Drugging the pain epigenome

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Abstract | More than 20% of adults worldwide experience different types of chronic pain, which are frequently associated with several comorbidities and a decrease in quality of life. Several approved painkillers are available, but current analgesics are often hampered by insufficient efficacy and/or severe adverse effects. Consequently, novel strategies for safe, highly efficacious treatments are highly desirable, particularly for chronic pain. Epigenetic mechanisms such as DNA methylation, histone modifications and microRNAs (miRNAs) strongly affect the regulation of gene expression, potentially for long periods over years or even generations, and have been associated with pathophysiological pain. Several studies, mostly in animals, revealed that inhibitors of DNA methylation, activators and inhibitors of histone modification and modulators of miRNAs reverse a number of pathological changes in the pain epigenome, which are associated with altered expression of pain-relevant genes. This epigenetic modulation might then reduce the nociceptive response and provide novel therapeutic options for analgesic therapy of chronic pain states. However, a number of challenges, such as nonspecific effects and poor delivery to target cells and tissues, hinder the rapid development of such analgesics. In this Review, we critically summarize data on epigenetics and pain, focusing on challenges in clinical development as well as possible new approaches to the drug modulation of the pain epigenome.

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Pain is one of the leading characteristics of many diseases and presents a major health burden worldwide[1,2]. Despite the large number of approved analgesics, many patients who are in pain are inadequately treated, with a consequent reduction in their quality of life. Effective and safe analgesics are still urgently needed, and the development of such drugs depends on detailed knowledge of the cellular and molecular mechanisms of pain processing. Although ongoing inflammation or tissue and nerve damage are well known to cause molecular changes in the CNS and PNS that are associated with pain hypersensitivity (caused by central and peripheral sensitization mechanisms, respectively), the detailed sensitization mechanisms, particularly with regard to pain chronification, remain unclear.

Epigenetics has been defined as the cellular mechanisms that enable "the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states" (REF. 3), with regard to modulated gene expression. Moreover, this altered activity state is sustained, even when the original stimulus has faded and, consequently, adaptations can be long-lasting, and are thought to have a role in processes such as memory formation[4–7]. Accordingly, epigenetic adaptations probably regulate pain sensitization, which could result in chronically increased nociceptive responses to noxious stimulation (that is, hyperalgesia) or nociceptive reactions to non-noxious stimuli (that is, allodynia)[8,9]. Without pharmacological treatment, such pathophysiological pain can become chronic or develop into stimulus-independent pain.

The field of epigenetics has attracted increasing attention over the past decade[10], not only in relation to general epigenetic mechanisms, but also in relation to translation of epigenetic concepts to other research fields, such as pain and pain therapy[11–15]. In this Review, we critically discuss the status of epigenetic research in pain. This research largely consists of preclinical proof-of-concept studies, predominantly in models of acute or subacute pain. In contrast to previous reviews in this field, we address the lack of clinical studies and the challenges to the development of well-tolerated epigenetic modifiers for clinical therapies of chronic pain states.

Introduction to epigenetic processes

Epigenetics refers to long-lasting adaptations to environmental conditions that can be passed on to daughter cells[16]. In mammals, some changes in epigenetic states can even be observed in the subsequent generation, but this observation does not mean that they will be readily transmitted to further generations[17].

One key issue with epigenetic changes is that, despite their possible long-lasting and persistent nature, they can be further modified or reversed at a later time. In this

Key points

- Pathophysiological pain such as inflammatory, neuropathic and cancer pain is characterized by increased pain sensitivity
- Despite a number of approved analgesics, pain therapy is often unsatisfactory because of low efficacy and adverse effects
- Pathophysiological pain is associated with a number of different types of epigenetic modulation
- Future therapeutic interventions in patients who experience pain might target tissue-specific and cell-specific epigenetic mechanisms with histone deacetylase inhibitors, DNA methyltransferase inhibitors, and microRNA mimics or inhibitors

way, the permanent exposure of an organism to environmental factors presents an inexhaustible source of external stimuli, each of which could influence the activity states of genes in corresponding organs, tissues and cells.

The underlying cellular mechanisms of epigenetics involve the concerted action of transcription factors, chromatin-modifying enzymes and chromatin-remodelling complexes (FIG. 1) as well as non-coding RNAs (FIG. 2)[18–22]. Although the whole machinery is far from being completely understood, several key enzymes and their modifications have been identified, and have attracted substantial attention as hallmarks of specific gene expression states[23,24].

Histone modifications

In eukaryotes, DNA is wrapped around two copies each of the histone proteins H2A, H2B, H3 and H4 to form nucleosomes. Several post-translational histone modifications have been identified so far[19], which are involved in regulation of gene expression, DNA repair and DNA replication[25,26]. These modifications are dynamic, reversible, interconnected and can have either a short-term or long-term duration. To date, changes in histone acetylation regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs; BOX 1) have been the focus of many studies on pain. So far, 18 mammalian HDAC genes have been identified, which are grouped into four phylogenetically derived classes (classes I to IV)[27,28]. Importantly, HDACs are not only responsible for deacetylation of histones but are also able to deacetylate other cytoplasmic target proteins[29]. HATs are classified into several structural families; the three major classes are the GCN5-related *N*-acetyltransferase family, the p300–CREB-binding-protein (CBP) family and MYST proteins[30,31]. The neutralization of the positive charge of the lysine side chain through acetylation by HATs leads to a relaxation of the chromatin structure, facilitating binding of transcription factors and leading to an increase in gene expression[20]. By contrast, deacetylation by HDACs stabilizes the positive lysine charge, enhances the binding of DNA to the histones and thereby hinders transcription factor activity. Deregulation of HDACs is associated with the initiation and progression of cancer, which has led to the development of a number of HDAC inhibitors as treatments for different tumours[32].

Four HDAC inhibitors have been approved for use in patients with T-cell lymphoma. For the cutaneous form of the disease, vorinostat (also known as SAHA) was approved in 2006 (REF. 33) and romidepsin was approved in 2009 (REF. 34). For peripheral T-cell lymphoma, belinostat and panobinostat were approved in 2015 (REF. 35). In the same year, panobinostat was approved for combination therapy in patients with multiple myeloma[36,37]. As these agents are nonspecific inhibitors of HDACs, they could interfere with activation of genes involved in non-oncological processes and might, therefore, also have potential in therapeutic drug repurposing for other diseases, including pain states, neurodegenerative or inflammatory disturbances[28,31,38,39].

Less is known about histone methylation than histone acetylation in relation to the onset and regulation of pain. Histone methylation is controlled by histone methyltransferases (HMTs) and histone demethylases (HDMs), and is involved in positive or negative regulation of gene expression depending on the type of residue (lysine or arginine), the amino acid position and degree of methylation[25,31] (BOX 1).

DNA modifications

DNA itself can be modified epigenetically by methylation (BOX 2). DNA methyltransferases (DNMTs) are responsible for adding a methyl group to cytosine at the 5′-carbon position of the pyrimidine ring. In mammals, this modification occurs predominantly to cytosines that are followed by a guanosine, at so-called CpG sites. Maintenance of methylation occurs during DNA replication when the methylation mark of the mother strand is copied to the daughter strand. Moreover, *de novo* methylation plays an important part in development and cellular differentiation[40]. In most cases, DNA methylation leads to inhibition of gene transcription, either by suppression of transcription factor binding to the DNA or by recruitment of a repressor complex consisting of methyl-CpG-binding protein 2 (MeCP2) and different HDACs[41]. By contrast, several incompletely elucidated, direct and indirect mechanisms control DNA demethylation[42,43]. Dysregulation of DNA methylation is associated with cancer, schizophrenia or addiction to opioids[44–46]. Two DNMT inhibitors, 5-azacytidine and decitabine (5-aza-2′-deoxycytidine) were approved in 2004 and 2006, respectively, for the treatment of patients with myelodysplastic syndromes[47–49].

microRNAs

microRNAs (miRNAs) are short (~19–25 nucleotides), non-coding RNA molecules that are generated in a multistep process from miRNA genes (FIG. 2). The initial miRNA transcript is processed (via primary miRNA and precursor miRNA intermediates) into mature double-stranded miRNA by the endonucleases ribonuclease 3 (also known as Drosha) and Dicer. The guide strand from this double-stranded miRNA is sequestered into the miRNA-induced silencing complex (miRISC), which controls the binding of miRNAs to their target mRNAs. Perfect binding of the miRNA to its target mRNA sequence leads to RNA degradation, whereas incomplete binding leads to inhibition of mRNA processing; both of these scenarios are associated with inhibition of target gene expression[50]. miRNAs are

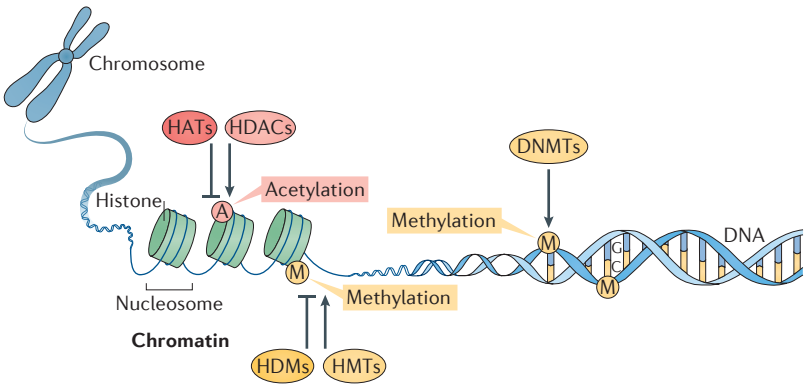

Figure 1 | **Epigenetic mechanisms.** In chromosomes, the DNA is wrapped around individual histone proteins, forming the basic unit of a DNA packaging — the nucleosome. Epigenetic modifications occur at the histones where differential acetylation is controlled by histone deacetylases (HDACs) and histone-acetyltransferases (HATs) and methylation by histone methyltransferase (HMTs) and histone demethylase (HDMs) enzymes. Methylation of the DNA is performed by DNA methyltransferases (DNMTs). A, acetyl group; M, methyl group.

particularly important in the fine-tuning of gene expression and are involved in developmental as well as physiological and pathophysiological processes. Each miRNA can regulate the expression of multiple target genes; conversely, each target gene can be regulated by a number of miRNAs[22]. miRNA dysregulation is well established in the pathogenesis of cancer, cardiovascular diseases and viral infections[51–53]. Moreover, miRNA regulation is involved in the initiation and processing of different pain types, and investigation of the functional roles of miRNAs and their regulation of target genes only began to develop within the past few decades. Direct miRNA involvement in pain was first shown in 2010 using conditional knockdown of Dicer, which led to modification of a panel of pain-relevant target genes and alteration of the pain response[54]. This study was followed by several other reports of functional effects of miRNAs[55,56].

Epigenetic mechanisms in pain

Many human pathological processes are under the influence of environmental factors; consequently, epigenetic changes are clearly associated with the onset and progression of several diseases, such as cancer, inflammation, neuropsychiatric disturbances and pathophysiological pain. A variety of factors have been identified that confer, in humans, vulnerability or resilience towards painful signals. These factors include genetic predisposition, epigenetic processes, priming effects on cells and alterations in brain networks concerned with the descending pain modulatory system (DPMS)[57]. Changes in the DPMS seem to be closely associated with the persistence of chronic pain and a predisposition towards such changes might develop in early life, as described for other neurological conditions such as autism spectrum disorder and schizophrenia[58–61]. For instance, in rats, increased maternal care resulted in demethylation of a CpG-island in the *IL10* gene, which increased its mRNA expression in glial cells in the nucleus accumbens, and thereby reduced subsequent morphine-induced glial activation and prevented abuse behaviour[62]. The possibility that long-term pain sensitivity might be epigenetically regulated by early or previous exposure to a noxious stimulus is very intriguing and might represent a unique indication for epigenetic drugs. However, to date, this option has received little attention and further studies are needed before it could become a therapeutic option.

Epigenetic regulation occurs in developing and in mature non-dividing cells in the nervous system[63], and contributes to neuronal development and differentiation, synaptic plasticity and learning and memory processes through the modulation of a number of genes[4–6]. These mechanisms are similar to those seen in central and peripheral sensitization of the nociceptive system including increased activation of excitatory neurotransmitter receptors or regulation of the same genes, such as inducible nitric oxide synthase or cyclooxygenase (COX)2 (also known as prostaglandin G/H synthase 2) (REF. 8). Epigenetic mechanisms are involved in sensitization of the nociceptive system and in the transition from acute to chronic pain via the direct modulation of pain genes and the nociceptive response. This phenomenon was demonstrated in a genome-wide methylation analysis of monozygotic twins with differing pain sensitivities, which included a follow-up study that indicated that epigenetic regulation of *TRPA1* affects thermal and mechanical pain sensitivity in humans[64,65]. However, chronic pain is a complex and multifactorial disease, and the level at which epigenetic modification occurs is not currently defined. Such changes could act as primary triggers of pain, downstream effectors of pain or could play both of these parts. Furthermore, epigenetic modifications might also modulate downstream consequences of pain itself. Indirectly, epigenetics can control inflammatory and pain signalling pathways by modulation of inflammatory mediators such as cytokines[38,57,66,67]. Most experimental studies have examined short-term pain or nociception, from hours up to a maximum of a few weeks, and addressed the issue of whether or not epigenetic mechanisms are involved, but not when they occur. However, the results of a study published in 2016 suggested that long-term epigenetic alterations in enhancer regions in microglial cells might contribute to long-lasting pain[68]. In addition, the promoter region of *Syt2*, the gene coding for synaptotagmin-2, a synaptic regulatory calcium sensor for fast neurotransmitter release, remained discretely hypomethylated in the prefrontal cortex 6 months after nerve-injury induction of neuropathic pain in mice[69]. Furthermore, epigenetic mechanisms might decrease endogenous synaptic inhibitory pathways for nociceptive signalling — for instance via epigenetically reducing the inhibitory action of GABAergic neurons. Downregulation of this inhibitory pathway would tend to enhance excitatory pronociceptive gene expression, leading to sensitization of the nociceptive system and potentially to chronification of pain[70]. If this indirect sensitization occurs, epigenetic modulators might reverse pronociceptive epigenetic marks and reduce pain hypersensitivity. In addition, standard analgesics

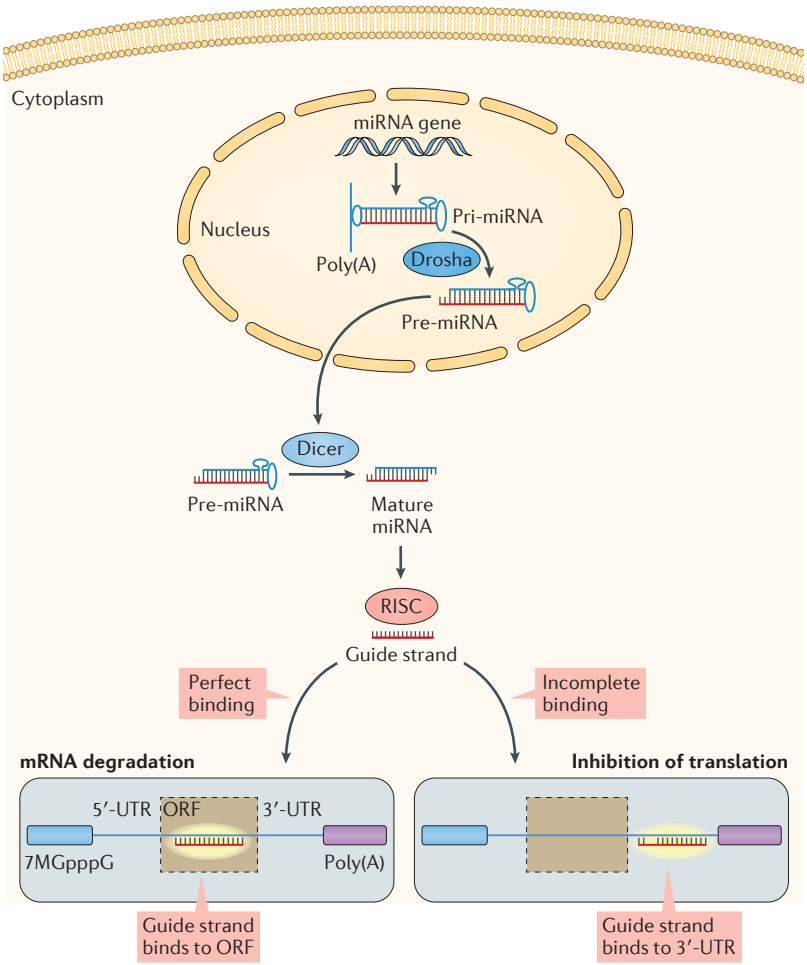

Figure 2 | **miRNA processing.** microRNAs (miRNAs) are encoded by miRNA genes, which are transcribed into double-stranded long-chain primary-miRNAs (pri-miRNAs). These pri-miRNAs are processed by endonuclease ribonuclease 3 (Drosha) to shorter, precursor miRNAs (pre-miRNAs). After translocation of the pre-miRNA from the nucleus to the cytoplasm, endonuclease Dicer cuts the molecule into mature short-chain miRNAs that are loaded onto an RNA-induced silencing complex (RISC). The guide strand of the miRNA binds to the target sequence in the RNA and leads to mRNA degradation after perfect binding and to translational repression after incomplete binding. 7MGpppG, 7-methylguanylate cap; ORF, open reading frame; poly(A), polyadenylation; UTR, untranslated region.

are thought to be able to modify epigenetic mechanisms, including histone acetylation, DNA methylation and miRNAs[71], which might contribute to their analgesic activity. Again, the stage at which these drugs might act has not been identified.

Analgesics as epigenetic modulators

Approved analgesics that exert pain-relevant epigenetic effects fall into several different classes. These include COX inhibitors, opioids and other analgesic drugs, each of which we discuss here.

Cyclooxygenase inhibitors

Aspirin and other NSAIDs, including the COX2 inhibitors celecoxib and sulindac, modulate miRNAs and suppress DNA methylation of several growth genes in human gastric mucosa, rat colon or cultured human colon carcinoma cells[72]. Long-term administration of aspirin and other NSAIDs might, thus, exert therapeutically relevant effects by epigenetic modulation. By contrast, a study in women who regularly used NSAIDs failed to find any substantially increased global CpG methylation levels in the blood[73]. However, in human coronary artery endothelial cells, aspirin inhibited DNA methylation of the *FGF2* promoter and protected the cells from LDL-induced apoptosis[74]. Clearly, an evaluation is needed of the effects of NSAIDs on the selective DNA methylation of specific pain-related genes, as opposed to global methylation alone, before conclusions can be drawn on the mechanisms of their analgesic activity.

Celecoxib is an analgesic and anti-inflammatory drug for long-term therapy of inflammatory pain in musculoskeletal diseases and shows benefit in patients with familial adenomatous polyposis. In rats and patients with colon cancer, celecoxib reversed tumour-induced hypermethylation of *ESR1*, which encodes the oestrogen receptor, and decreased expression of oestrogen receptor mRNA[75,76]. In addition, celecoxib increased histone H3 and H4 acetylation, resulting in reduced tumour growth in liver carcinoma[77], and also altered the expression of different miRNAs in a variety of cancer types such as human colorectal cancer, or breast cancer[78–80]. A number of celecoxib-mediated effects, including inhibition of tumour growth, cannot be explained exclusively by COX2 inhibition, and consequently epigenetic changes could provide insight into possible mechanisms for COX2-independent effects of the drug.

Opioids

Opioids, the most frequently administered analgesics for severe pain, are associated with a variety of effects on epigenetic mechanisms. Morphine reduces expression of histone-lysine *N*-methyltransferase EHMT2 (also known as G9a), which leads to decreased histone H3 lysine 9 dimethylation in the nucleus accumbens of mice, possibly contributing to development of morphine addiction[81]. In white blood cells of patients with opioid addiction who received a methadone substitution, methylation of global DNA and of *OPRM1*, which encodes μ-type opioid receptor, was increased. An increase in global methylation in patients treated with opioids might, therefore, contribute to opioid-induced hyperalgesia[82]. These observations are supported by the finding that treatment of human breast cancer cells with methadone *in vitro* was associated with an increased probability of DNA hypermethylation[83]. Opioids are also reported to have a role in miRNA regulation[84]. Several miRNAs are either upregulated or downregulated after opioid treatment of cells, animals or patients, and might contribute to opioid tolerance or opioid-induced hyperalgesia by regulating μ-type opioid receptors[85]. Thus, opioids are involved in diverse types of epigenetic regulation, and potentially contribute to analgesic effects but also to unwanted effects such as opioid-induced hyperalgesia or addiction. Further research is needed to determine the relative contributions of these epigenetic changes to opioid actions.

Other analgesic drugs

Treatment of chronic neuropathic pain remains a challenge, as common analgesics (such as NSAIDs) are ineffective in this condition. In addition to opioids, current evidence-based therapeutic strategies involve certain antidepressants (such as fluoxetine and amitriptyline) that act at least partially as noradrenaline reuptake inhibitors[86,87], and some antiepileptic drugs[88,89], which can be administered either as monotherapy or combination therapy[90]. Responses to these groups of drugs (outside the context of pain) correlate with several epigenetic effects in animal and cell models, including changes in histone acetylation and DNA methylation[91–94].

These data clearly show that classic analgesics are associated with modulation of epigenetic processes (TABLE 1) — although, until now, this association has not been reported in the context of nociceptive signalling pathways. On the basis of current data, mostly generated in cell culture or in the field of cancer research, the epigenetic effects of these drugs are likely to be direct rather than secondary effects or a consequence of pain relief. However, to clarify this issue, further assessment of the epigenetic properties of these drugs is desirable in pain studies.

Epigenetic modifiers

Modulation of histone modifiers in pain

Histone modifications have been reported to contribute to initiation and processing of pathophysiological pain. However, only a few preclinical studies have focused on histone methylation. For instance, studies have shown that differential methylation of histone amino acids in the promoter region of *CCL7* (encoding C-C motif chemokine 7, also known as monocyte chemotactic protein 3) or *PANX1* (encoding pannexin 1) contributes to neuropathic pain[95,96] and differential methylation of CCL genes (*CCL2* and *CCL3*) contributes to inflammatory pain[97,98]. Long-lasting reduction of K^+ ion channel expression after nerve injury in rats results from histone H3 lysine 9 dimethylation (but not DNA methylation) and silencing of the K^+-channel promoters by G9a. Selective knockdown of G9a in dorsal root ganglion neurons from these rats completely inhibited K^+-channel repression as well as the development of chronic neuropathic pain. The expression of more than 600 regulated genes was also restored[99], indicating a prominent role of G9a-induced dimethylation of histone H3 lysine 9 in the development of neuropathic pain. This assumption is further supported by a study in mice, which showed that *Kcna* (encoding potassium voltage-gated channel subfamily A member 1) is methylated and downregulated in primary sensory neurons by G9a after nerve injury[100].

Many other studies, mainly performed in animal models, have provided evidence that pharmacological modulation of acetylation by inhibitors of HDACs or HATs also modifies the nociceptive response (TABLE 2). In addition, a number of HDAC and HAT inhibitors have been developed for research purposes and for treatment of various malignancies[101] that show efficacy in inflammatory diseases[102], which indicates that they might also affect inflammatory pain. Here, we focus on previously approved drugs, drugs used in clinical studies or very common compounds used for research.

HDAC modulators in preclinical studies of nociception. To date, most studies on histone modification in pain were performed in nociceptive animal models (TABLE 3). In models of inflammatory pain, knockout or inhibition of HDAC is mainly associated with antinociceptive responses. For example, conditional *Hdac4* knockout in sensory neurons of mice was associated with reduced sensitivity to inflammatory pain. Surprisingly, although HDAC inhibition is usually associated with an increase in gene expression, knockout mice exhibited an unexpected reduction in the expression of several pain-related genes, including *Trpv1*, thereby contributing to a decreased pain phenotype in these animals. The downregulation of pain-related genes owing to *Hdac4* knockout must, presumably, be indirect and secondary to upregulation of other genes[103]. In further studies, increased expression of *Hdac2* was observed in the spinal dorsal horn of mice during inflammatory joint nociception. Intrathecal injection of HDAC inhibitors selective for class I and II HDAC enzymes (for example vorinostat, valproic acid or trichostatin A), delayed the onset and reduced the pain behaviour[104], an effect also described in formalin-induced inflammation[105]. A role of histone acetylation in pain sensitization is further supported by the demonstration that trichostatin A statistically significantly reduced stress-induced visceral nociception in rats. Promoter methylation of *CRH* (encoding corticoliberin) and *NR3C1* (encoding glucocorticoid receptor) was also altered, suggesting that various epigenetic modifications regulate stress-induced visceral pain[106].

Box 1 | **Histone modifications**

Acetylation was the first histone modification discovered[166] and is thought to relax chromatin to a more accessible structure for transcription[26] by dissociating DNA from nucleosomes and weakening nucleosomal interactions. Moreover, the acetyl groups can serve as interaction marks, as they are recognized by bromodomains found in several human proteins with diverse functions (for example, histone acetyltransferases (HATs), HAT-associated proteins, and histone methyltransferases)[167]. HATs and histone deacetylases (HDACs) are thought to be recruited to actively transcribed genes by RNA polymerase II[168].

Other histone modifications include methylation, ubiquitylation and phosphorylation. Some of these histone marks are correlated functionally with specific activity states of the gene locus involved, and crosstalk between modifications can arise. For instance, trimethylation of histone H3 at lysine 4 (H3K4me3) is a hallmark of active promoter sites and antagonizes promoter silencing associated with histone H3 lysine 27 trimethylation (H3K27me3). H3K27me3 is associated with unmethylated DNA, whereas histone H3 lysine 9 (H3K9) methylation, a mark for heterochromatin formation and gene silencing, is strongly connected with DNA methylation through recruitment of DNA methyltransferases[169].

Interestingly, histone methylation and DNA methylation can also negatively interact, as H3K4me3 blocks *de novo* methylation[170]. Moreover, some histone H3 lysine 4 (H3K4)–methyltransferase complexes contain zinc-finger domains, facilitating the binding of unmethylated CpG sites (BOX 2) and enabling H3K4 methylation at these sites[171].

To date, four HDAC inhibitors have been approved for the treatment of cancer[118]. Moreover, effort is increasingly being put into the development of drugs that specifically target other classes of histone modifying proteins, such as the bromodomain-containing readers of histone acetylation as well as histone methyltransferases and demethylases[172].

Box 2 | **DNA modifications**

The human genome roughly comprises 28 million CpG sites, of which ~75% are methylated throughout the DNA sequence in somatic cells[173]. By contrast, DNA stretches with unexpectedly high CpG frequencies and up to 1 kb in length, named CpG islands, are generally not methylated[174]. CpG islands often overlap with gene promoters and methylation of these sites can result in promoter silencing, for example, during embryonic development. Aberrant methylation or demethylation has been observed in pathological contexts (for example, in malignant cancer cells), in which these modifications can cause silencing or reactivation of gene expression[175].

Whole-genome methylation analyses revealed that the overall methylation landscape is stable in human somatic cells and tissues[176]. Roughly 20% of the CpG sites have a dynamic methylation state. These regions, so-called differently methylated regions, are predominantly located distant to promoter sites and overlap with regulatory elements (for example, enhancer elements and response elements), and probably play a part in the regulation of gene expression[64,177,178].

In mammals, CpG methylation is carried out by DNA methyltransferases (DNMTs). However, demethylation can occur in several ways[43]. One way is by passive dilution of the methylation mark during DNA replication, which occurs when methylation maintenance on hemimethylated DNA is hampered (for example, owing to reduced DNMT1 activity). The second way is thought to occur mainly through repetitive oxidation of 5-methylcytosine to 5-hydroxymethylcytosine, 5-formylcytosine, and finally to 5-carboxylcytosine by the ten–eleven translocation (TET) dioxygenases (also known as also known as methylcytosine dioxygenases). During DNA replication, CpG sites exhibiting these modified 5-methylcytosines are not available for maintenance methylation, which results in passive wash-out of the methylation mark. Moreover, 5-formylcytosine and 5-carboxylcytosine can be replaced by unmodified cytosines through thymine DNA glycosylase excision followed by base-excision repair.

HDAC inhibitors also showed analgesic efficacy in models of traumatic or drug-induced peripheral neuropathy[107]. Chronic pain induced by dysregulation of GABA-mediated antinociception is associated with hypoacetylation and decreased expression of glutamate decarboxylase 2 (GAD2, also known as GAD65). In models of neuropathic pain in mice, treatment with HDAC inhibitors, including vorinostat, restored GABA-mediated synaptic function and relieved mechanical hyperalgesia[108,109]. Importantly, even long-standing changes could be modulated by pharmacological intervention. For example, hypoacetylation of histones, observed in early life maternal-separation-induced visceral hypersensitivity in mice, was rectified by treatment with vorinostat, which also significantly reduced the extent of pain behaviour[110]. These results are encouraging, as they suggest that chronically enhanced sensitivity to pain and the associated neuronal plasticity are potentially open to drug treatment with HDAC inhibitors and could represent a unique advantage of epigenetic drugs.

In contrast to these findings, hyperacetylation, particularly in the CNS, has been reported as a trigger for increased nociception. In the chronic constriction injury (CCI) rat model of neuropathic pain, reduction of acetylation resulting from inhibition of HAT p300 reduced pain hypersensitivity as well as COX2 expression in the spinal cord[111]. Furthermore, administration of the pan-HDAC inhibitor quisinostat induced mechanical hypersensitivity in mice, which was associated with spinal upregulation of the voltage-dependent calcium channel subunit α2/δ1. This drug is being considered for cancer therapy; consequently, the authors suggested that nonspecific inhibition of HDACs might increase the risk of chemotherapy-induced peripheral neuropathy[112].

In addition to histones, HDACs modify acetylation of other genes, such as those encoding the transcription factor p65 or the glutamate receptor mGluR2, which are involved in nociceptive processing. Modulation of HDAC activity, therefore, also induces changes in the nociceptive response by regulation of these genes[105,113,114].

Histone acetylation also plays a part in analgesic therapy and might be involved in the development of opioid tolerance or opioid-induced hyperalgesia (OIH). In mouse models of OIH, morphine treatment increased acetylation of histone H3 and decreased HDAC activity in the spinal dorsal horn, whereas HAT activity remained unchanged. The HAT inhibitor curcumin counteracted OIH, whereas administration of the HDAC inhibitor vorinostat facilitated OIH even when the drug was given after the termination of opioid administration[115]. The extent to which modification of histone acetylation might enhance opioid analgesia in humans is unknown and needs careful clinical evaluation.

Although these preclinical studies provide a number of hints on epigenetic gene regulation in pain, they are only proof-of-concept experiments that suggest involvement of epigenetic mechanisms in pain. In particular, clinical studies are needed to provide a robust rationale for the use of epigenetic modifiers in chronic pain.

HDAC inhibitors in clinical studies of pain therapy. Few clinical studies have dealt with effects of HDAC inhibitors on pain. In an open-label clinical study, oral administration of the HDAC inhibitor givinostat effectively reduced symptoms of systemic-onset juvenile idiopathic arthritis, a painful inflammatory disease in adolescents[116]. Disease progression was slowed significantly by the drug, as indicated by a reduction of the number of affected joints with active disease and severe motor restriction. Adverse effects affecting the respiratory and the gastrointestinal tract were of only moderate severity and self-limiting. However, the open-label design and small patient population preclude a valid conclusion to be drawn on the effectiveness of the therapy[116]. Romidepsin, a cyclic peptide that inhibits HDACs, was used in a phase II clinical trial in patients with refractory multiple myeloma. After intravenous administration, no obvious response was observed, but several patients reported improvements in bone pain and resolution of hypercalcaemia, indicating that single patients might benefit from the epigenetic analgesic effects[117]. Clearly, conclusive translational clinical studies on HDAC inhibitors in pain conditions are greatly needed.

The safety profile of epigenetic drugs is crucial if they are to be considered as potential therapies for chronic pain. An initial impression of the general safety of epigenetic drugs can be gained from drugs currently on the market. The histone acetylation machinery is certainly highly complex, and consists of not only different HDAC classes and types that can modify histones in the nucleus, but also many non-histone proteins in the nucleus as well as the cytoplasm[29], making the activity profile of HDAC

Table 1 | **Pain-relieving compounds that exert epigenetic effects**

Compound	Pain condition	Analgesia epigenetic link	Refs
Celecoxib	Inflammatory, musculoskeletal	Possible	75–80
Opioids	Cancer pain, acute pain; opioid induced hyperalgesia	Likely	81,82,84,85
Fluoxetine	Neuropathic pain	Likely	91,92
Amitriptyline	Neuropathic pain	Possible	93,94
Valproic acid	Neuropathic pain	Possible	93

modulatory drugs unclear. Currently, most HDAC inhibitors show neither specificity for organs and cell types nor for HDAC classes or single HDACs, and so risk causing a range of adverse effects, such as fatigue, nausea, anaemia, anorexia, hyperglycaemia and thrombocytopenia[36,118].

HDAC inhibitors with an increased specificity for certain isoforms are under development[39] and could facilitate correlation of biological effects with a specific HDAC, at least *in vitro*. For instance, HDAC inhibitors have been reported to cause QT interval prolongation and heart diseases[119]. Class II HDACs are highly expressed in the heart and control several cardiac genes[120]; consequently, use of selective HDAC inhibitors that are inactive against class II HDACs could be a way to reduce cardiac adverse effects. On the other hand, HDACs often form multimeric protein complexes *in vivo*, including with other HDACs, and so selective inhibitors might indirectly influence the activity of several distinct HDAC isoforms[121]. This nonspecificity might also affect normal physiological pathways and lead to adverse effects. Although HDAC inhibitors show therapeutic effects in inflammatory diseases[122], these drugs can also compromise host defence[123] and aggravate atherosclerosis[124]. The same is true for HAT inhibitors, which have so far received less interest than HDAC inhibitors. Further studies, therefore, are needed on tissue-specific or cell-specific targets in standard models of inflammatory and neuropathic pain under differing painful conditions. Techniques such as chromatin immunoprecipitation sequencing (ChIP-Seq) applied to cells and tissues of interest (such as spinal cord, dorsal root ganglion neurons and sensory neurons) could reveal whole-genome histone modification states with resolution down to specific gene loci, which might then enable discrimination between histone modifications associated with beneficial and detrimental effects on pain states.

Although DNA methylation strongly modulates gene expression, insufficient data are available on the role of DNA methylation in pain and its modulation as an approach to analgesic therapy (TABLE 2). A correlation has been reported between the methylation of different gene promoters[125,126] and nociception, but only a minority of researchers used drugs that modulate methylation. Identification of the specific genes that are affected by changes in DNA methylation patterns is crucial — particularly with DNA methylation, which is usually associated with gene silencing — and will enable increased precision in the determination of potential drug targets, whether pronociceptive or feedback regulatory genes.

In the rat CCI model of peripheral neuropathy, increased *Mecp2* promoter methylation, as well as increased global methylation, was observed in the spinal cord. Intrathecal injection of the DNMT inhibitor 5-azacytidine reversed methylation responses and reduced mechanical and thermal hyperalgesia[127]. The extracellular matrix protein SPARC is involved in bone remodelling and its downregulation has been associated with painful disc degeneration[128,129]. In ageing mice and patients with disc degeneration, increased methylation of the *SPARC* promoter was correlated with increased pain or pain behaviour and was counteracted in mice by treatment with 5-azacytidine. The patients in this study were not treated with the DNMT inhibitor[130]. The data suggest that age-dependent downregulation of SPARC contributes to painful disc degeneration and that methylation of the gene promoter is involved in this process. However, the patient data must be considered with care as samples were taken post mortem and SPARC expression could not be evaluated.

Decreased morphine analgesia in neuropathic pain is associated with increased methylation of the *OPRM1* promoter in dorsal root ganglia leading to reduced levels of *OPRM1* mRNA and μ-type opioid receptor protein. Treatment with decitabine reduced promoter methylation, restored *OPRM1* expression levels and increased morphine-mediated analgesia[131]. Decitabine treatment also restored sensitivity to the chemotherapy drug cisplatin in painful head and neck squamous cell carcinoma (HNSCC), and decitabine–cisplatin combination treatment inhibited mechanical allodynia and tumour growth in a mouse HNSCC model[132] and decreased nociception in a model of oral cancer pain in mice. Mice with tumours and patients with oral dysplasia and cancer showed a strong increase in DNA methylation of *OPRM1*, indicating that epigenetic downregulation of this gene might contribute to the development of cancer pain[133].

Similarly to modulators of HDAC, DNMT inhibitors are associated with a number of problems that hinder their use in clinical studies. Inhibitors of DNMT are already approved by the FDA for the treatment of cancers[36]. Over the past decade, 5-azacytidine has been proven to be an effective treatment of myelodysplastic syndromes, even in elderly patients who are at increased risk of the disease, and treatment is recommended beyond the initial response[48,134]. An oral formulation is in development. 5-azacytidine causes neutropenia, thrombocytopenia, nausea and emesis at the high doses used in patients with myelodysplastic syndromes, and these adverse effects need to be carefully controlled as the onset of action is slow. Much lower doses are widely thought to be needed to selectively inhibit DNMT, but whether a nontoxic dose can be achieved is debatable[134]. Decitabine is less effective than 5-azacytidine in elderly patients with myelodysplastic syndromes, does not substantially increase survival and has similar but more severe adverse effects than those of 5-azacytidine[134,135]. A reason for the development of adverse effects might be the fact that neither 5-azacytidine nor decitabine exhibit isoform specificity but target all DNMTs.

A further problem with these drugs is that they must be incorporated into the DNA to function[136] and so are most effective in dividing rather than non-dividing cells such as neurons, despite preclinical effects *in vivo*. Decitabine is incorporated into DNA whereas 5-azacytidine can be integrated into DNA as well as RNA, which results in a diminished capacity for inhibition of DNA methylation by 5-azacytidine. However, because of these properties, 5-azacytidine can influence dividing and nondividing cells, which might provide additional opportunities for pain therapy[137]. New, specific modulators of DNMTs are under development,

Table 2 | **Epigenetic modulators in nociception**

Modulators of epigenetic marks	Specificity	Study type	Effect on pain	Regulated genes	Refs
HDAC inhibitors					
Trichostatin A	HDAC class I, II, IV	Preclinical	Inflammatory nociception (CFA-induced) reduced in mice; intrathecal administration	NR	104
			Visceral pain (water avoidance stress-induced) reduced in rats; intracerebroventricular administration	NR	106
			Corticosteroid-induced pain reduced in rats; administration by bilateral infusion in central amygdala	*Crh*, glucocorticoid receptor genes, *Sirt6*, *Rela*	179
			Chronic pain (CFA-induced, SNL-induced) reduced in rats and mice; intraperitoneal administration	*GAD2*	108
Vorinostat	HDAC class I, II, IV	Preclinical	Inflammatory nociception (CFA-induced, formalin-induced) decreased in mice; intrathecal or subcutaneous administration	*Rela*, *Grm2*	104, 105
			Visceral pain (caused by colorectal distension) reduced in rats; intrathecal administration	*Grm2*	114
			Visceral pain (stress-induced) reduced in rats; intraperitoneal administration	NR	110
			Chronic pain (CFA-induced, SNL-induced) reduced in rats and mice; intraperitoneal administration	*GAD2*	108
			Spared nerve injury, mechanical hyperalgesia reduced in mice; intrathecal administration	*GAD2*	109
			Opioid-induced hyperalgesia increased in mice; intraperitoneal administration	NR	115
Givinostat	HDAC class I, II	Phase II	Juvenile idiopathic arthritis in humans; oral administration	NR	116
Quisinostat	HDAC class I, II, IV	Preclinical	Spontaneous mechanical allodynia in mice; subcutaneous administration	*Cacna2d1*	112
Romidepsin	HDAC class I	Phase II	Multiple melanoma (pain reduced) in humans; intravenous administration	NR	117
HAT inhibitors					
Anacardic acid	p300	Preclinical	Partial nerve ligation, neuropathic pain reduced in mice; intraperitoneal administration	*Cxcl2* and *Cxcr2*	180
DNMT inhibitors					
5-Azacytidine	All DNMTs	Preclinical	CCI-induced neuropathic pain reduced in rats; intrathecal administration	*Mecp2*	127
			Disc degeneration (pain reduced) in mice; intravenous administration	*Sparc*	130
Decitabine	All DNMTs	Preclinical	CCI, morphine tolerance reduced in mice; intraplantar administration	*Oprm1*	131
			HNSCC xenograft (pain reduced) in mice; intraperitoneal administration	NR	132
			Oral squamous cell carcinoma xenograft (pain reduced) in mice; intraperitoneal administration	*Oprm1*	133

CCI, chronic constriction injury; CFA, complete Freund's adjuvant; DNMT, DNA methyltransferase, HAT, histone acetyltransferase; HDAC, histone deacetylase; HNSCC, head and neck squamous cell carcinoma; NR, not reported; SNL, spinal nerve ligation.

which are likely to act on nondividing cells. Further progress requires tissue or even cell-type specificity. In cancer, mutations of epigenetic genes such as *DNMT3A* can alter the effectiveness of demethylating agents[138]. Currently, whether this interaction is also relevant for pain is unclear. However, such mutations are likely to present further challenges to clinical analgesic use.

microRNA regulation and pain

Several animal studies have revealed the involvement of neuronal miRNA regulation in inflammatory, neuropathic and cancer pain. The results are supported by clinical studies that show differential miRNA expression levels in patients with fibromyalgia, rheumatic diseases, irritable bowel syndrome, painful endometriosis, interstitial cystitis or psoriasis[55,56,139,140]. As with histone acetylation and DNA methylation, miRNAs are also involved in the development of opioid tolerance[85]. The mechanisms of epigenetic regulation by miRNAs and their target genes might, therefore, suggest options for miRNA-specific analgesic therapies.

The first functional study into the potential of miRNAs in pain modulation, published in 2010 (REF. 54), substantiated the therapeutic potential of these molecules: deletion of the miRNA processing endonuclease Dicer caused a reduction of all miRNAs in sensory neurons and reduced inflammatory nociception owing to the modification of pain-relevant genes. Complete Freund's adjuvant (CFA)-induced chronic inflammatory pain also reduced the expression of miRNA-219 in spinal neurons in mice and increased spinal levels of the validated miRNA-219 target calcium/calmodulin-dependent protein kinase type II subunit γ (CaMKIIγ). Experimental downregulation of spinal miRNA-219 in wild-type mice resulted in a nociceptive response whereas miRNA-219 overexpression in the spinal cord counteracted pain hypersensitivity and reversed the increased expression of spinal CaMKIIγ[141]. An increase in miRNA-219 levels, which was associated with reduced pain behaviour and spinal neural sensitization, was also induced by 5-aza-deoxycytidine in the CFA-induced chronic inflammatory pain model, suggesting that this action is associated with hypermethylation of CpG islands in the miRNA-219 promoter[141].

Our group showed that expression of the neuron-specific miRNA-124a is reduced in the mouse spinal cord after peripheral inflammatory stimulation. Experimental overexpression of miRNA-124a led to a significant reduction of the nociceptive response[142], which was also found in a mouse model of neuropathic pain[143]. miRNA-103 was downregulated in the spinal nerve ligation model of neuropathic pain, which induced upregulation of

Table 3 | **Effects of epigenetic modification in animal models of pain**

Model	Method	Effect	Epigenetic modulations
Inflammatory nociception			
CFA-induced paw inflammation	Injection of CFA into one hind paw	Paw inflammation, oedema formation[181,182]	Histone acetylation (spinal cord, nucleus raphe magnus)[104,108]
Formalin-induced paw inflammation	Injection of formalin into one hind paw	Short-term paw inflammation[183]	Histone acetylation (spinal cord)[105,113]
Visceral pain	Colorectal distension	Visceral hypersensitvity[114]	Histone acetylation (spinal cord, amygdala)[114]
	Stress (water avoidance)	Psychological stress, visceral hypersensitvity[184]	Histone acetylation (spinal cord, amygdala)[106,110]
Carrageenan-induced muscle inflammation	Injection of carrageenan into the gastrocnemius muscle	Muscle hyperalgesia[185]	microRNA (muscle)[185]
Neuropathic pain			
Spinal nerve ligation	Unilateral ligation of L5 or L5 and L6 spinal nerves	Long-lasting allodynia and hyperalgesia[186,187]	Histone acetylation (nucleus raphe magnus, spinal cord)[108,126,188]
Chronic constriction injury	Constriction of the sciatic nerve by loose ligatures	Nerve inflammation, loss of fibres, spontaneous pain, long-lasting hyperalgesia and allodynia[189]	Histone acetylation (sciatic nerve, spinal cord); DNA methylation (spinal cord, dorsal root ganglion)[190,191,111,127,131]
Partial nerve ligation	33–50% of the sciatic nerve is unilaterally ligated	Long-lasting allodynia and hyperalgesia[192]	Histone acetylation (spinal cord)[180]
Spared nerve injury	Section and ligation of two of the three peripheral branches of the sciatic nerve	Long-lasting allodynia and hyperalgesia[193]	Histone acetylation (spinal cord)[109]
Diabetic neuropathy	Streptozotocin-induced diabetes mellitus	High glucose levels, hyperalgesia, allodynia[194]	microRNA (dorsal root ganglion neurons)[145]
Bone cancer	Implantation of Walker 256 rat mammary gland carcinoma cells into the tibial cavity	Allodynia and ambulatory pain[195]	microRNA (sensory neurons)[147]

CFA, complete Freund's adjuvant.

the voltage-dependent L-type calcium channel subunit α1C (also known as Cav1.2-LTC), which is often associated with neuropathic pain. Intrathecal injection of a miRNA-103 inhibitor led to mechanical hypersensitivity in healthy animals, whereas miRNA-103 overexpression inhibited $Ca_V1.2$-LTC expression in the spared nerve injury model of neuropathic pain and reduced the nociceptive response almost to basal levels[144]. An association between miRNA-induced ion channel modification and pain hypersensitivity was also observed in a disease model of painful diabetic neuropathy in rats[145].

Interestingly, several studies have indicated crosstalk between miRNA regulation and other epigenetic mechanisms in pain. miRNAs can be regulated by epigenetic modulation and, conversely, miRNAs are also involved in the expression of genes that are important for epigenetic mechanisms. For instance, *Mecp2* expression is modified by miRNA-124a in the mouse spinal cord in inflammatory pain[142] and *Dnmt3a* expression is modified by miRNA-200b/429 in the mouse nucleus accumbens in neuropathic pain[146]. Consequently, despite the relative selectivity of miRNAs, their potential use in pain therapy is likely to be confounded by such complex interactions.

In a model of bone cancer pain, miRNA-1a was upregulated in sensory neurons. Previous studies had shown that this miRNA was downregulated in the context of inflammatory and neuropathic pain, and so upregulation of miRNA-1a was suggested to be specific for cancer-associated pain. Treatment with an miRNA-1a inhibitor reduced nociceptive behaviour in a mouse model of bone cancer pain and increased levels of the pain-relevant chloride channel H^+/Cl^- exchange transporter 3 (encoded by *Clcn3,*), indicating that miRNA-1a and its target genes might constitute therapeutic targets in cancer pain[147]. These preclinical data show that modulation of different miRNAs has an effect on the nociceptive response and that these molecules could serve as potential targets in the treatment of inflammatory, neuropathic and cancer pain.

Functional clinical studies on the role of miRNAs and their modulation in pain have not yet been reported. Future studies such as these should shed further light on the usefulness of miRNA modulators in clinical practice. miRNAs can be modulated by different types of oligonucleotides[148–151], which are fairly stable *in vivo,* show long-lasting efficacy[152] and can be further stabilized with locked nucleotide acids[153]. However, cellular uptake and tissue-specific delivery of these oligonucleotides is poor and needs to be improved with the use of carriers, such as lipid emulsions or cholesterol conjugates[154]. In addition, short RNA sequences tend to stimulate the immune system, and these oligonucleotides could, therefore, provoke adverse effects[155]. These challenges undoubtedly contribute to the lack of clinical studies investigating miRNAs in pain.

Challenges in clinical translation

Before epigenetic modulators can be used for therapy of chronic pain, the requirements of these treatments must be considered — including the necessity for long-term administration, selective targeting of neuronal tissue and acceptable tolerability. Researchers are clearly reluctant to perform clinical studies of epigenetic modifiers, which is almost certainly a result of the (often severe) challenges that are expected with the use of such drugs. As previously indicated, currently available and approved epigenetic agents act as genomic medicines that potentially target the epigenome as a whole[156], but might not distinguish between epigenetic marks and patterns arising in a physiological or pathological setting. Furthermore, most of the drugs are not specific to individual cell types or tissues, and induce systemic adverse effects. These nonspecific actions can unleash uncoordinated changes in a number of signalling cascades that lead to severe, unpredictable and long-lasting epigenetic dysregulation, as well as life-threatening adverse effects.

In addition, the drugs must cross the blood–brain barrier to enter the CNS and modulate pain. This action is only possible for lipophilic substances or hydrophilic agents formulated for targeted delivery to the brain, which would otherwise not be able to gain access to a number of interesting targets important in pain signalling (for example, the anterior cingular cortex or dorsal posterior insula). In animals, this problem can be circumvented by altering the route of drug administration — for example, using direct injections into the spinal cord or the brain, which is not feasible in patients. Regardless of this limitation, as shown in TABLE 2, varied administration routes and investigated tissues mean that the results of animal studies show a high degree of variability, which limits comparability and translation to humans, as well as comparison between pain states that are reversible (such as inflammatory pain) or irreversible (such as neuropathic pain). Few publications have compared different pain states in a single study, and most have focused on one specific pain type.

Instability of the approved DNMT and HDAC inhibitors is also a problem. Cytidine deaminase (the enzyme that metabolizes both 5-azacytidine and decitabine) is highly expressed in human liver and spleen, which results in a shorter half-life of these drugs than that found *in vitro*. The HDAC inhibitor vorinostat is also rapidly metabolized (half-life <2 h). As epigenetic modulation requires time to induce therapeutic effects, these short half-lives can be overcome by repeated or continuous injection of drugs, which could further increase adverse effects. These challenges might be circumvented by the use of prodrugs, which would be activated more slowly by metabolism, or modified delivery strategies, which would be capable of targeting drugs to desired sites of action and, thus, reduce toxicity[157].

The fact that some epigenetic drugs show cytotoxic activity at high doses presents a further challenge, as HDAC inhibitors have only weak effects on chronic pain, which necessitates use of high doses for sufficient analgesic activity. Cytotoxicity is only tolerable to a limited extent in cancer patients to sustain an acceptable quality of life. Whether such severe adverse effects are ethically acceptable for patients with pain is unclear, but new, more potent compounds, either repurposed from other indications or designed specifically for use

in chronic pain, will probably need to be developed. Here, we would like to emphasize that the underlying rationale for using epigenetic drugs in the treatment of cancer differs in some important respects to their use for the treatment of pain. Cancers are frequently characterized by systemic epigenetic aberrations, and often exhibit mutations in genes involved solely in epigenetic mechanisms (for example, chromatin remodelling, histone acetylation and methylation, and DNA methylation)[158]. Hence, one therapeutic approach to cancer is to counteract the effects of these mutations on the same global level. In addition to this epigenetic reprogramming of tumour cells, cytotoxic and cytostatic effects of the drugs — such as those mediated by DNA damage, upregulation of apoptosis-inducing tumour suppressor genes, or activation of endogenous retroviruses that trigger a tumour-inhibiting immune response[159] — are easier to tolerate, as they might also contribute to clinical benefit. However, these global approaches are clearly not feasible in pain therapy.

Perspectives for future research

Little is known about the time course of epigenetic changes in relation either to their functional outcome or to their generational inheritance. Most epigenetic changes can be classified into two categories. Context-dependent changes result from direct and continuing exposure to an environmental stressor and might be sustained in somatic cell lineages. Germline-dependent epigenetic inheritance arises when the germline is directly affected by an epigenetic change, and phenotype changes persist across generations in the absence of the causative agent. Few studies have examined the time courses of these changes, as most investigators have studied whether such epigenetic phenomena are present rather than the dynamics of their onset or wash-out, either in relation to the stressor or over several generations[160]. The same also applies to studies on epigenetic changes and pain. Although many associations between epigenetics and pain have been demonstrated, most have been context-dependent, with little appreciation of the timescale involved. Chronic pain and neuropathic pain conditions usually develop over time, and so the time factor in pharmacological effects on nociceptive epigenetics poses an important consideration. The association of local epigenetic changes with long-term neuronal plasticity during chronic pain indicates that reversal of enhanced sensitivity — owing to a past noxious stimulus, received potentially years before the time of treatment — could represent an exceptional indication for epigenetic drugs.

The fact that drugs approved for multiple diverse therapies from different chemical classes can also affect the epigenetic machinery indicates that structure-based selectivity for a specific epigenetic target is likely to be achieved, and drug repurposing might be possible[161]. Context-dependent epigenetic changes and their consequences have been investigated both in preclinical models and in patients. Direct epigenetic modifications of DNA, histones or miRNAs in either cell culture or organs in both animal and clinical investigations have revealed associations between target gene regulation and differential nociceptive responses. However, clear definition of the aspect of the nociceptive machinery at which epigenetic drugs exert their main effect remains a challenge for future research.

Investigation of epigenetics during drug screening is important, particularly with regard to possible inheritance of epigenetic modifications, as shown for several drugs described in this Review. With regard to the different epigenetic modulations and their regulation by different drugs in the context of pain, development of effective drugs for pain could be facilitated by focusing on one specific aspect of epigenetics, such as a class of enzymes, a certain mechanism of action or tissue-selective mechanisms. Some HDAC inhibitors can cross the blood–brain barrier and have shown potential in neurodegenerative diseases such as Huntington disease, amyotrophic lateral sclerosis, Parkinson disease and Alzheimer disease by restoring transcriptional dysfunction and ameliorating symptoms[162,163], which is a distinct advantage of this drug class. HDAC and DNMT inhibitors can both correct pathological changes in the genome and suppress symptoms of neurodegenerative diseases. On the other hand, miRNA mimics or inhibitors can target several pathological gene modifications simultaneously and are open to derivatization owing to their oligonucleotide structure. However, at this early stage, empirical evidence remains too inconsistent to consider DNA methylation, histone modifications or miRNAs as the most promising targets in analgesic therapy. Future approaches that might help to elucidate pain-relevant modifications could include epigenome-wide assays for methylation or acetylation pattern. Selected modifications could then be targeted using epigenome editing, including CRISPR–Cas9-based techniques, by manipulating the chromatin state at defined genomic target regions[164] and thereby providing functional information on their chromatin states[165]. Individual epigenome-editing therapy for patients with pain, which might arise from these developments, ideally combined with specific targeting of affected cells, would provide promising options in the long term.

Conclusions

Epigenetic mechanisms contribute to expression of pain genes and their subsequent signal transduction, and pharmacological modulation of DNA and histone marks or miRNA expression provides analgesic options. Research on epigenetic mechanisms and modulators in pain states is only beginning to emerge, whereas epigenome modulators have already been approved for cancer therapy. The slow speed of research on pain epigenetics is related to the fact that long-term adverse effects of the drugs remain to be clarified and suitable clinical biomarkers are still needed. Initial studies on pain have revealed promising analgesic activities for some drugs, but as epigenetic changes can be transmitted to further generations, potential effects on germ cells must be assessed.

Genome-wide analyses of histone and DNA modifications and miRNA regulation of pain-relevant target genes could shed light on potential long-term effects

and provide specific biomarkers for certain pain types. Moreover, studies in conditional knockout mice should provide insight into the epigenetic actions and targets of candidate agents. The development of specific modulators of disease-associated changes would be a substantial step forward.

In conclusion, epigenetic marks need to be studied in greater detail and under different conditions, particularly in specific pain conditions and nociceptive models. New drugs with increased specificity are needed that are directed to precise molecular targets and have improved delivery to diseased tissues, which would reduce adverse effects. Available data suggest that epigenetic modulators might be combined with traditional drugs to improve analgesic efficacy. However, a great deal more experimental and clinical research is necessary before epigenetic modulators can be considered for the clinical treatment of pain.

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All authors, wrote, edited and discussed content for the article. E.N. and E.R carried out the literature search.

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References for this Review were identified by searching the PubMed database for publication from January 1980 to February 2017, using the terms "pain", "nociception", "hyperalgesia", "epigenetics", "DNA methylation", "histone acetylation", "microRNA", "analgesics", "pain gene", including combinations thereof. Articles were also identified through the reference lists of key papers. The final reference list was generated on the basis of originality, quality and relevance to the topic of this Review.