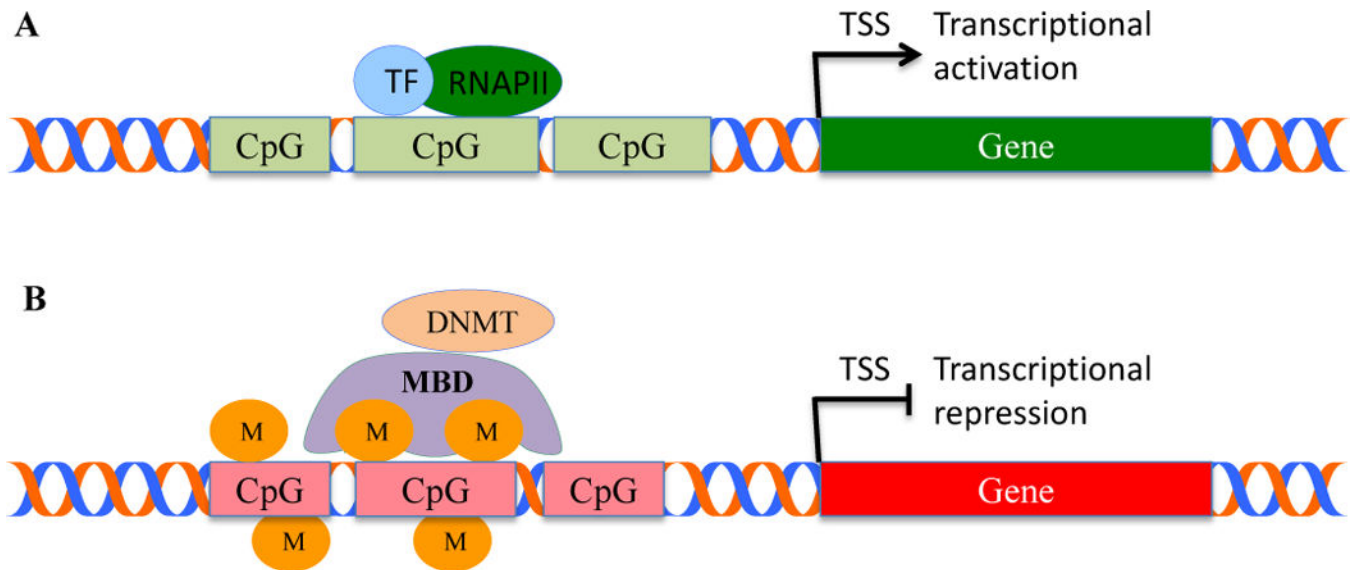
#### DNA methylation and chronic pain

- DNMT inhibitors blocks inflammatory pain.
- DNMT inhibitors attenuates neuropathic pain
- Alterations of DNA methylation have been found in patients with some painful diseases.





**Fig. 1.** Histone modification regulates gene expression. **(A)** Methylation (Me) of histones and deacetylation of histones with HDAC results in a condensed chromatin. Under this condition, transcription factors (TF) cannot bind to the promoter region of the gene, thereby gene transcription is repressed. **(B)** Histone acetylation (Ac) with HAT results in loose chromatin that allows the transcription factor (TF) to bind to the promoter region of the gene. Consequently, gene transcription is activated.



**Fig. 2.** DNA methylation represses gene transcriptional processes. (A) Without methylation at the CpG sites of the gene promoter, the transcription factor (TF) and RNA polymerase II (RNAPII) bind to the promoter region of the gene, thereby gene transcription is activated. (B) When methyl groups are added at the CpG islands by methyl-CpG-binding domain protein (MBD)-mediated DNA methyltransferases (DNMTs), the transcription factor (TF) and RNA polymerase II (RNAPII) cannot bind the promoter region of the gene, resulting in the repression of gene transcription. TSS: Transcription start site.

Table 1

## Summary of studies on histone acetylation and deacetylation

Pain model	Changes of acetylation or enzyme expression	Tissue	Inhibitors	Noiceptive behavior response to inhibitors	Target genes	Ref
Formalin	na	DRG	MS-275, SAHA (s.c.)	2 <sup>nd</sup> phase (↓)	mGluR2/3	Chiechio, 2009 [21]
CFA	HDAC4, 5, 7, 9 (↑), HDAC1, 2, 3 (-)	spinal dorsal horn	SAHA, TSA, LAQ824, VPA, 4-PB (i.t.)	Thermal (↓)	na	Bai, 2010 [20]
	Global histone H3 and H4 acetylation (↑)	NRM	TSA and SAHA (NRM)	Thermal (↓)	GAD65	Zhang, 2011 [22]
Water avoidance stress	na	na	TSA(i.c.v.)	Visceral hypersensitivity(↓)	na	Tran <i>et al.</i> , 2013 [23]
CCI	na	sciatic nerve	Sodium butyrate(oral)	Thermal (↓), mechanical (↓), cold(↓)	TNF-α	Kukkar, 2013 [24]
Traumatic nerve injury and stavudine-induced peripheral neuropathy	global H3K9ac(↑)	spinal cord	MS-275, MS-275 or MGDC0103 (i.t.)	Thermal (↓), mechanical (↓)	na	Denk <i>et al.</i> , 2013 [25]
SNL	HDAC1(↑) Acetyl H3 (↓)	Spinal dorsal horn	Baicalin	Thermal (↓), mechanical (↓)	na	Cheng, 2014 [41]
	Acetylation changes on promoter of genes	DRG	na	na	mu receptor, Nav1.8, Kv4.3, BDNF	Uchida, 2010; Uchida, 2013 [27-29]
	H3K9 ac(↑) on promoter of MIP-2 and CXCR2	injured sciatic nerve	Anacardic acid (i.p.)	Thermal (↓), mechanical (↓)	MIP-2 and its receptor CXCR2	Kiguchi, 2012; Kiguchi, 2013 [31;32]
CCI	p300 (↑)	spinal cord	p300 shRNA or C646 (i.t.)	Thermal (↓), mechanical (↓)	COX-2	Zhu, 2012; Zhu, 2013 [33;34]
	H4 acetylation at Cdk5 promoter (↑)	spinal cord	na	na	Cdk5	Li, 2014 [30]
Incision	Sirt (↓), Acetyl H3(↑)	spinal cord	Resveratrol	Thermal (↓), mechanical (↓)	na	Yin, 2013 [35]
	na	na	anacardic acid (i.p.)	Mechanical (↓), thermal (-)	na	Sun, 2013; Sun, 2013 [36;37]
	na	na	SAHA	Mechanical (↓), thermal (-)	na	
Morphine	Sirt (↓), Acetyl H3(↑)	spinal cord	Resveratrol	↓Tolerance	na	He, 2014 [40]
	na	na	Curcumin	↓mechanical allodynia, thermal hyperalgesia, tolerance, and physical dependence	na	Liang, 2013 [39]
	na	na	SAHA	↑mechanical allodynia, thermal hyperalgesia, tolerance, and physical dependence	na	

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na: not applicable; DRG: dorsal root ganglion; CFA: complete Freund's adjuvant; NRM: nucleus raphe magnus; CCI: chronic constriction injury; SNL: spinal nerve ligation; COX-2: cyclooxygenase-2; MIP-2: macrophage inflammatory protein 2; CXCR2: chemokine CC motif receptor 2; TSA: Trichostatin A; SAHA: suberoylanilide hydroxamic acid; i.t.: intrathecal; i.p.: intraperitoneal injection; s.c.: subcutaneous injection; i.c.v.: intracerebroventricular injection