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Review

Histone post-translational modifications as potential therapeutic targets for pain management

Jose V. Torres-Perez [1,*] Jahanzaib Irfan [2,4] Muhammad Rizki Febrianto [2,4] Simone Di Giovanni [3,*] and Istvan Nagy [2,*]

Effective pharmacological management of pain associated with tissue pathology is an unmet medical need. Transcriptional modifications in nociceptive pathways are pivotal for the development and the maintenance of pain associated with tissue damage. Accumulating evidence has shown the importance of the epigenetic control of transcription in nociceptive pathways via histone post-translational modifications (PTMs). Hence, histone PTMs could be targets for novel effective analgesics. Here, we discuss the current understanding of histone PTMs in the modulation of gene expression affecting nociception and pain phenotypes following tissue injury. We also provide a critical view of the translational implications of preclinical models and discuss opportunities and challenges of targeting histone PTMs to relieve pain in clinically relevant tissue injuries.

Highlights

Epigenetic changes play a decisive role in the transition from acute to chronic tissue-injury-associated pain by supporting maladaptive molecular changes in neurons and glial cells.

PTMs at histone tails are of particular importance. They modulate pain-induced gene transcription by regulating DNA–histone interactions and the binding of other proteins to the chromatin.

A shift in HAT/HDAC interactions has been observed in the transition from immediate to lasting pain.

Targeting EZH2, a histone methyltransferase enzyme, could serve as a potential novel analgesic treatment for neuropathic pain by preventing microglial activation and the expression of proinflammatory mediators.

Transient phosphorylation of histone H3 has recently been identified at spinal neurons following noxious stimuli. Blockage of its effector kinases prevents the development of inflammatory heat hyperalgesia.

The epigenetic landscape of nociception

Physical or chemical stressors that have the potential to damage tissue integrity induce the immediate development of a pain experience. This type of pain is essential for survival, develops through the activity of the naïve pain-signalling (nociceptive) pathways, and ceases within seconds if the tissues are removed from the stressor and no damage occurs. In the case of tissue damage, a qualitatively and quantitatively different pain experience develops that lasts until the tissue integrity is restored although it often persists beyond damage resolution. While the development of lasting pain on tissue damage constitutes a fundamental component of an adaptive response to restore homeostasis, when the pain experience persists beyond serving any biological function it becomes a pathological condition, a disease on its own, and is considered maladaptive [1,2]. Importantly, lasting pain has a devastating effect on quality of life. However, analgesics, which would reduce pain effectively and potently to an acceptable level, are lacking.

Lasting pain arises following plastic changes in cellular signalling that lead to increased neural excitability and activity, known as sensitisation [3]. When no neuronal tissues are damaged, sensitisation is driven by the inflammatory reaction, which follows the tissue damage [4]. If the damage involves peripheral sensory nerve fibres, injury-induced signals in primary sensory neurons are typically the driving force for sensitisation. Sensitisation in primary sensory neurons is termed peripheral sensitisation, while central sensitisation refers to increased excitability in the central nervous system [5].

Sensitisation occurs all along the nociceptive pathway. However, it has been best studied in nociceptive primary sensory neurons, which reside in dorsal root ganglia (DRG), and the spinal dorsal horn [2,3,5]. Sensitisation is described by three mechanistically and temporarily distinct molecular changes that underpin the transformation of the initial noxious experience into a lasting

[1]UK Dementia Research Institute at Imperial College London and Department of Brain Sciences, Imperial College London, 86 Wood Lane, London W12 0BZ, UK
[2]Nociception Group, Division of Anaesthesia, Pain Medicine and Intensive Care, Department of Surgery and Cancer, Imperial College London, Chelsea and Westminster Hospital Campus, 369 Fulham Road, London SW10 9FJ, UK
[3]Division of Neuroscience, Department of Brain Sciences, Imperial College London, E505, Burlington Danes, Du Cane Road, London W12 ONN, UK
[4]Contributed equally

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pain associated with tissue injury [6]. Immediate events occur instantly after sustained stimulation of primary sensory neurons or nociceptive input into the spinal dorsal horn and are due to self-amplification of currents carried by various ion channels [7]. The early signals occur within seconds to minutes after the start of a sustained noxious stimulus and are mediated by PTMs such as phosphorylation of various membrane and cytoplasmic molecules [8,9]. Finally, the third type of events that are fundamental for the long-term maintenance of the sensitised state is characterised by transcriptional changes that occur from hours to days after the injury in neuronal and glial cells [10,11]. As a result, their phenotype is significantly altered for a prolonged period of time.

Epigenetic mechanisms are fundamental in controlling transcription and, in the nervous system, they are strongly regulated by the activity of the cells [12,13]. Hence, epigenetic alterations in neurons and glial cells are considered as a mechanism via which those cells reprogram themselves in an activity-dependent manner [14,15]. In the nociceptive system, epigenetic regulation has been suggested to be pivotal in the development and maintenance of tissue-injury-associated pain through supporting long-lasting molecular changes [16].

Epigenetic modifications include inducible marks on chromatin (Box 1) such as DNA methylation or PTMs of histones. Histone PTMs, which are among the best-studied epigenetic mechanisms, are particularly interesting as, via the regulation of the myriad enzymes that add (writers), recognise (readers), or remove (erasers), these marks may provide specific control of gene expression. Although still in their infancy, the number of basic and clinical studies on the role of histone PTMs in pain has increased considerably in the past several years. Hence, there is an urgent need to critically review the available data to realistically evaluate the analgesic potential of controlling histone PTMs. This will help in finding the most appropriate avenues for further studies and ways to utilise the analgesic potential of controlling histone PTMs. Accordingly, subsequent

*Correspondence:
j.torres-perez@imperial.ac.uk
(J.V. Torres-Perez),
s.di-giovanni@imperial.ac.uk
(S. Di Giovanni), and
i.nagy@imperial.ac.uk (I. Nagy).

Box 1. The structure of chromatin

Chromatin: a complex of DNA and proteins found in the nucleus of eukaryotic cells. DNA is compacted and wrapped around proteins to physically fit in the nucleus.

Nucleosome**:** the smallest unit of chromatin; a globular unit comprising intranucleosome DNA wrapped around a histone octamer.

Intranucleosome DNA**:** 145–147 bp of DNA running one and three-quarter turns around the histone octamer.

Histone octamer: contains two copies of each of the core histones H2A, H2B, H3, and H4. These proteins assemble in pairs (H2A with H2B, H3 with H4) and merge to form the octamer.

Beads-on-a-string structural model: each nucleosome is joined to adjacent nucleosomes by the linker DNA, a portion of 10–70 bp. In that conformation, the genes within can be transcriptionally active.

Histone H1: an outer-nucleosome histone that stabilises the nucleosome. It can interact to further package the chromatin into a 30-nm fibre.

Euchromatin: open chromatin. It is loosely packed and easily accessed by the transcriptional machinery.

Heterochromatin: densely compacted chromatin, consequently closed to transcription. Heterochromatin is classified as two types: facultative heterochromatin, which differs by cell type; and constitutive heterochromatin, which is similar in all cell types and has a role in structural integrity (e.g., centromeres, telomers).

Nucleosome sliding: nucleosomes can move translationally onto adjacent DNA sequences. The nucleosome remodels through time due to passive movements or by active processes involving remodelling proteins.

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sections of this review discuss histone PTMs (Figure 1) in the context of the development and maintenance of lasting pain experiences (outlined in Table 1) and critically evaluate preclinical and clinical data on the analgesic effects of controlling various writers, readers, or erasers (outlined in Figure 2 and Table 2). While this review focuses on PTMs in the nociceptive pathway, readers should note that epigenetic changes also occur in injured/inflamed non-neuronal tissues and are fundamental in maintaining the drive for peripheral sensitisation through the production of agents acting on primary sensory neurons. However, a discussion of epigenetic changes in injured/inflamed tissues is beyond the scope of this review.

Histone PTMs in the nociceptive system

Nucleosomes, the basic units of chromatin (Box 1 and Figure 1), are dynamic structures involved in the regulation of gene expression. Their stability is achieved via histone–DNA interactions. Histone tails are positively charged protein domains that bind to the negatively charged DNA [17]. Those tails are susceptible to PTMs and covalent modifications that affect the structure of the chromatin [18].

The four core histone proteins have similar conformations and are among the most evolutionarily conserved proteins in eukaryotes, particularly at the C-terminal portions and the core globular domains, which are critical for their assembly into an octamer and interaction with the DNA. However, the most N-terminal part that forms the tail and accounts for 25–30% of the protein mass do not take part in the assembly [17]. Instead, the tails protrude from the core. Hence, the histone tails are easily accessible and especially suited to interactions with the adjacent nuclear environment and thus being subject to PTMs. The tails of histones H3 and H4 are more prone to PTMs than H2A and H2B due to their positioning at the exit point of the DNA from the nucleosome and their greater length [19].

To date, different types of PTMs have been described in histones of which (mono-, di-, or tri-) methylation, acetylation, and phosphorylation are the best known and characterised (Box 2). PTMs can occur in many combinations leading to a variety of functional outcomes. This combinatorial effect of histone modifications is referred as the Histone Code hypothesis [20].

Histone methylation

Altered histone methylation during neuropathic pain

Histone methylation can drive gene expression changes at either the DRG or the spinal cord level during the development of neuropathic pain. Zhang and colleagues found that **spinal nerve ligation (SNL)** (see Glossary) led to cumulative effects of both increases of the permissive mark H3K4me2 (and H3K9ac; see below) and reduction of the repressive marks H3K9me2 and H3K27me3 at the promoter region of Pannexin-1 (*Panx1*), a large-pore membrane channel involved in ATP release and nucleotide permeation, in the DRG but not in the spinal cord [21]. By contrast, Yadav and Weng found increased levels of both enhancer zeste homolog-2 (EZH2), a histone methyltransferase (HMT) enzyme, and H3K27me3 at the spinal dorsal horn of rats with partial SNL at 3–10 days post-injury. These changes were found in both spinal cord neurons and microglia and were associated with reduced levels of the cytokines tumour necrosis factor alpha (TNF-α) and interleukin (IL)-1β and the chemokine monocyte chemoattractant protein-1 (MCP-1) [22].

Writers of histone methylation during pain processing

The histone-lysine *N*-methyltransferase 2 G9a (encoded by the gene *Ehmt2*) has been indirectly linked to pain in humans. Chen and colleagues identified mutations in PR domain zinc-finger protein 12 (PRDM12), a transcription factor, in 11 rare human pedigrees of congenital insensitivity

Glossary

Brain-derived neurotrophic factor (BDNF): a growth factor important for neuronal survival and differentiation during development. BDNF is also implicated in plastic changes associated with learning and memory and the acute-to-chronic-pain transition.
Chronic constriction injury (CCI): a rodent model used to study peripheral neuropathy where one of the sciatic nerves is partially injured (loosely ligated). It produces long-lasting pain hypersensitivity.
Cleavage under targets and release using nuclease (CUT&RUN): laboratory methodology to study DNA–protein interactions. An antibody is used to direct a micrococcal nuclease (MNase) to cleave the DNA surrounding a certain DNA–protein interaction. DNA fragments can be extracted, sequenced, and analysed. It represents an improvement over ChIP-seq as the cleavage is produced *in situ*, can be performed without crosslinking, and produces a significantly lower background. Well suited to assess the binding of transcription factors.
Complete Freund's adjuvant (CFA): a solution comprising an inactivated mycobacterium. It is used as an inflammation-induced model in rodents – a subcutaneous injection of CFA diluted in saline induces a swelling that peaks at 24 h and persists for at least 1 week.
CUT and tagmentation (CUT&Tag): an improvement from CUT&RUN. It uses the Tn5 transposase, an Mg^{2+}-dependent enzyme, to control the cleavage instead of MNase. CUT&Tag does not require chromatin lysate and can be achieved at single-cell resolution. Conjugation of the next sequencing adapters to the Tn5 allows simultaneous chromatin cleavage and library preparation.
Mitogen- and stress-activated kinase 1 and 2 (MSK1/2): two nuclear kinases from the RSK family. They are activated (phosphorylated) by ERK1/2 and/or p38 MAPKs. Their substrates include histone H3 and several transcription factors including ATF1, CREB, and NF-κB. MSK1/2 has been implicated in many neuronal functions, including proliferation and survival, neurodegeneration, addiction, memory, and inflammatory pain.
Neuron-restrictive silencer factor (NRSF): also known as RE1-silencing transcription factor (REST). NRSF is a

to pain (CIP). Although PRDM12 lacks intrinsic writer activity, it recruits G9a to dimethylate histone H3 at lysine 9 (H3K9me2) [23]. Additionally, rodent models of axotomised DRG neurons suggested that G9a-dependent methylation of H3K9me2 and H3K27me3 might silence genes encoding voltage-gated potassium channels (*Kv1.2* and *Kv1.4*), which indirectly leads to neuronal hyperexcitability, and *Oprm1*, causing reduced opioid sensitivity [24–26].

The arginine-specific demethylase JMJD6, which can act on arginine 2 on histone H3 and on arginine 3 on histone H4, has also been involved in nociception in animal models of neuropathic pain. The spinal levels of JMJD6 are supressed following the induction of **chronic constriction injury (CCI)** in rats, which in turn resulted in the upregulation of proinflammatory cytokines via the transcription factor nuclear factor kappa B (NF-κB) [27]. When JMJD6 was overexpressed in the spinal cord, there was a marked reduction of CCI-induced behavioural and gene expression changes, suggesting that it may have a function in the development of neuropathic pain.

Could blocking histone methylation be used to attenuate neuropathic pain?

Blockage of the methyltransferase EZH2 has been suggested as a potential novel analgesic treatment for neuropathic pain. In their study, Yadav and Weng showed that local administration of 3-deazaneplanocin (DZNep) or GSK126, two EZH2 inhibitors, could decrease the expression of EZH2 and H3K27me and block microglial activation, thus attenuating neuropathic pain [22]. Additionally, *in vitro* studies by Arifuzzaman and coauthors found that EPZ-6438, a selective EZH2 inhibitor currently in Phase II trials to treat integrase interactor 1 (INI1)-negative tumours or relapsed/refractory synovial sarcoma [28], could prevent microglia activation and modulate the expression of inflammatory mediators including interferon regulatory factor (IRF) 1, IRF8, and signal transducer and activator of transcription 1 (STAT1) [29].

Histone acetylation

Altered histone acetylation during pain

Changes in histone acetylation have been reported in both inflammatory (somatic and visceral [30]) and neuropathic pain conditions at different levels of the nociceptive pathway. Lian and Tao observed a global acetylation increase at histones H3 and H4 (H3ac and H4ac) in both neurons and glial cells of the DRG, but not of the spinal cord, following SNL, while the injection of **complete Freund's adjuvant (CFA)**, used to induce inflammatory pain, increased them in the spinal dorsal horn but not in the DRG [31]. After **trigeminal inflammatory compression (TIC) injury**, a transient global increase in the acetylation of H3K9 was observed in the trigeminal ganglia, which lasted until day 21 post-injury and correlated with the first of two transient peaks of differential expression of 34 genes involved in nerve regeneration [32]. Similarly, a gradual increase of H3ac was observed in the rat nucleus raphe magnus (NRM), which is important for lasting pain modulation, 1 day after CFA administration [33]. By contrast, deacetylation of H3K9 has been observed in the central amygdala following exposure to elevated corticosteroids, which affects both anxiety and pain processing and ultimately accounts for the increase of corticotropin-releasing factor (CRF) [34].

However, global histone acetylation changes might not reflect the actual interplay of modifications tailoring the expression of certain pro- and anti-nociceptive genes during pain progression. Uchida and colleagues found that partial SNL increased H4ac, but not H3ac, at the promoter region of the **neuron-restrictive silencer factor (NRSF)** in DRG neurons 1–14 days post-injury [35]. In turn, NRSF recruited class I histone deacetylase (HDAC) to deacetylate both histone H3 and H4 at the promoters of *Oprm1* and $Na_v1.8$ to reduce their expression, thus leading to C-fibre dysfunction. Deacetylation of histones H3 and H4 seems to correlate with reduced spinal expression of potassium-chloride co-transporter 2 (KCC2), which causes hyperexcitability,

transcription factor that functions both as a neuronal transcription repressor by binding a DNA sequence called the neuron-restrictive silencer element (NRSE) and as an activator by inducing neural differentiation. Among others, NRSF modulates the expression of the μ-opioid receptor.

Spinal nerve ligation (SNL): a preclinical rodent model of peripheral neuropathic pain in which a lumbar segmental spinal nerve is unilaterally ligated. It leads to stable, long-lasting pain hypersensitivity for over 1 week post-surgery. There are many variations of this model, including a partial SNL.

Transient receptor potential cation channel subfamily V member 1 (TRPV1): a nonselective cation channel activated by multiple stimuli including temperature (>43°C), capsaicin, or the endocannabinoid anandamide. TRPV1 is mainly expressed in nociceptors, although it has also been identified in other tissues.

Trigeminal inflammatory compression (TIC) injury: an orofacial preclinical neuropathic pain model where a chromic gut suture is surgically implanted in the skull of the animal. TIC leads to both chronic facial pain and increased anxiety.

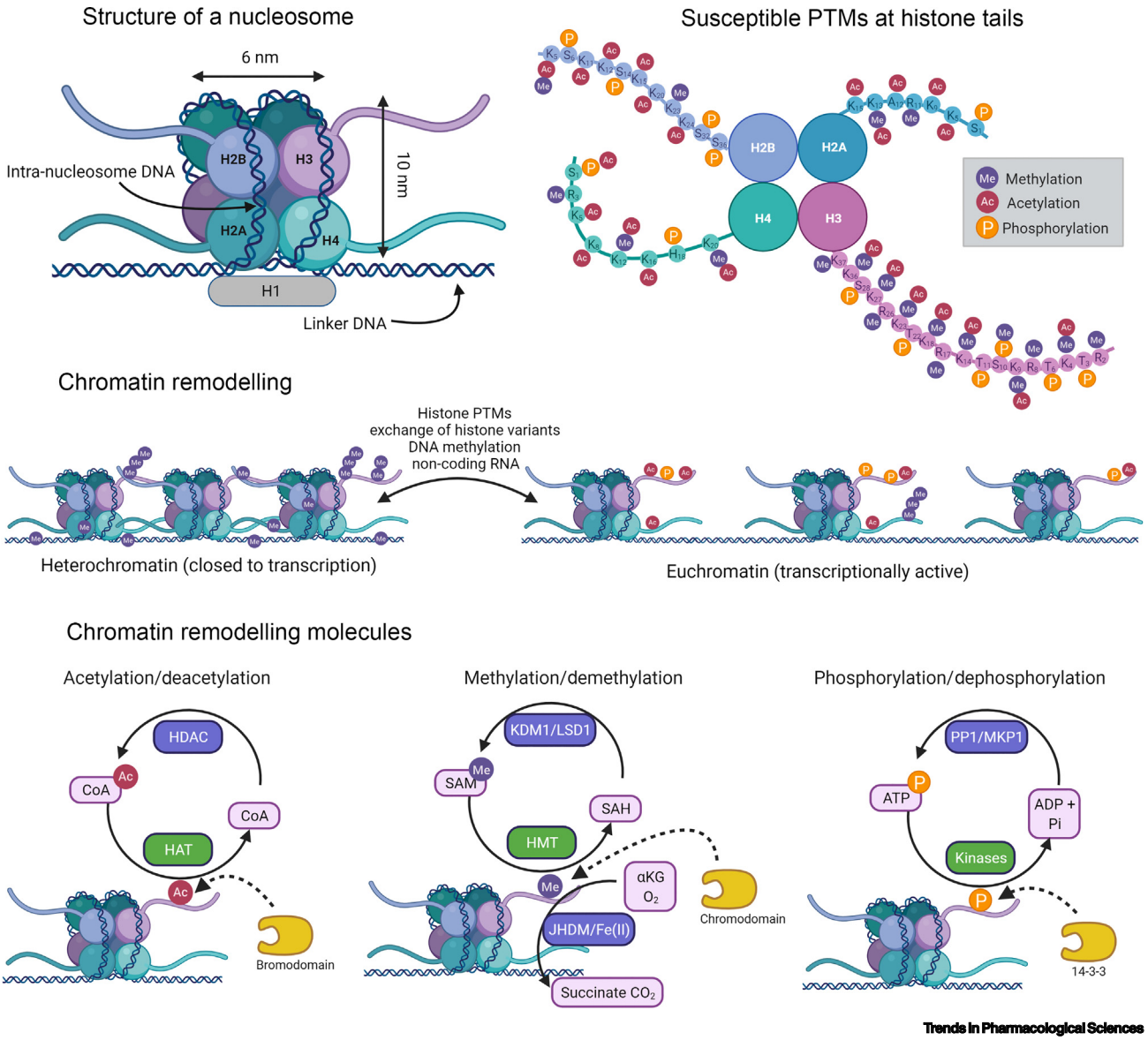

Figure 1. Post-translational modifications (PTMs) at histone tails and their impact on chromatin remodelling. The nucleosome, the smallest unit of chromatin, comprises intranucleosome DNA, core histone proteins, and the outer histone H1 (Box 1). Histone tails undergo PTMs at different amino acids and play a role in chromatin remodelling and DNA accessibility to transcription. Acetyl, methyl, and phosphoryl are added to those amino acids by writer enzymes (green), recognised by reader proteins (yellow), and removed by eraser enzymes (purple). For more details, see Box 2. Created with BioRender.com.

following CFA injection [36]; nonetheless, the lack of change in gene expression for this co-transporter might suggests that its reduction is due to proteolysis or phosphorylation and not causally linked to the changes in histone acetylation. Similarly, H3ac and H4ac at the **brain-derived neurotrophic factor (BDNF)** promoter have been reported to be enriched in DRG neurons following SNL [37]. By contrast, CCI led to a significant spinal increase in H3ac at the promoter of *Wnt3a* [38].

Despite the global hyperacetylation reported in the NRM, Pan's group detected a decrease of H3ac at the promoter of glutamic acid decarboxylase 65 (*Gad65*), a GABA-synthesising enzyme

Table 1. List of changes in histone PTMs and associated proteins observed in nociceptive models

PTM/enzyme	Animal	Model	Site	Behaviour assessed	Target (gene or enzyme)	Refs
H3K4me3, H3K9me2, H3K27me3	Rat	SNL	DRG	Mechanical	*Panx1*	[21]
H3K27me3	Rat	Partial SNL	SC neurons, microglia	Thermal, mechanical	*EZH2*	[86]
H3K9me2, H3K27me3, G9a	Rat, mouse	SNL	SC neurons, DRG	Thermal, mechanical	*Kcna4*, *Kcnd2*, *Kcnq2*, *Kcnma1*	[24]
H3K9me2, G9a	Mouse	SNL, CCI	DRG	Thermal, mechanical, cold	*Kcna2*	[26]
G9a, C/EBPβ	Mouse	CCI	DRG	Thermal, mechanical, cold	*Oprm1*, *Kcna2*	[25]
JMJD6	Rat	CCI	SC neurons	Thermal, mechanical	*NF-κB p-p65*	[27]
H3ac, H4ac	Rat	SNL, CFA injection	DRG neurons and glial cells	Mechanical	–	[31]
		CFA injection	SC neurons	Thermal, mechanical	*KCC2*	[36]
	Mouse	SNL	DRG neurons	–	*bdnf*	[37]
H3ac	Rat	CCI	SC neurons	Thermal, mechanical	*Wnt3a*	[38]
	Rat, mouse	CFA injection, SNL	NRM	Thermal	*Gad2*, *Gad65*	[33]
H3K9a	Mouse	TIC	Trigeminal ganglia	Mechanical	34 various genes	[32]
	Rat	SNL	DRG	Mechanical	*Panx1*	[21]
		Elevated corticosteroids (CORTs)	Central amygdala (CeA)	Somatic and visceral hypersensitivity and anxiety-like behaviours	*crf*	[34]
H3K27a	Mouse	CFA, CCI	DRG	Mechanical	*Grm2*	[48]
NRSF, H4ac	Mouse	SNL	DRG	Thermal, mechanical	*MOP*, *Nav1.8*	[35]
p300-CBP	Rat	CCI	SC neurons	Thermal, mechanical	*COX2*, *BDNF*	[39]
	Mouse	Formalin injection	DRG	–	*mGluR-2*	[41]
HDAC4	Mouse	CFA injection	DRG	Thermal, mechanical	*Calca*, *TRPV1*	[46]
H3S10p	Rat	Burn injury, carrageenan, capsaicin injection, electrical stimulation	SC neurons	Thermal	*cFos*	[65]
		Formalin, capsaicin injection		Nocifensive behaviour	*Zif268*	[66]

important for synaptic inhibition [33], and an increase of H3K9ac at the promoter of *Panx1* in the DRG following SNL [21].

Histone acetyltransferase (HAT) and HDAC imbalances during pain processing

The expression and function of enzymes with HAT/HDAC activity have also been shown to play important roles in the transition from immediate to lasting pain. Both of the transcription coactivator proteins p300 [or E1A-binding protein p300 (EP300)] and CREB-binding protein (CREBBP or CBP) have HAT activity as well as bromodomains to read acetylated lysines. They are important in the downstream acetylation of p65/RelA, which in turn activates the expression of NF-κB-target genes. Accordingly, p300-CBP has been shown to upregulate the expression of cyclooxygenase-2 (COX-2) and BDNF in the spinal cord of rats 14 days after the induction of CCI [39,40]. p300-CBP also upregulates the expression of type 2 metabotropic glutamate receptors (mGlu2Rs) in the DRG of mice after ~20 min following formalin injection, which is used to model acute inflammatory pain [41]. A shift toward enhanced histone acetylation, via decreased HDAC activity, in the spinal cord has also been linked with opioid-induced long-lasting neuroplasticity changes in mice [42,43].

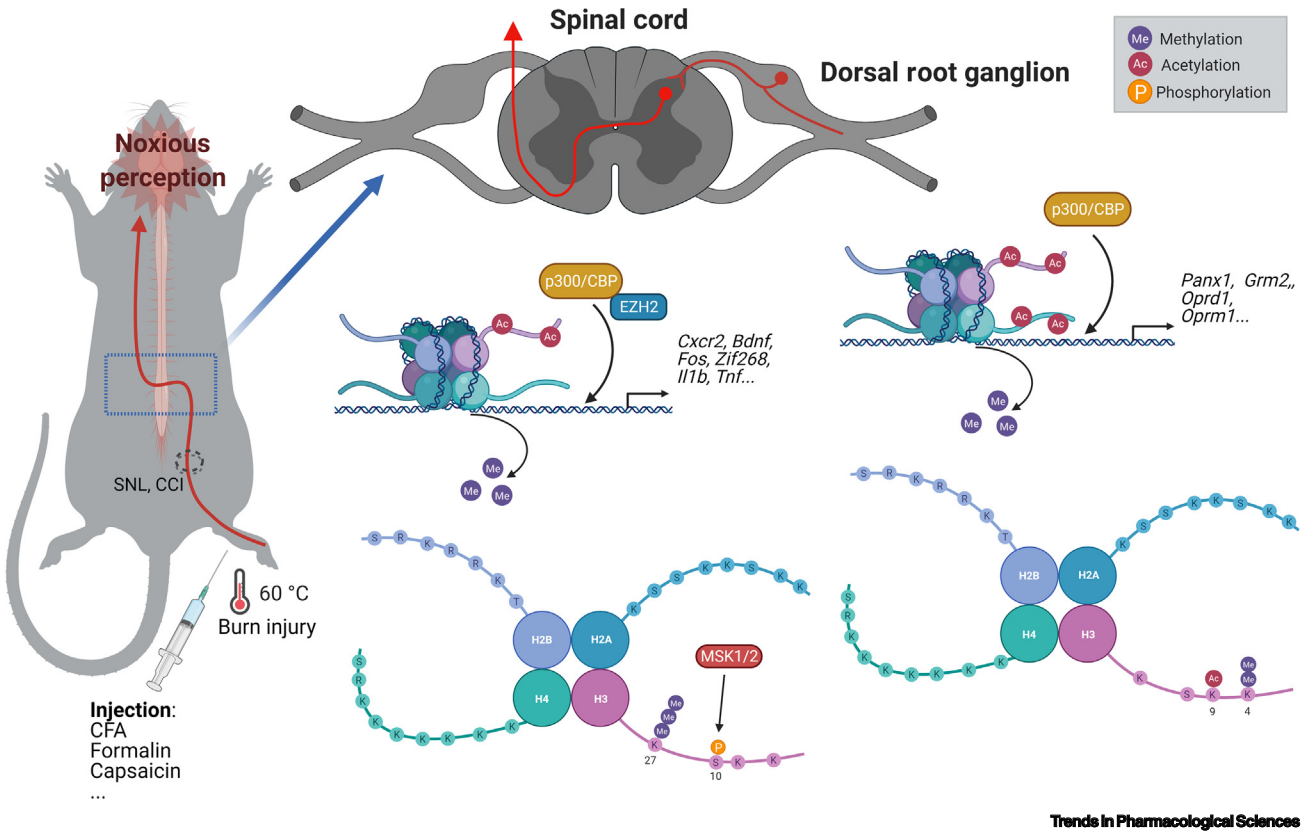

Figure 2. Changes in histone post-translational modifications (PTMs) associated with pain processing in preclinical models.

For a Figure360 author presentation of Figure 2, see the figure legend at https://doi.org/10.1016/j.tips.2021.08.002.

In vivo pain models include scalding-type burn injury, orofacial or intraplantar injections (complete Freund's adjuvant, formalin, carrageenan, or capsaicin), spinal nerve ligation (SNL), and chronic constriction injury (CCI). Once activated, nociceptors send signals to the spinal cord and, ultimately, the brain, where the noxious activation is perceived. Nociceptors reside in the dorsal root ganglion (DRG) (right column). The figure summarises histone PTMs associated with changes in gene expression observed during pain paradigms at the spinal cord (central column) and DRG (right column) level. Created with BioRender.com.

A study by Bai and coauthors analysing the levels of acetylated H3 lysine 9/18 (H3K9/18a) demonstrated that the spinal cord activity of class II HDACs (HDAC4, HDAC5, HDAC7, HADC9), and not class I HDACs (HDAC1, HDAC2, HDAC3), is critical for the induction and maintenance of inflammatory heat hyperalgesia after CFA [44]. Similarly, HDAC4, which was previously associated with synaptic plasticity, was shown to play a crucial role in acute thermal nociception [45] and the development of thermal hyperalgesia following CFA injection [46] in mice. HDAC4 has been suggested to indirectly lead to increased expression of genes involved in heat sensitivity, including *Calca* and **transient receptor potential cation channel subfamily V member 1 (*Trpv1*)** [46]. In addition, the reduced expression of *Gad65* at the NRM was found to be associated with increased activity of HDAC4 and the class I HDACs HDAC1 and HDAC2 [33].

Could modulation of histone acetylation be a novel target for pain management?

The first evidence that HDAC inhibitors (HDACIs) could induce analgesia was provided by the teams of Chiechio [41] and Bai [44]. Both groups reported impaired development of thermal hyperalgesia when animals were treated intrathecally with class I and II HDACIs, suberoylanilide hydroamic acid (SAHA), trichostatin A (TSA), or LAQ824, or class IIa HDACIs, valproic acid

Table 2. Summary of pharmacological targets and mechanisms of action for various epigenetic drugs targeting histone PTMs during pain paradigms

Preclinical						
Epigenetic modifier	Chemical name	Target/selectivity	Animal model	Administration	Pain modality	Refs
HATI	ACA	HAT	Mice: PSL	i.p.	Hypersensitivity	[87]
			Mice: incision in hind paw skin	i.p.	Mechanical and thermal hypersensitivity	[88]
	C646	HAT (p300/CBP)	Rats: CCI	i.t.	Thermal and mechanical hypersensitivity	[39]
	Curcumin (diferuloylmethane)	HAT (p300/CBP)	Rats: CCI	i.p.	Hypersensitivity in neuropathy	[40]
			Mice: OIH	i.p.	Thermal and mechanical hypersensitivity	[43]
HDACI	4-PB	HDAC class IIa	Mice: CFA	i.t.	Thermal hypersensitivity	[44]
	Baicalin	HDAC1	Rats: SNL	i.t.	Thermal and mechanical hypersensitivity	[89]
	LAQ824	HDAC class II	Mice: CFA	i.t.	Thermal hypersensitivity	[44]
	MS-275	HDAC (1 and 3 selectively)	Mice: formalin	s.c.	Persistent inflammatory pain second phase	[41]
	MS-275 and MGCD0103	HDAC class I	Rats: SNL, PSL, and peripheral neuropathic pain induced by d4T	i.t.	Thermal and mechanical hypersensitivity	[51]
	SAHA	HDACs class I and II	Rats and mice: CFA, SNL	i.p. infusion into NRM	Chronic pain, thermal hypersensitivity	[33]
			Mice: PSL	Local application i.pl.	Hypersensitivity of C-fibres and injury-induced thermal and mechanical hypersensitivity	[35]
			Mice: incision in hind paw	i.p.	Mechanical hypersensitivity exacerbated, no impact on thermal hypersensitivity	[88]
			Mice: formalin	s.c.	Persistent inflammatory pain second phase	[41]
			Mice: CFA	i.t.	Thermal hypersensitivity	[44]
			Mice: OIH	i.p.	Thermal and mechanical hypersensitivity	[43]
	Resveratrol (Sirt1 activator)	HDAC class III	Rat: CCI	i.t.	Thermal and mechanical hypersensitivity	[90]
	Sodium butyrate	HDAC class I and II	Rats: CCI	Orally	Neuropathic pain hypersensitivity (including cold, mechanical, and thermal hypersensitivity)	[50]
	TSA	HDAC class I and II	Mice: CFA	i.p.	CFA-induced changes in WT mice but no hypersensitivity developed in *GAD2*$^{-/-}$ mice	[33]
			Mice: CFA	i.t.	Thermal hypersensitivity	[44]
			Mice: PSL	Local application i.pl.	Hypersensitivity of C-fibres and injury-induced thermal and mechanical hypersensitivity	[35,91]
			Female adult rats: endometriosis	s.c.	Thermal hypersensitivity	[92]
			Rats: repeated water-avoidance stress	i.c.v. cannula	Stress-induced visceral hypersensitivity	[93]
	VPA	HDAC class I (1–3 and 8) and class IIa (4, 5, 7, and 9)	Rats: SNL	Orally	Neuropathic pain hypersensitivity	[94]
			Mice: PSL	Local application i.pl.	Hypersensitivity of C-fibres and injury-induced thermal and mechanical hypersensitivity	[35,91]

Table 2. (continued)

Preclinical						
Epigenetic modifier	Chemical name	Target/selectivity	Animal model	Administration	Pain modality	Refs
			Female adult rats: endometriosis	Orally	Thermal hypersensitivity	[95]
			Mice: CFA	i.t.	Thermal hypersensitivity	[44]
			Rat: SNL	Orally	Thermal hypersensitivity	[96]
			Rat: forced swim	i.p.	Stress-induced somatic hyperalgesia and visceral hypersensitivity	[97]
HPI	SB727651A	MSK1 inhibitor	Mice: i.pl. formalin, i.pl. capsaicin	i.t.	Thermal and mechanical hypersensitivity	[66]
			Rats: i.pl. capsaicin, sustained electrical activation	Incubating SC slices in the drug solution	Thermal and mechanical hypersensitivity	[65]
Clinically tested/approved						
Epigenetic modifier	Chemical name	Target	Clinical status	Administration	Pain modality	Refs
HDACI	Givinostat (ITF 2357)	HDAC class I and II	Phase II	Orally	Idiopathic form of juvenile arthritis	[98]
	Ricolinostat	Selective HDAC6	Phase II	Orally	Diabetic neuropathic pain	[54]
	Romidepsin	Selective HDAC6	Approved for use in clinic against CTCL and PTCL; Phase II for multiple myeloma	i.v. infusion	N/A Some multiple myeloma patients reported alleviated bone pain	[99]
	VPA	HDAC class I (1–3 and 8) and class IIa (4–5, 7, and 9)	Failed clinical trial	Orally	Chronic pain after amputation surgery	[53]
EZH2I	Tazemetostat (EPZ-6438)	EZH2	Phase II, multicentre study in adult subjects	Orally	INI1-negative tumours or relapsed/refractory synovial sarcoma	[28,29]

(VPA) or 4-phenylbutyrate (4-PB) [44], or intraperitoneally with SAHA or the class I HDACI 2-aminobenzamide (MS-275) [47].

Notartomaso and colleagues showed that repeated administration of L-acetylcarnitine (LAC), an epigenetic drug that acetylates both histones and p65/relA and is currently prescribed for fibromyalgia-associated neuropathic pain, can reduce mechanical allodynia in both CFA and CCI models [48,49]. They reported that LAC injection, through increased H3K27a at the promoter of *Grm2* (a mGlu2 receptor-encoding gene) in the DRG, led to the upregulation of mGluR2 in the spinal dorsal horns. Similarly, Kukkar's group found that repeated administration of sodium butyrate, a class I and II HDAC inhibitor, attenuated hyperalgesia and allodynia, while it increased levels of TNF-α, in a CCI model [50].

Denk and colleagues showed that repeated intrathecal injections of the class I HDACI MS-275 reduced both mechanical and thermal sensitivity in models of neuropathic pain, but only if treatment started before the induction of pain [51]. They suggested that MS-275 altered the expression of various proinflammatory mediators, including IL-1β and TNF-α [52]. The TIC injury study mentioned above also demonstrated that HDACIs could prevent persistent hypersensitivity in the orofacial neuropathic pain model. Treatment with SAHA and MS-275 before injury reduced whisker-pad mechanical hypersensitivity and prevented the development of a persistent pain after the injury [32].

Box 2. PTMs of histones

Acetylation: adding an acetyl group (C_2H_3O) to the NH_3^+ of the amino acid lysine (K).

- Neutralises positive charge, which weakens the interaction between histones and DNA, relaxes the chromatin structure, and promotes gene expression.
- HATs are the writer enzymes that transfer the acetyl groups from a molecule of acetyl coenzyme A (acetyl-CoA).
- HATs can also have other domains that interact with different epigenetic motifs.
- HDACs are the eraser enzymes. HDACs are grouped in four classes (I–IV) according to their sequence homology.
- A dynamic HAT/HDAC balance is required to deliver a specific pattern of gene expression.
- Bromodomain-containing proteins recognise and bind to acetylated histones.

Methylation: addition of methyl groups ($-CH_3$). Both K and arginine (R) are susceptible to mono-, di-, and trimethylation.

- Does not alter histone charge but affects the ability of proteins to recognise/bind to the nucleosome or specific DNA motifs (e.g., CpG islands).
- Most seen on K of H3 and H4 histones.
- Correlates with either transcriptional repression or activation depending on the PTM; for example, dimethylation at lysine-9 (H3K9me2) and trimethylation at lysine-27 (H3K27me3) of histone H3 favour gene silencing, while trimethylation at lysine-4 of H4 (H3K4me3) is considered permissive.
- Written by HMTs, which are either R or K specific (KTMs).
- Reader proteins with specific 'reader' domains, chromodomains, can interact, including the malignant brain tumour (MBT), PHD finger, and Tudor domains.
- Histone K can be demethylated by two families of K-specific histone demethylases (KDMs): FAD-dependent amine oxidase (KDM1 or LSD1) and Fe (II)- and 2OG-dependent JmjC-domain-containing proteins.
- Only two histone R demethylases have been identified so far, PAD4 and Jumonji domain containing 6 (JMJD6).

Phosphorylation: addition of phosphoryl groups ($P^+O_3^{2-}$).

- Increases negative charges (may disrupt histone–DNA interactions). They might also interfere with the ability of other proteins to bind/recognise specific DNA motifs.
- All core histones are susceptible at different residues, including serine (S), threonine (T), tyrosine (Y), and histidine (H).
- Phosphorylated histones can establish crosstalk with other histone PTMs and serve as recruiting platforms for other effectors; for example, histone H3 at S10 (H3S10p), S28 (H3S28p), and T11 (H3T11p) have been associated with H3 acetylation.
- The writer enzymes are kinases, which mediate the transfer of a phosphate moiety from the high-energy molecule ATP.
- The main readers belong to the 14-3-3 protein family, with seven members in mammals.
- The proteins with phosphatase activity (erasers) include protein phosphatase 1 (PP1) and MAPK phosphatase 1 (MKP1).

One clinical study has recently reported that perioperative administration of the HDACI VPA failed to prevent the development of chronic pain after amputation surgery [53]. Currently, there is one ongoing trial in Phase II for the use of the HDACI ricolinostat, a drug also tested for cancer, as a potential treatment for diabetic neuropathic pain [54]. However, while HDACIs have proved to be safe to use, the relative lack of specificity of their mechanisms and their controversial effect on pain management make their use still problematic [55]. Some studies have shown that HDACIs can increase pain sensitivity in naïve animals [56], which adds to their controversial use as therapeutics.

Histone phosphorylation

Histone phosphorylation during tissue injury/inflammatory pain

Phosphorylation of histone H3 at serine 10 (H3S10p) is associated with transcriptional activation in response to external stressors, growth factors and cytokines in post-mitotic cells. This process is directed to a small set of genes, which includes the immediate early genes (IEGs) *c-fos*, *c-jun*, and *c-myc* [57,58]. Interestingly, the kinases involved are distinctive for each process [59,60].

A stimulus-specific increase of H3S10p has been identified in hippocampal and prefrontal neurons of rodents during events of synaptic plasticity and memory consolidation [61–63]. In post-mitotic neurons, this epigenetic tagging is mediated via glucocorticoid, α-amino-3-hydroxy-5-methyl-4-

isoxazolepropionic acid (AMPA), and *N*-methyl-D-aspartate (NMDA) receptors and involves transcriptional activation of the IEGs *c-fos* and *zif-268* [61–64]. Therefore, H3S10p has been considered to play an important role during neuronal plasticity and to have a possible involvement during pain sensitisation.

We, together with Tochiki and colleagues, described a rapid and transient upregulation of H3S10p in a subpopulation of dorsal spinal cord neurons following inflammation and tissue injury in rodents [65,66]. The induction of scalding-type burn injury, intraplantar injection of the inflammation-inducing agent capsaicin or carrageenan, or electrical stimulation of C-fibres led to a specific upregulation of H3S10p at the nuclei of a group of neurons in the superficial spinal dorsal horn. That upregulation peaks minutes after the injury and lasts at least for several hours. By contrast, low-frequency electrical stimulation or brief noxious stimulations, which do not induce tissue damage, failed to induce this upregulation [65]. Similarly, peripheral formalin injection increased H3S10p expression in a group of superficial spinal dorsal horn neurons with a maximal peak at 30 min post-injury [66].

The upregulation in H3S10p expression appears to be the result of NMDA receptor activation, as the NMDA inhibitor D-APV blocked the increase in H3S10p expression [65]. It has been hypothesised that NMDA activation leads to phosphorylation of the mitogen-activated protein kinases (MAPKs) extracellular signal-regulated 1 and 2 (pERK1/2) and, following ERK1/2 nuclear translocation, subsequent phosphorylation of **mitogen- and stress-activated kinase 1 and 2 (MSK1/2)**, the writers of this epigenetic modification. In turn, H3S10p induced transcriptional activation of the IEGs *c-Fos* [65] and *Zif268* [66]. H3S10p has also been associated with the expression of *Cox-2* in murine epidermal cells following UVB stimulation [67].

Recently, we have investigated the type of spinal cord neurons that express H3S10p after burn injury [68]. Surprisingly, only a few projecting neurons exhibited phosphorylation of H3S10. The majority of the H3S10p-expressing neurons were dynorphin-expressing excitatory neurons. However, a few inhibitory neurons expressing this PTM were also seen. H3S10p is expected to be involved in pronociceptive mechanisms, as deletion of its writers MSK1/2 results in a lack of inflammatory heat hyperalgesia [65]. Hence, the inhibitory neurons exhibiting H3S10p expression could inhibit other inhibitory neurons in the spinal dorsal horn.

Could histone phosphorylation be targeted to mitigate or block inflammatory pain?

It has been suggested that H3S10p serves as the rate-limiting step during signal integration and chromatin remodelling [64,67,69,70]. This epigenetic tagging, affecting a small population of nucleosomes, seems to facilitate and direct the fast activation of other chromatin remodellers to shift the transcriptional balance between gene activation and repression [64,71,72]. For instance, the activity of some HATs, including Gcn5, which induces H3K14a and thus enhances gene transcription, seems to be promoted by the presence of H3S10p [69,73]. Therefore, in addition to its role during initial pain processing, H3S10p can be important for pain maintenance by altering the expression of nociceptive-relevant genes at later timepoints. Thus, stopping this phosphorylation might prevent a series of transcriptional changes accompanying tissue injury and inflammation.

Importantly, our study showed that deletion of MSK1/2, in addition to blocking inflammation-induced upregulation in H3S10p, also prevents the development of inflammatory heat hyperalgesia [65]. Similarly, animals that received an intrathecal injection of the MSK1 inhibitor SB727651A prior to the formalin test failed to develop nocifensive behaviour associated with sensitisation [66]. Thus, these preclinical data seem to suggest that blocking MSK1/2-mediated H3S10p might be of

therapeutic value for the treatment of heat hyperalgesia in tissue injury/inflammation. This approach might be particularly useful as MSK1/2, in addition to H3S10, could also phosphorylate other targets in neurons, including the cAMP response element-binding protein (pCREB) and NF-κB, transcription factors previously implicated in the expression of pronociceptive genes [74]. However, MSK1/2's ubiquitous expression patterns significantly narrow the therapeutic potential of these enzymes. Nonetheless, elucidating the changes in gene expression regulated by this epigenetic writer may identify crucial regulatory molecules involved in the development of inflammatory heat hyperalgesia [74].

Concluding remarks and future directions

Possible issues when translating epigenetic findings to the clinic

Elucidating the influences that PTMs, together with other epigenetic motifs, pose for gene transcription during pain signalling must be done with caution. Simple correlational studies can be misinterpreted as causal interactions. The mechanisms by which different changes in PTMs and their writers, readers, and erasers crosstalk to deliver a specific expression pattern at a specific moment of time are not completely understood. Therefore, the functional regulatory effect of a certain epigenetic transformation must be linked to the changes of expression in a certain gene, or group of genes, in a specific cell type in a tissue and at a particular time during pain progression [75]. Further, it must be considered that epigenetic mechanisms are also at play during the recovery from pain syndromes after injury. For example, sensitisation-associated chronic itch, which manifests after wound healing is completed, seems to also be subject to epigenetic control [76].

Additionally, the functional transcriptional outcome of a certain epigenetic modification can be influenced by adjacent interactions including the combinatorial effects of multiple epigenetic tags or the recruitment of various chromatin remodellers [15,20].

A drawback of the use of immunohistochemical analysis to assess the combinatorial effect of PTMs is the issue of epitope occlusion [77]. When different PTMs are present in the same histone tail or in close proximity to one another, they could prevent the recognition and binding of specific antibodies used for detection.

The experimental models used to assess epigenetic changes in the context of pain can also lead to contradictory findings. While *in vitro* models may seem a feasible approach to assess changes in a cell-type-specific manner, thus avoiding tissue heterogeneity, the observed interactions in such experiments may not reflect those occurring in an *in vivo* model [20]. Animal models are the best suited to assess epigenetic changes during pain processing. They offer a feasible scenario in which to assess the transcriptional changes occurring during pain processing in relevant tissues such as DRG or spinal cord. However, researchers must also be aware that different organisms might favour different mechanisms to regulate the same set of epigenetic modifications and that epigenetic processing is dynamic and affected by other variables such as sex and age [78–80].

Additionally, the relationships between epigenetic modifications and transcriptional changes might not be as linear as some researchers imply or correlations might be misinterpreted as causations [81]. Importantly, the quantification methods used to assess epigenetic changes might differ in efficacy or rigorousness between studies, all of which add to the incongruences observed between investigations. Therefore, it is difficult at times to extrapolate these observations to the clinical setting.

Future perspective on epigenetic research for pain processing

The development and use of novel approaches, including high-resolution imaging and single-cell sequencing coupled with multiomics and refined computational analysis, will complement the

Outstanding questions

How do epigenetic changes develop and interact during the transition from acute to long-lasting pain? How do they correlate with changes in gene expression?

Can we separate the epigenetic signatures associated with pain progression and consolidation from those associated with wound healing and functional recovery?

How can specific correlations between epigenetic modifications and changes in gene expression be causally investigated?

Could novel technological advances, including CUT&RUN, CUT&Tag, and ATAC-seq, serve to elucidate cell-type-specific changes in the nociceptive pathway?

What are the challenges for the assessment of the epigenome in the human nociceptive system?

How could next-generation epigenetic drugs be designed to tailor specific changes selectively at nociceptive-relevant cell types? What drug delivery methods could be implemented?

already established techniques in the field, such as flow cytometry, quantitative mass spectrometry, and ChIP combined with gene sequencing (ChIP-seq). The implementation of **cleavage under targets and release using nuclease (CUT&RUN)** and/or **CUT and tagmentation (CUT&Tag)**, which can precisely identify the binding sites of certain DNA-associated proteins (e.g., specific histone PTMs, transcription factors) even at (nearly) single-cell resolution, and assay for transposase-accessible chromatin using sequencing (ATAC-seq), able to elucidate open regions of the genome even at single-cell-type resolution (scATAC-seq), are examples of techniques that promise to dramatically enhance our understanding of the cell-specific mechanisms underpinning pain [82].

An important goal would be to complete the epigenome map of pain signals with cellular and temporal resolution after specific tissue injuries to establish an exhaustive description of the epigenetic modifications that regulate gene expression throughout pain processing leading to its persistent state in relevant tissues and within each cellular type at individual time points (see Outstanding questions). Completion of this pain epigenomic atlas will allow the identification of targets for molecular understanding of the transition from acute to chronic pain and thus the elucidation of better ways to reverse this process with the objective of delivering tailored therapeutic interventions. To that end, initiatives such as the NIH Roadmap Epigenomics Mapping Consortium (http://www.roadmapepigenomics.org/), the PsychENCODE Consortium (http://www.psychencode.org/), and the BluePrint Epigenome (https://www.blueprint-epigenome.eu/), aiming to produce a public resource of epigenomic data to assess basic and disease-oriented research, should be encouraged.

Last, most of the readers, writers, and erasers of histone PTMs are likely to be involved in myriad cellular processes, including the state of non-histone proteins or the methylation of nucleic acids [55,83]. Thus, although the amount of preclinical and clinical data describing epigenetic interventions for pain management is constantly increasing, alternatives to prevent this limitation are still needed. Those could include the use of local or cell-specific delivery, whenever possible, and the design of more targeted and specific inhibitors with cell-type and temporal selectivity as well as spatial regulation [6,84,85].

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Author contributions

J.V.T-P.: manuscript editing and writing, preparation of tables and figures, references; M.R.F.: manuscript writing, Table 1 and figure preparation; J.I.: manuscript writing, Table 2 and figure preparation; S.D.G.: writing, editing, and supervision; I. N.: manuscript editing, writing, and supervision.

Declaration of interests

The authors have no interests to declare.

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