Epigenetic regulation in opioid induced hyperalgesia

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ABSTRACT

About 25 million American adults experience pain daily and one of the most commonly prescribed drugs to treat pain are opioids. Prolonged opioid usage and dose escalations can cause a paradoxical response where patients experience enhanced pain sensitivity. This opioid induced hyperalgesia (OIH) is a major hurdle when treating pain in the clinic because its underlying mechanisms are still not fully understood. OIH is also commonly overlooked and lacks guidelines to prevent its onset. Research on pain disorders and opioid usage have recognized potential epigenetic drivers of disease including DNA methylation, histone modifications, miRNA regulation, but their involvement in OIH has not been well studied. This article discusses epigenetic changes that may contribute to pathogenesis, with an emphasis on miRNA alterations in OIH. There is a crucial gap in knowledge including how multiple epigenetic modulators contribute to OIH. Elucidating the epigenetic changes underlying OIH and the crosstalk among these mechanisms could lead to the development of novel targets for the prevention and treatment of this painful phenomena.

Introduction

Opioids remain the standard of care to manage pain in patients experiencing post-operative pain, neuropathic pain, cancer associated pain and musculoskeletal pain, among others (Roeckel et al., 2016). However, patients who are continuously prescribed opioids to control pain can develop opioid induced hyperalgesia (OIH). OIH is the paradoxical phenomenon in which prolonged opioid use results in increased pain. Thus, instead of providing relief to individuals grappling with various forms of pain, opioids escalate pain sensitivity. OIH differs from opioid tolerance where the same dose of drug administered over time produces less analgesic effect. Tolerance is thus a pharmacological phenomenon resulting in the rightward shift in the dose–response relationship that is not unique to opioids and can be overcome by dose escalation (Silverman, 2009). The heightened pain response in OIH is not merely a matter of tolerance but results from interaction between opioids and the neurobiological pathways involved in pronociceptive activity leading to lowering of pain thresholds. Development of

tolerance can be independent of OIH, and though not everyone who develops tolerance will develop OIH, tolerance may contribute to the development of OIH (Chen et al., 2014; Chu et al., 2008). Prolonged use or higher doses of opioids results in tolerance and OIH leading to diminished pain control requiring additional analgesics (Chen et al., 2014; Lee et al., 2011; Stoicea et al., 2015). OIH can be induced by multiple commonly prescribed and used opioids including fentanyl, remifentanil, heroin, oxycodone and morphine (Angst & Clark, 2006). Importantly, different opioid molecules affect OIH onset and duration based on their kinetic action, metabolism, and potency depending on the individual drug and patient characteristics (Hayhurst & Durieux, 2016; Roeckel et al., 2016; Silverman, 2009). Fig. 1 illustrates OIH onset with increasing opioid doses or prolonged opioid treatment.

OIH is a complication that is often overlooked, as clinicians are more often cautioned to monitor signs of addiction, abuse, and diversion when administering opioids (Lee et al., 2013). OIH clinical symptoms include waning of opioid effects in the absence of disease progression, unexplained pain, severe allodynia unassociated with the original pain stimuli and increased levels of pain with increased opioid doses. These symptoms can be debilitating for some who progress to chronic pain and must not be ignored. OIH is also commonly detected in people with an opioid use disorder (OUD) and can linger even after recovery begins. This can prompt an individual's relapse, in hopes to decrease pain hypersensitivity (Lee et al., 2011). Nevertheless, not all patients or those with OUD develop OIH, and those who do, often show differences in opioid dosing, sex, genetic background and age (Roeckel et al., 2016; Vargas-Schaffer et al., 2020). The underlying mechanisms and risk factors of OIH are not completely understood (Chu et al., 2008) but are thought to be multifactorial and could include age, prior opioid usage, type of opioid, sex, race, etc. (Averitt et al., 2019; Ganguly et al., 2021). A multidisciplinary approach is currently pursued in attempts to treat OIH in clinic (Lee et al., 2011; Mercadante, 2023; Wilson et al., 2021). These include (1) opioid rotation by switching to a different opioid with a distinct receptor profile; (2) opioid tapering with gradual reduction of opioid dosage under medical supervision;(3) incorporating non-opioid analgesics such as NSAIDs, or acetaminophen; (4) N-methyl-D-aspartate (NMDA) receptor antagonists (eg. ketamine) to modulate pain pathways and counteract OIH. Other strategies pursued include non-
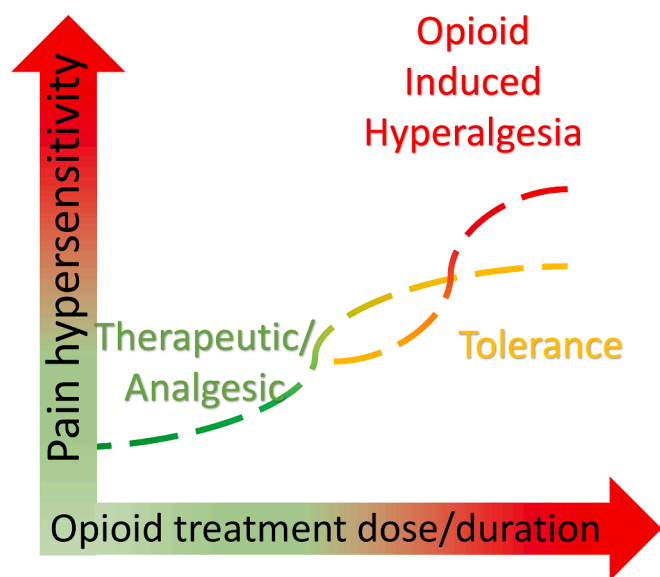

Fig. 1. Schematic representation of how an increase in duration or dose escalation can lead to the progression from an analgesic effect of opioids to tolerance and OIH. Tolerance can develop independently of OIH, and not everyone who develops tolerance will necessarily experience OIH. However, tolerance may contribute to the development of OIH and tolerance may be in part due to the lower nociceptive thresholds associated with OIH.

pharmacological interventions like physical therapy and rehabilitation programs, psychological interventions such as cognitive-behavioral therapy to help manage pain, improve function, and decrease reliance on opioids (Colvin et al., 2019; Mercadante, 2023).

The field requires reliable and translatable animal models to study OIH's underlying mechanisms and possible treatment options. Various studies have reported mechanical allodynia and/or thermal hyperalgesia following the acute administration of opioids such as heroin and fentanyl, chronic (week long) administration of intrathecal or intraperitoneal morphine, local peripheral administration of morphine or chronic administration of several systemic opioids (Liang et al., 2006). Another study implanted morphine pellets into mice to continuously expose them to opioids for 6 days, and after removing the pellets, they observed thermal hyperalgesia and mechanical allodynia (Li et al., 2001). They also noticed that prior morphine treatment increased formalin-induced licking behavior, and these behaviors were exaggerated by intermittent administration of the opioid receptor antagonist naloxone during morphine treatment. Pain hypersensitivity in animal models is commonly assessed using techniques like von Frey (mechanical), Hargreaves and tail flick (thermal), cold pain, electrical stimulation, and ischemic pain testing (Krishnan et al., 2012). Importantly, the cold pain test is reportedly one of the most reliable and translatable tests suited to detect OIH in opioid-dependent patients (Krishnan et al., 2012). Animal models of OIH have also been used to assess mechanisms and potential drug targets, notably including knockout studies of genes such as mu opioid receptor Oprm1 (Gregory Corder et al., 2017; Roeckel et al., 2017; Sun et al., 2019a) and other drug treatments (Araldi et al., 2018) with subsequent behavioral pain assays. Overall, hyperalgesia caused in animal models by administering opioids are beneficial models in investigating OIH during or after opioid treatment.

Current studies on mechanisms of OIH

There are many hypothesized mechanisms of OIH and several excellent reviews address these (Chu et al., 2008; DuPen et al., 2007; Higginbotham et al., 2022; Lee et al., 2011; Liang et al., 2006; Roeckel et al., 2016; Silverman, 2009). We will briefly mention a few potential mechanisms of OIH below.

The N-methyl D-aspartate receptor (NMDAR) is the excitatory neurotransmitter receptor thought to contribute to OIH.Some opioids and their metabolites can increase NMDA activity, with the notable exception of methadone that can be an antagonist (Chen et al., 2022; Hewitt, 2000). The NMDAR is located presynaptically on the central terminals of primary afferent neurons in addition to postsynaptically on spinal dorsal horn neurons (Roeckel et al., 2016). The NMDAR binds the excitatory amino acids glutamate and aspartate causing a state of sensitization within the central nervous system (CNS) (Hewitt, 2000). Prolonged administration of opioids over-activates NMDAR producing an influx of glutamate and calcium leading to excitotoxicity and enhanced excitability of neurons, contributing to hypersensitivity and OIH (Silverman, 2009). Specifically, NMDARs expressed in primary sensory neurons are shown to contribute greatly to OIH since knockout of GluN1, an essential subunit of the NMDAR, in mice prevented OIH development compared to control (Chen et al., 2022). Further, the NMDAR antagonists ketamine and dextromethorphan protect against OIH after chronic opioid treatment in rats (Arout et al., 2015; Stoicea et al., 2015), further suggesting that NMDARs can promote OIH, though their exact role needs further investigation.

OIH may also be affected by long term potentiation (LTP), a process in which synaptic connections between neurons become more robust after repeated activation in response to experiences such as learning and memory (Shors & Matzel, 1997). LTP can occur in pain pathways and lead to hypersensitivity at synapses between nociceptive C fibers and neurons in the spinal dorsal horn (Drdla et al., 2009). LTP and OIH have been shown to be regulated by similar signaling pathways (Comelon et al., 2016; Drdla et al., 2009). For example, brain-derived neurotropic

factor (BDNF) positively modulates LTP (Rex et al., 2007) and also activates the mitogen-activated protein (MAP) kinases ERK (extracellular signal-regulated protein kinase) and p38 (Ying et al., 2002). It has been shown that ERK signaling in the CNS is involved in both the analgesic and hyperalgesic outcomes of morphine, whilst, JNK and p38 are involved in the morphine-induced delayed hyperalgesia (de Freitas et al., 2019). This confirms the overlapping involvement of ERK and p38 signaling in LTP and OIH pathways.

The dysregulation of ion channels and transporters has been implicated in OIH pathophysiology. A microglia-specific subtype of Ca2+ -activated K+ (BK) channel was shown to be necessary for OIH. These channels were activated at the onset of OIH by arachidonic acid (AA) in the mouse spinal cord, and silencing BK channels prevented OIH (Hayashi et al., 2016). Moreover, morphine induced hyperalgesia in rats also downregulated the K(+)-Cl(−) co-transporter KCC2, dysregulating Cl- homeostasis. The restoration of Cl- balance by blocking BDNF-TrkB signaling reversed hyperalgesia (Ferrini et al., 2013). Hv1, the proton-selective ion channel, is also upregulated in peripheral sensory neurons after peripheral neuroinflammation and its inhibition via YHV98-4 diminished morphine-induced hyperalgesia in rodents (Zhang et al., 2022).

A recent study on remifentanil induced hyperalgesia in a rodent postoperative pain model showed that OIH may be driven by hyperexcitable thalamocortical networks (Jin et al., 2022). Glutamatergic neurons in the thalamic ventral posterolateral nucleus (VPL) showed an increase in burst firing and the change in neuronal excitability was due to upregulation in expression and activity of the $Ca_V3.1$ isoform of T-channels. Repression of $Ca_V3.1$-dependent burst firing or chemogenetic inhibition of VPL terminals projecting to the somatosensory cortex before surgery prevented the onset of hyperalgesia after remifentanil administration (Jin et al., 2022). Therefore, upregulated thalamic T-channels could be a target to prevent remifentanil induced hyperalgesia.

Inflammatory mechanisms have also been shown to play a large role in OIH onset. Opioid administration can activate astrocytes and microglia, as determined by increased expression of the markers glial fibrillary acidic protein (GFAP) and ionized calcium binding adaptor molecule 1 (Iba1) (Zhang et al., 2020b). Neurons can also promote astrogliosis (Liu et al., 2022a), in particular through wingless type 5a (Wnt5a), which triggers pro-inflammatory signaling cascades and associates with receptor tyrosine kinase-like orphan receptor 2 (ROR2) to form a complex (Zhang et al., 2020a). Genetically ablating astrogliosis in animals hindered the onset of OIH and knockout of Wnt5A or ROR2 in astrocytes effectively blocked OIH in mice (Liu et al., 2022b). Furthermore, OIH can be facilitated by pro-inflammatory cytokines like IL-1β, IL-6, IL-18, tumor necrosis factor-α (TNFα), interferon (IFN)-α and IFN-γ (Prieto & Cotman, 2017). A study of mice that developed OIH found enhanced skin levels of IL-1β, IL-6 and TNFα, and blocking transcription of these cytokines with pentoxifylline abolished OIH (Liang et al., 2008). Another study utilizing propentofylline, a modulator that reduces glial activation and proinflammatory cytokine production in the spinal cord, also attenuates OIH (Raghavendra et al., 2004). Therefore, preventing pro-inflammatory mechanisms could aid in OIH prevention.

Opioids contribute to analgesia by modulating descending control of pain mediated by serotonin (5-HT) and norepinephrine systems, thereby suppressing nociceptive transmission in the spinal cord. Prolonged administration of opioids can shift the balance from inhibitory control of pain towards pro-nociception. A recent study showed the 5-HT3 receptor antagonist ondansetron and the serotonin synthesis inhibitor *para*-chlorophenylalanine attenuated OIH and astrocyte activation associated with repeated morphine use, in the spinal dorsal horn of OIH model mice (Sasaki et al., 2021). Similar to 5HT, norepinephrine can promote or inhibit pain through binding to α- and β-adrenergic receptors (Pertovaara, 2006). Therefore, the 5-HT and norepinephrine systems could serve as targets to prevent OIH.

The family of opioid receptors is large and includes the mu, delta, and kappa opioid receptors, as well as the nociceptin receptors and opioid-receptor-like receptor 1. The mu opioid receptor (MOR) has been shown to play an outsized role in OIH (Gregory Corder et al., 2017; Roeckel et al., 2017; Sun et al., 2019a) The MOR is the most highly expressed opioid receptor along the pain pathway, making it a key player responsible for opioid binding and subsequent analgesia and anti-nociceptive properties. MORs are G-protein coupled receptors that are embedded in the outer membrane of neurons, immune and glial cells throughout the dorsal horn of the spinal cord and in different brain regions that process nociceptive information (Herman et al., 2022; Machelska & Celik, 2020). MORs couple to an inhibitory G protein G_i, and intracellular signaling pathways are activated after an endogenous or exogenous opioid ligand binds to the extracellular N-terminal domain of the receptor (Herman et al., 2022). MOR activity and downstream signaling has been highly implicated in OIH outcomes, as OIH models were protected from hypersensitivity by the MOR antagonists naloxone, methylnaltrexone bromide and D-Phe-Cys-Tyr-D-Orn-Thr-Pen-Thr-NH2 (CTOP) (Corder et al., 2013; G. Corder et al., 2017; Roeckel et al., 2016). On the other hand, MOR agonists, (D-ALA2,N-ME-PHE4,GLY5-OL)-enkephalin acetate (DAMGO), PZM21 and TRV130, induce OIH more rapidly (Ana Rita Costa et al., 2020; Araldi et al., 2018). Moreover, mice with completely ablated MORs in the dorsal root ganglion (DRG) and reduced MOR levels in the spinal cord, or with MOR deletion in nociceptors do not develop hyperalgesia from chronic opioid treatment (G. Corder et al., 2017; Sun et al., 2019a).

Although multiple mechanisms contribute to OIH, this review will focus on the epigenetic aspects that underpin OIH. This includes how various epigenetic factors like microRNAs could shed light on the underlying molecular landscape and how they may be novel targets to prevent OIH. Since we lack studies that mechanistically link OIH and epigenetics, we will highlight some of those gaps and propose potential additional studies to help understand how epigenetic regulation contributes to OIH.

Epigenetics

Epigenetics implies "both heritable changes in gene activity and expression (in the progeny of cells or of individuals) and also stable, long-term alterations in the transcriptional potential of a cell that are not necessarily heritable" according to the definition put forth by the NIH Epigenomics Roadmap Project (Gibney & Nolan, 2010). While initial interest in epigenetic alterations emphasized the degree of heritability of many epigenetic alterations, there has been a shift to a broader understanding of epigenetic mechanisms as the biological embedding of experience at a cellular level. In this way, modifications to how DNA and RNA are spatially organized, chemically modified, and transcribed in the context of various stimuli can be thought of as biologically embedded memory of these prior stimuli that modify the nature, magnitude or duration of their response (Aristizabal et al., 2020). At a cellular level, there are various epigenetic mechanisms that encode or embed this memory response within a cell, including DNA and RNA methylation, histone modifications (methylation, acetylation etc.), chromatin alterations and spatial organization, and non-coding RNA (microRNA, long non-coding RNA, piwi-interacting RNA, short-interfering RNA, repeat-associated RNA).

Each level of epigenetic control has its own regulatory and control mechanisms that allow changes to be made selectively. Key epigenetic regulatory proteins can modify DNA, RNA, and histones by writing (deposition of modifications), reading (recruiting other transcriptional machinery or regulatory proteins to that modification), and erasing (involved in removal of modifications) (Biswas & Rao, 2018). DNA methyltransferases, for example Dnmt3a and Dnmt3b, add methyl groups to DNA that block other proteins like transcription factors from binding to DNA and driving gene expression. Typically, methylation turns genes "off" and demethylation turns genes "on" (Gibney & Nolan, 2010). DNA is wrapped around histone proteins, which can also be modified to make genes more tightly or loosely packed to control their

expression. Histone acetylases add acetyl marks on specific lysine residues, such as H3K9ac and H3K27ac, and are known to turn genes "on". Alternatively, methylation of a histone's lysine residues can either activate or repress genes, as H3K4me1 activates whereas H3K9me2 represses transcription (Gupta et al., 2010). Lastly, small non-coding RNAs also epigenetically regulate gene expression by binding mRNA strands to degrade or repress translation (Bartel, 2004). Non-coding RNA can also recruit histone modifying proteins to turn genes "on" or "off". These epigenetic modifications occur in response to factors such as aging, infections, pregnancy, and cancer to aid the body's response to future stimuli. Epigenetic changes are characterized by how persistently they alter gene expression. Importantly, many epigenetic modifications are long lasting but also have the potential for reversal, which allows a proper stimulus to alter or reverse embedded experiences or epigenetic memories (Alvarado et al., 2015). This key feature of reversibility suggests a targetable mechanism for treating pain conditions, as studies have shown reversibility of gross anatomical changes such as cortical thinning in chronic low back pain patients who received effective pain management (Seminowicz et al., 2011). In addition, epigenetic control of transcriptional states has been shown to be both ubiquitous and cell specific suggesting that cell types that drive OIH could be targeted with epigenetic therapies.

Epigenetic regulation underlying OIH

Studies suggest that epigenetic changes resulting from repeated opioid administration as a possible mechanism that may contribute to OIH.

DNA methylation

Methylation of DNA is a well-studied epigenetic modification that involves the deposition of a methyl group on a cytosine residue within the DNA converting them to 5-methylcytosine. These cytosine residues are often concentrated within areas that contain repeats of a cytosine-phosphate-guanine motif, known as CpG islands, and methylated CpG islands repress transcription of other proximal genes. Methylation of cytosine residues is mediated by DNA methyltransferases (DNMTs), a class of enzymes (writers) with different members that perform different roles. For example, DNMT3a/b generates *de novo* methylation and DNMT1 maintains methylation during DNA replication (Gibney & Nolan, 2010). Proteins that read DNA methylation are called methyl CpG binding proteins (MBPs). They can be subdivided into three subfamilies, including methyl CpG binding domains (MBDs), SET and RING-associated domains (SRAs), and zinc finger proteins (ZnFs). Methylation marks are removed by eraser proteins such as the ten-eleven translocation (TET) family, which catalyze the conversion of 5-methylcytosine to 5-hydroxymethylcytosine (Biswas & Rao, 2018).

There are very few reports investigating changes in DNA methylation in the context of opioid use. One study of leukocytes from opioid users showed an increase in methylation at a CpG rich island in the *OPRM1* gene coding for the mu opioid receptor (MOR) and at the long interspersed nuclear element 1 (LINE-1) compared to control donors (Doehring et al., 2013). LINEs are non-long terminal repeats that are a part of the retrotransposon family, which are mobile genetic elements that utilize germ-line copy mechanisms to spread throughout the genome (Briggs et al., 2018). Global methylation sites are especially prevalent at these LINE-1 elements. Although the OPRM1 methylation had no direct effect on MOR transcription levels, high levels of chronic pain were substantially correlated with the global DNA methylation at LINE-1. This finding links opioid use to increased genome-wide DNA methylation and suggests a novel epigenetic mechanism underlying OIH (Doehring et al., 2013), though additional work must uncover the functional outcomes of LINE-1 methylation. DNMT3 is significantly upregulated in DRG and spinal cord in animal models of neuropathic pain (Pollema-Mays et al., 2014), and DNMT3a and DNMT3b isoforms

were elevated after chemotherapy induced and inflammatory pain in humans (Abzianidze et al., 2014). DNMT1 is also upregulated in DRG after peripheral nerve injury (Sun et al., 2019b), further suggesting a role for DNMTs in various painful ailments. Another study found changes in global DNA methylation correlate with decreased DNMT expression in patients who underwent breast surgery under opioid-based general anesthesia (Caputi et al., 2021), suggesting DNA methylation is an important epigenetic mechanism that could promote OIH.

Histone modification

Histone proteins control access to DNA and spatially organize the genome by regulating chromatin structure and dynamics. Chromatin is fundamentally organized into nucleosomes, which are made of a hetero complex of four different histone proteins: H2A, H2B, H3, and H4 (in addition to linker histones H1 and H5). DNA is wound around the nucleosome units, leaving some accessible portions available for transcription. This accessibility can be modified by lysine residues on the tail portions of the histone proteins, in particular histone H3 (Gibney & Nolan, 2010). Lysine residues are positively charged, and any changes in this charge can alter the binding properties of the negatively charged DNA and spatially rearrange the genome. Histone modifications most often consist of acetylation or methylation at lysine or arginine residues. Acetylation is carried out by the writer family of histone acetyltransferases (HATs), and erasure is mediated by histone deacetyl transferases (HDACs). Histone acetylation often activates transcription, while deacetylation often represses transcription by compacting chromatin (Liang et al., 2015). Additionally, histones can be methylated by histone methyl transferases on either lysine (HKMTs) or arginine (PRMTs) residues. Unlike DNA methylation in promoter regions which usually represses genes, histone methylation is more contextual and can include mono-, di- or tri-methylation modifications. Several histone methylation positions can activate transcription, such as H3K4, H3K36, and H3K79. Transcriptional repression is more commonly associated with methylation at H3K9, and H3K27 (Biswas & Rao, 2018).

Few reports link histone modifications to OIH. One study found that mice that received chronic morphine treatment showed differential effects on OIH development when also treated with the HAT inhibitor curcumin or the selective HDAC inhibitor suberoylanilide hydroxamic acid (SAHA). Curcumin significantly reduced OIH mechanically and thermally, whereas SAHA augmented OIH symptoms even four weeks after morphine treatment ended (Liang et al., 2013). The same study reported that OIH was associated with increased histone H3 acetylation and decreased HDAC activity in mouse dorsal horn of the spinal cord (Liang et al., 2013), suggesting this epigenetic mechanism could enhance development of OIH. Thus, preventing deacetylation could lead to OIH symptoms. Chronic morphine in combination with SAHA can also upregulate gene expression in the spinal cord. These treatments elevated aceH3K9 levels (acetylated histone H3 lysine9) on promoter regions of Bdnf and Pdyn (prodynorphin), and this chromatin activation marker is also upregulated in OIH (Liang et al., 2014). Morphine treated mice also showed increased spinal dynorphin levels, which were amplified by SAHA. In OIH model mice, the increased BDNF expression was related to a higher number of BDNF + cells in the spinal dorsal horn that co-localized with aceH3K9 in neuronal cells (Liang et al., 2014). In mice that recovered from OIH, low dose intrathecal administration of BDNF or dynorphin induced pain hypersensitivity compared to controls. BDNF acts mainly via binding to tropomyosin-related kinase B (TrkB) receptor and blocking TrkB with its antagonist ANA-12 reduced OIH (Sahbaie et al., 2016). Furthermore, coadministration of HAT inhibitor, anacardic acid, with morphine impeded the progression of OIH and attenuated post-operative incision-induced hyperalgesia in mice (Sahbaie et al., 2016). These results signify epigenetic modifications alter protein expression in OIH and blocking the acetylation of histones or BDNF/dynorphin signaling could reduce OIH outcomes.

Non-coding RNAs

Non-coding RNAs (ncRNAs) do not encode for proteins and have different functions than messenger RNAs (mRNAs), which carry genetic information from DNA for protein synthesis. ncRNAs comprise about 98 % of all transcriptional output in humans, and they regulate gene expression in various ways (Mattick, 2001; Rinn & Chang, 2012). There are several types of ncRNAs, including transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), and microRNAs (miRNAs). While tRNAs and rRNAs are crucial components of the protein synthesis machinery, snRNAs and snoRNAs help splice and process RNA (Esteller, 2011). Other ncRNAs like long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs) can regulate gene expression at different levels (Qin et al., 2020). The discovery of ncRNAs has revolutionized our understanding of regulation of gene expression and opened new avenues for therapeutic intervention (Kopp & Mendell, 2018). Many diseases, including cancer and neurological disorders, have been linked to dysregulation of ncRNA expression or function (Grimm et al., 2022; Yao et al., 2019). Though research on ncRNAs could be used to develop new diagnostic and therapeutic strategies, this avenue remains mostly unexplored regarding underlying factors that drive OIH.

miRNAs

miRNAs are a class of small, non-coding, single stranded, endogenous RNAs that are essential gene regulators. They are typically 18–24 nucleotides long, and they bind complementary sequences in the 3′ untranslated region (3′ UTR) of target mRNAs to repress their translation or degrade the target mRNA (Bartel, 2004). miRNAs bind to mRNA via a complementary seed sequence at positions 2–7 from the miRNA 5́-end. miRNAs are produced through a multistep process. In the nucleus, primary miRNA transcripts, called pri-miRNAs, are transcribed by RNA polymerase II and processed by the Drosha-DGCR8 complex into ~70 nucleotide precursor miRNAs called pre-miRNAs (O'Brien et al., 2018). The pre-miRNAs are then exported to the cytoplasm and processed by Dicer to generate mature miRNAs (Bartel, 2004). Mature miRNAs are then incorporated into the RNA-induced silencing complex (RISC), where they guide RISC to target mRNAs (Fabian et al., 2010). miRNAs are involved in a wide range of biological processes, including development, differentiation, apoptosis, and immune response (Bartel, 2004), and dysregulation of miRNA expression has been implicated in a variety of diseases (Kosaka & Ochiya, 2011). Further, miRNAs are being investigated as potential therapeutic targets, and miRNA-based therapy is an active area of research (Hanna et al., 2019). Recent work has identified circulating miRNAs as promising biomarkers for disease diagnosis and prognosis due to their stability in various bodily fluids, including blood, urine, and saliva (O'Brien et al., 2018). Several studies have demonstrated the potential of circulating miRNAs as biomarkers for various diseases, including cancer, cardiovascular disease, and neurological disorders (Mitchell et al., 2008). Importantly miRNAs are transported in circulation by small extracellular vesicles (sEV) that are secreted into the extracellular space by various cells. sEVs carry diverse sets of cargo to recipient cells, and the miRNAs transported by sEVs differ depending on the physiological state of the cell (Luo et al., 2021b). Therefore, it is possible that the miRNA signature in sEVs could be altered by chronic morphine exposure, which may lead to the different outcomes including OIH. Overall, miRNAs are important regulators of gene expression with diverse functions in normal cellular processes and disease pathology, and they have promise as diagnostic biomarkers or therapeutic targets for opioid use and OIH.

Recent studies have identified several miRNAs that may regulate pain. Notably, miRNA levels have been assessed in many animal models that mimic various pain states. miRNAs negatively regulate expression of genes associated with pain by translational repression or degradation of the target mRNA, thereby impacting pain signaling,

neuroinflammation, and neuronal plasticity. There are reports of miRNA regulation of ion channels, receptors, and signaling molecules associated with pain pathways. By regulating gene expression modulating inflammatory responses and neuronal plasticity, miRNAs exert a functional role in the development and maintenance of pain states. Changes in miRNA expression can affect synaptic plasticity, altering the strength and efficiency of neuronal connections in pain pathways contributing to the persistence and amplification of pain signals. Some miRNAs participate in bidirectional regulation, influencing both pro-nociceptive and anti-nociceptive pathways. It is also known that miRNAs contribute to the fine-tuning of gene expression by regulating multiple target genes within a signaling pathway. This allows for precise control over the expression of key molecules involved in pain processing (Andersen et al., 2014; Dai et al., 2018; Kalpachidou et al., 2020; Morchio et al., 2023; Sabina et al., 2022; Sakai & Suzuki, 2015; Zhang et al., 2023; Zhao et al., 2023).

MOR related miRNAs

MOR are primary drivers of analgesic tolerance and OIH (Gregory Corder et al., 2017) and therefore, we will discuss miRNAs targeting mainly the MOR as they are more likely implicated in opioid tolerance and onset of OIH.

Let-7 miRNA

The let-7 (lethal-7) family of miRNAs are implicated in opioid tolerance via their binding to 3′-UTR of MOR mRNA (He et al., 2010). This study showed that Let-7 translationally repressed MOR expression after morphine administration such that an upregulation of let-7 was associated with a downregulation of the MOR. Additionally, chronic morphine upregulated let-7 levels in mice, and knockout of let-7 reversed the tolerance effect (He et al., 2010). It is possible that tolerance and OIH may share mechanisms, as proteins or molecules upregulated in tolerance may be downregulated in OIH and *vice versa*. Therefore, the let-7 family of miRNA could be absent or downregulated in those who experience OIH, which may hypersensitize the MOR and lead to hyperalgesia. Further studies show that spinal cord injury (SCI) induced hyperalgesic states are also associated with increased levels of let-7 miRNAs and lower MOR and opioid response levels, again suggesting an important role for let-7 miRNAs in OIH (Callahan et al., 2016).

miR-190

miRNA levels can also be regulated bidirectionally, as studies show that MOR signaling can in turn regulate miRNAs. The MOR was shown to regulate miR-190 in an agonist-dependent fashion, as fentanyl decreased miR-190 levels in rodents. Fentanyl inhibited transcription of the miR-190 host gene *talin2* by disrupting the transcription factor Ying Yang 1 (YY1) from binding the promoter region that normally induces miR-190 expression (Zheng et al., 2010a). miR-190 has intriguingly been correlated with opioid action and addiction via inhibitory control of neurogenic differentiation 1 (NeuroD), which in turn is regulated by opioid receptor agonists and contributes to the addictive properties of opioids. Fentanyl treatment increased NeuroD levels by reducing miR-190 expression, which promoted addiction and potentiated opioid actions (Zheng et al., 2010b). However, NeuroD responds differentially to various opioids, and its activity decreases after morphine treatment. Decreased NeuroD was associated with anti-nociceptive effects after opioid administration, which was reversed after its activity was restored. Decreased NeuroD also inhibited development of tolerance, which was modulated through the expression of miR-190 driven by the *nestin* promoter (Li et al., 2014). Since NeuroD protects against tolerance under homeostatic conditions, it is possibly upregulated in OIH due to an opioid-mediated downregulation of miR-190. NeuroD1-null mice

have fewer sensory neurons (Quintanilla et al., 2014), so overexpression of NeuroD could sensitize these neurons and lead to OIH. These studies suggest miR-190 as a novel target to consider when exploring anti-OIH therapies and prevention methods.

miRNA regulation of MOR splice variants

The MOR is encoded by the *OPRM1* gene, which produces different splice variants and subtypes of the receptor (MOR-1A through MOR-1X) (Pasternak & Pan, 2011). Different MOR isoforms are thought to drive the opposing effects of analgesia, tolerance and hyperalgesia, as some may conversely excite cells by activating G_s instead of G_i proteins (Gris et al., 2010). *OPRM1* gene splicing may produce some differential effects seen in patients that are homozygous for the gene, where they experience less morphine analgesia compared to heterozygous patients (De Gregori et al., 2012). Therefore, OIH may be affected by splice variants of the MOR and differential miRNA binding that depends on the 3′UTR of the splice variants.

miR-103/107

The 3′-UTR of human MOR-1A contains conserved binding sites for miR-103 and miR-107 (Lu et al., 2014). These miRNAs were upregulated by chronic morphine treatment in mice, and they subsequently downregulated polyribosome-associated MOR-1A. Therefore, chronic opioid administration may produce OIH via the miR-103/107 "intron-retention carboxyl terminal splice variant" of the *OPRM1* gene, MOR-1A (Lu et al., 2014). Targeting miR-103/107 could be a novel mechanism to increase MOR-1A levels and alleviate OIH symptoms.

miR-23b and miR-339

Another study indicates that miR-23b decreases MOR1 association with polysomes after morphine treatment. miR-23b binds to the K-box motif in the 3′-UTR of MOR-1 mRNA, which suppresses translation by inhibiting the polysome – mRNA interaction (Wu et al., 2009). Chronic morphine treatment was shown to increase miR-23b levels and thus, repress this association further to downregulate MOR-1 activity (Wu et al., 2009). This group also showed that miR-339 can downregulate the MOR in response to opioids. Chronic morphine or fentanyl administration amplified levels of miR-339-3p and inhibited MOR activity by binding to the 3′-UTR target site. miR-339 functioned to degrade MOR transcripts and suppress endogenous MOR expression after chronic opioid usage (Wu et al., 2013). Though the group did not study specific effects of miR-23b or miR-339 on behavioral outcomes, their role in MOR activation suggests they could be potential targets for developing preventive or therapeutic intervention strategies.

MOR-1 K

Although some *OPRM1* splice variants may protect against OIH, the splice variant MOR-1 K has explicitly been shown to contribute to OIH. For example, a study reported mice with lower MOR-1 K gene expression levels displayed normal analgesic responses and behavior after chronic opioid administration, whereas mice with increased MOR-1 K gene expression levels showed hyperalgesia (Oladosu et al., 2015). siRNA knockdown of MOR-1 K prevented OIH after chronic opioid administration and also unmasked morphine associated analgesia (Oladosu et al., 2015). MOR-1 K has been shown to couple to G_s instead of G_i proteins, leading to increased cAMP and calcium levels that excite cells. As G_s coupling likely enhances central sensitization and OIH (Gris et al., 2010), degrading MOR G_s coupled variants with miRNAs could be a strategy to prevent OIH.

miR-212/132

The miR-212/132 cluster also regulates MOR expression after prolonged opioid administration. Morphine upregulates miR-212 via MOR-mediated signaling through cAMP response element-binding protein (CREB), which mediates actions of opioids and addiction and has also been implicated in pain pathways (Garcia-Concejo et al., 2016). CREB activation also regulates miR-212 expression after cocaine administration in animals (Hollander et al., 2010). Notably, miR-212 was found to cluster with and modulate miR-132, and this cluster subsequently inhibits *Oprm1* expression by binding to its 3′-UTR (Garcia-Concejo et al., 2016). This study also showed that miR-212/132 regulates other important factors in pain and addiction, including BDNF and MeCP2. They suggest that morphine-induced miR-132/212 downregulates MeCP2 expression, and since MeCP2 is an inhibitor of BDNF, Bdnf levels are increased and cause neuronal aggregation (Garcia-Concejo et al., 2016). Neuronal aggregation has been associated to increased pain states as overexpression of progranulin, which reduces protein aggregates, attenuates neuropathic pain in mice (Altmann et al., 2016). Many studies have shown Bdnf as a biomarker for pain hypersensitivity, further supporting the miR-212/132 cluster as a mechanism for hyperalgesia after prolonged opioid exposure (Thakkar & Acevedo, 2023).

Another study showed a role for miR-132 in opioid addiction, as opioid-induced upregulation of miR-132 potentiated morphine-seeking behavior and helped differentiate neural stem cells in rodent brains (Jia et al., 2019). These studies correlate both miR-212 and miR-132 in opioid signaling pathways after chronic usage, especially by regulating MOR expression and activity. Therefore, it is plausible that miR-212/132 contributes to OIH which may be context and tissue dependent and should be explored further.

miR-124 and miR-146

miR-124 is another miRNA has been implicated in OIH. A study found that injection of miR-124 and miR-146a intrathecally for four days reversed the persistent pain sensitization in chronic constriction injury (CCI) model rats that develop OIH after chronic morphine treatment. miR-124 injections successfully reversed persistent pain states for 6 h after the last dose and the rats returned to baseline pain levels pre-CCI 72 h afterwards (Grace et al., 2018).

miR-124 also alleviated nociceptive hypersensitivity in mouse models of formalin induced inflammatory pain (Kynast et al., 2013). In models of cancer pain, miR-124 was downregulated in the spinal cord, and intrathecal injections of miR-124 mimics completely attenuated cancer related pain in the early phase of the cancer. miR-124 was also downregulated in cancer patients with pain, suggesting it promotes analgesic effects (Elramah et al., 2017). Furthermore, miR-124 was downregulated in CCI rat models and overexpression of miR-124-3p suppressed mechanical allodynia, thermal hyperalgesia, and inflammatory cytokine expression (Zhang et al., 2019).

miR-124 is thought to affect OIH by targeting toll-like receptor 4 (TLR4) and increasing markers of anti-inflammatory M2 polarization via reduced microglial activation. These are vital processes that contribute to morphine induced hypersensitivity, as microglial activation and TLR4 signaling processes maintain the allodynia produced by repeated morphine administration (Ponomarev et al., 2011). TLR4 is a transmembrane protein expressed in myeloid cells and nociceptive DRG neurons, and it is encoded by the TLR4 gene (Vaure & Liu, 2014). Activation of TLR4, by lipopolysaccharide (LPS) for example, causes cells to release inflammatory cytokines and chemokines that activate the innate immune system in response to invading pathogens (Vaure & Liu, 2014). Morphine was shown to induce TLR4 signaling which could provide a justification for opioid-induced proinflammatory outcomes such as OIH (Hutchinson et al., 2010). Pharmacological blockade of TLR4 signaling potentiated morphine analgesia and mitigated analgesic tolerance, hyperalgesia, and opioid withdrawal behaviors, suggesting

TLR4 contributes to OIH (Hutchinson et al., 2010). Though TLR4 may be involved in the microglial activation that occurs in the development of opioid tolerance, other findings suggest that microglial activation caused by a mechanism independent of TLR4 is involved in the development of morphine tolerance (Fukagawa et al., 2013) and TLR4 is not required for opioid-induced analgesic tolerance, hyperalgesia, or physical dependence (Mattioli et al., 2014). Another study found that LPS from *Rhodobacter sphaeroides*, a TLR4 antagonist, also prevented remifentanil induced OIH in post-operative pain (Chen and Wei, 2019). Further, treatment with oligodeoxynucleotide antisense to TLR4 mRNA (TLR4 AS-ODN) decreased TLR4 expression in the DRG and prevented OIH from low-dose morphine (Araldi et al., 2019). TLR4 on the cell surface associates with myeloid differentiation protein-2 (MD-2), which permits TLR4 to respond to LPS and enhances its expression (Dziarski & Gupta, 2000). The TLR4/MD-2 heterodimer complex can be activated by the morphine metabolite M3G in the CNS, and systemic injections of M3G produce OIH even though M3G has a limited affinity for the MOR and no inherent analgesic effect (Due et al., 2012). Similarly, compound 15, a small molecule inhibitor of the TLR4/MD-2 complex, eliminated the excitability of sensory neurons after LPS or M3G administration (Due et al., 2012). TLR4 KO mice did not exhibit the M3G-induced hyperalgesia seen in WT mice. Further, miR-124 inhibits the pro-inflammatory actions of TLR4 signaling (Yang et al., 2022), which prevents the upregulation of TLR4 after chronic cocaine administration (Periyasamy et al., 2018). These studies show that TLR4 regulates OIH outcomes and miR-124 control of TLR4 activity is one of the well-studied epigenetic modulations of OIH.

miR-124 was also found to repress CREB expression (Rajasethupathy et al., 2009). Spinal activation of cAMP pathway subsequently phosphorylates CREB, which induces mechanical hyperalgesia (Hoeger-Bement & Sluka, 2003). CREB is often used as a marker for pain-related plasticity after injury and has been implicated in the effects of morphine. Morphine treatment increased levels of p-CREB, whereby inhibiting the ERK/CREB signaling pathway lowers morphine analgesic tolerance (Wu et al., 2018). Therefore, miR-124 suppression of CREB (Rajasethupathy et al., 2009) and ability to relieve opioid induced pain states suggests this miRNA can improve OIH outcomes.

miR-365

Another miRNA, miR-365, has been linked to morphine tolerance by targeting β-arrestin. β-arrestin regulates opioid actions and outcomes by controlling opioid receptor internalization and desensitization (Wang et al., 2016). Targeting β-arrestin interactions with opioid receptors has been explored as a potential therapeutic to reduce opioid tolerance. When phosphorylated GPCRs bind β-arrestins, they uncouple from their associated G proteins and internalize into the cell (Zuo, 2005). β-arrestin knockout models have been shown to protect against opioid tolerance, but they also develop an enhanced nociceptive response to chronic morphine treatments (Connor et al., 2015), suggesting involvement in OIH. An opioid tolerance model downregulates miR-365, a miRNA that targets β-arrestin, and miR-365 overexpression prevents tolerance by at least partially decreasing β-arrestin expression (Wang et al., 2016). These studies suggest that miR-365 plays an important role in OIH and downregulating miR-365 such that β-arrestin expression levels are increased could be a preventative strategy for patients with OIH.

miR-133b

Studies on miR-133b have found correlations to opioids and pain pathways. For example, miR-133b levels in blood were significantly decreased in men on methadone maintenance therapy who had a history of OUD compared to healthy controls (Hsu and Tsai., 2019). miR-133b may also be involved in pain pathways of post-stroke rat models, as it was significantly downregulated in rats experiencing intense post-stroke pain. Overexpression of miR-133b reversed the allodynia in these rats

and treatment with gabapentin, an anticonvulsant used to treat neuropathic pain, restored miR-133b levels, suggesting miR-133b expression decreases in hyperalgesic states (Guo et al., 2022). Further, a miR-133b inhibitor eliminated the antiallodynic properties of gabapentin (Guo et al., 2022), suggesting that increased levels of miR-133b protect against allodynia and hyperalgesia. Opioids are also known to increase dopamine levels in the nucleus accumbens (NAc), and this pathway also involves miR-133b (Kosten & George, 2002). In this study, morphine decreased miR-133b expression, which increased the expression of its target, pituitary homeobox 3 (Pitx3). Pitx3 then activated the dopamine transporter (DAT) and increased dopamine levels (Sanchez-Simon et al., 2010). These studies suggest that miR-133b downregulation is a normal effect of opioid exposure, but prolonged usage of opioids chronically and considerably downregulates miR-133b, as seen in the cohort of men with OUD on methadone maintenance therapy (Hsu and Tsai., 2019). Since miR-133b protects against the development of hyperalgesia, its downregulation after chronic opioid usage may drive OIH, and its upregulation could protect against OIH. Table 1 summarizes the miRNAs discussed, their predicted targets and effects along with corresponding studies and organisms.

Human studies

A few studies show that opioid exposure and pain alter levels of several circulating miRNAs that correlated with OIH outcomes. One group reported plasma miRNA changes after healthy males were orally dosed hydromorphone or oxycodone. They found nine miRNAs upregulated after treatment, including let-7a-5p, miR-23b-3p, miR-146a-5p and miR-146b-5p, in addition to 17 downregulated, including miR-144-3p, miR-192-5p, miR-363-3p and miR-194-5p (Toyama et al., 2017). Many of these miRNAs are associated with the human MOR, including the let-7 family, miR-103a-3p, miR-339-3p, miR-146a-5p, miR-23b-3p, miR-23a-3p, and miR-181a-5p, all of which were differentially expressed following opioid administration (Toyama et al., 2017). The same investigators also studied novel biomarkers for analgesic efficacy of opioids based on blood samples from patients with cancer associated pain before treatment with hydromorphone. They tested levels of four highly up- and downregulated miRNAs based on their previous study and interestingly, found that the patients who had miRNA signatures correlating with lower MOR activities responded better to the opioid treatment (Kiyosawa et al., 2019). Their results suggest differential MOR activity in humans dysregulates opioid outcomes and leads to their negative effects, potentially including OIH.

Another study in humans who have chronically abused opium revealed differential miRNA signatures compared to healthy controls. The study observed a dose-dependent upregulation of miR-155-5p and miR-187-5p in those who chronically used opium with an associated increase in pro-inflammatory cytokines TNF-α and IL-10 and a decrease in anti-inflammatory IL-6 (Purohit et al., 2022). Prolonged opioid use leading to OIH could, therefore, be linked with aberrant expression of human miR-155 and miR-187 levels as well. Further, a study on postmortem brain and blood tissue from people with OUD revealed miRNA alterations associated with changes in endothelial cell function and tube development. Notably, miR-92a-3p was upregulated and miR-29a downregulated in the brain, both acting via the p38 MAPK signaling pathway (Grimm et al., 2022). p38 MAPK has been highly correlated with various pain states (Anand et al., 2011; Crown et al., 2008; Mai et al., 2020), so it is possible that differential expression of p38 MAPK associated miRNAs after prolonged opioid usage could potentiate pain and lead to OIH.

Another study investigating how prolonged heroin exposure affects miRNA signatures found significant decreases of CREB-targeting miR-NAs miR-582-5p and miR-590-5p in human monocytes as well as decreased TNFα levels, both of which have been implicated in pain (Long et al., 2016). Heroin dependent patients also had increased expression of miR-320a and let-7b-5p, which could also play a role in

Table 1
miRNAs implicated in opioid tolerance and the onset of OIH, and their predicted targets.

miRNA	Effect	Studied Targets	Ref	Organism Studied
miR-124	Reversed OIH in CCI model	TLR4	Grace et al., 2018 Elramah et al., 2017	Rat
	Repress TLR4 activation	CREB		Human
	Therapeutic in pain models			
	Repress CREB activation			
miR-146a	-Reversed OIH in CCI model		Grace et al., 2018	Rat
let-7	-Represses MOR activation	MOR	He et al., 2010 Callahan et al., 2016	Mice
	-Chronic morphine upregulates let-7			Rat
	-SCI hyperalgesia upregulates let-7			
miR-103	-Downregulates MOR-1A splice variant	MOR-1A splice variant	Lu et al., 2014	Mice
	-Chronic morphine upregulates miR-103			
miR-107	-Downregulates MOR-1A splice variant	MOR-1A splice variant	Lu et al., 2014	Mice
	-Chronic morphine upregulates miR-107			
miR-23b	-Downregulate MOR-1 activity	MOR-1	Wu et al., 2009	Mice
	-Chronic morphine upregulates miR-23b			
miR-339	-Downregulates MOR	MOR	Wu et al., 2013	Mice
	-Chronic morphine or fentanyl upregulates miR-339			
miR-212	-Chronic cocaine increases miR-212	MOR	Hollander et al., 2010 Garcia-Concejo et al., 2016	Rat
	-Chronic morphine upregulates miR-212	CREB		Zebrafish
	-Downregulates MOR	MeCP2		
	-Clusters with miR-132	BDNF		
miR-132	-Chronic opioid usage upregulates miR-132	MOR	Garcia-Concejo et al., 2016 Jia et al., 2019	Zebrafish
	-Downregulates MOR	CREB		Rat
	-Potentiates morphine-seeking behaviors	MeCP2		
	-Clusters with miR-212	BDNF		
miR-365	-Chronic opioids downregulate miR-365	B-arrestin	Wang et al., 2016 Connor et al., 2015	Rat
	-Decreases B-arrestin			Mice
miR-133b	-Chronic opioids decreases miR-133b	Dopamine Transporter	Hsu et al., 2019	Human
	-Downregulated in pain disorders		Guo et al., 2022 Sanchez-Simon et al., 2010	Rat
	-Reverses allodynia			Zebrafish
miR-190	-Fentanyl decreases miR-190	NeuroD	Zheng et al., 2010	Rat
	-Decreases NeuroD expression		Li et al., 2014	Mice

OIH development (Liu et al., 2021). Chronic opioid exposure alters many miRNAs in humans, some with known relations to pain states, and therefore their involvement in OIH should be studied further. Fig. 2 summarizes the epigenetic mechanisms discussed and highlights the associated studies.

Conclusions

Epigenetic regulatory pathways in pain are still not well studied and will require additional work to evaluate how these alterations are maintained throughout OIH. Most studies have utilized a handful of well characterized models to identify alterations in key ion channels, neuronal plasticity genes, and excitatory and inhibitory signaling, predominantly within the spinal cord. The current use of inhibitors to many of the enzymes that mediate epigenetic modifications (HDACs and DNMTs) are non-specific and were developed to treat cancer (Li & Seto, 2016). We do not fully understand the long-term effects of drugs that modulate global epigenetic modifications, and their lack of specificity likely makes them unsuitable for chronic use. However, they may be useful in the acute perioperative period to prevent OIH onset (Luo et al., 2021a). It will be critical to develop new approaches to more specifically target these epigenetic enzymes, such as viral vectors and gene therapies that take advantage of a dead Cas9 targeting system to deliver epigenetic modifying enzymes directly to the site of the NaV1.7 gene to regulate its expression (Moreno et al., 2021). Gene editing tool CRISPR/Cas system is used to target any region of interest in the genome by designing guide RNA that can recruit a CRISPR-associated protein to the loci of interest (Pickar-Oliver & Gersbach, 2019). Mutated Cas9 protein blocks nucleolytic activity of Cas9 but does not impact binding to its target. This transcriptional effector function of dead Cas9 is now used to deliver cargo that can induce epigenome remodeling to induce targeted transcriptional repression or activation of genes (Heidersbach et al., 2023; Kanafi & Tavallaei, 2022). Epigenetic modifications are often located at a distance from the gene promoter. Enhancer regions are DNA-regulatory elements which are located at a distance but come in physical proximity with the promoters of their target genes to regulate transcription. CRISPR/dCas9-based can be used to target enhancer region for epigenetic editing. Inducible enhancer-targeting by CRISPR/dCas9 epigenetic editing system was used in inhibiting or activating enhancer functions (Heidersbach et al., 2023; Li et al., 2020). Such studies will require elucidating the epigenetic marks associated with OIH and more importantly determining if these are cell specific so that neuronal (Giehrl-Schwab et al., 2022) or other CNS specific cells tools can be employed (Giehrl-Schwab et al., 2022).

It would be fruitful to see how opioid receptor subtype activation modifies non-coding RNA and other epigenetic alterations, and if they overlap with those that have been associated with the MOR dependent induction of OIH. This may also give insight into common targets and mechanisms driving OIH. Other potentially useful strategies include targeted protein degradation through the use of PROteolysis TArgeting Chimeras (PROTACs), which can be combined with targeted delivery to regulate the expression of methylation and histone altering enzymes in OIH (Nalawansha & Crews, 2020). PROTAC technology is an emerging field that allows the ubiquitin-proteasome system to be adapted to degrade a specific target protein. PROTACs are bifunctional molecules comprised of a target binding unit, a linker, and an E3 ligase binding moiety. Considered to be the new and exciting class of therapeutic agents, the underlying principle of PROTAC is to induce the polyubiquitination and proteasome degradation of the target proteins in cells, specifically aimed at proteins that cannot be trag22eted by traditional small molecules. However, only a handful of E3 ligases have been successfully utilized in PROTAC compounds (Burslem & Crews, 2020). It will be exciting to see what the future holds for our ability to regulate the epigenome in pain states and provide novel treatments for patients in the future.

OIH has been an unexpected yet quite devastating outcome of using opioids to treat pain. It is very unfortunate for patients and clinicians alike because it causes extreme pain, worse than what the opioid was prescribed to control, and lacks a surefire way to mitigate its effect. Thus far, many studies have tried to uncover various pieces of OIH on a
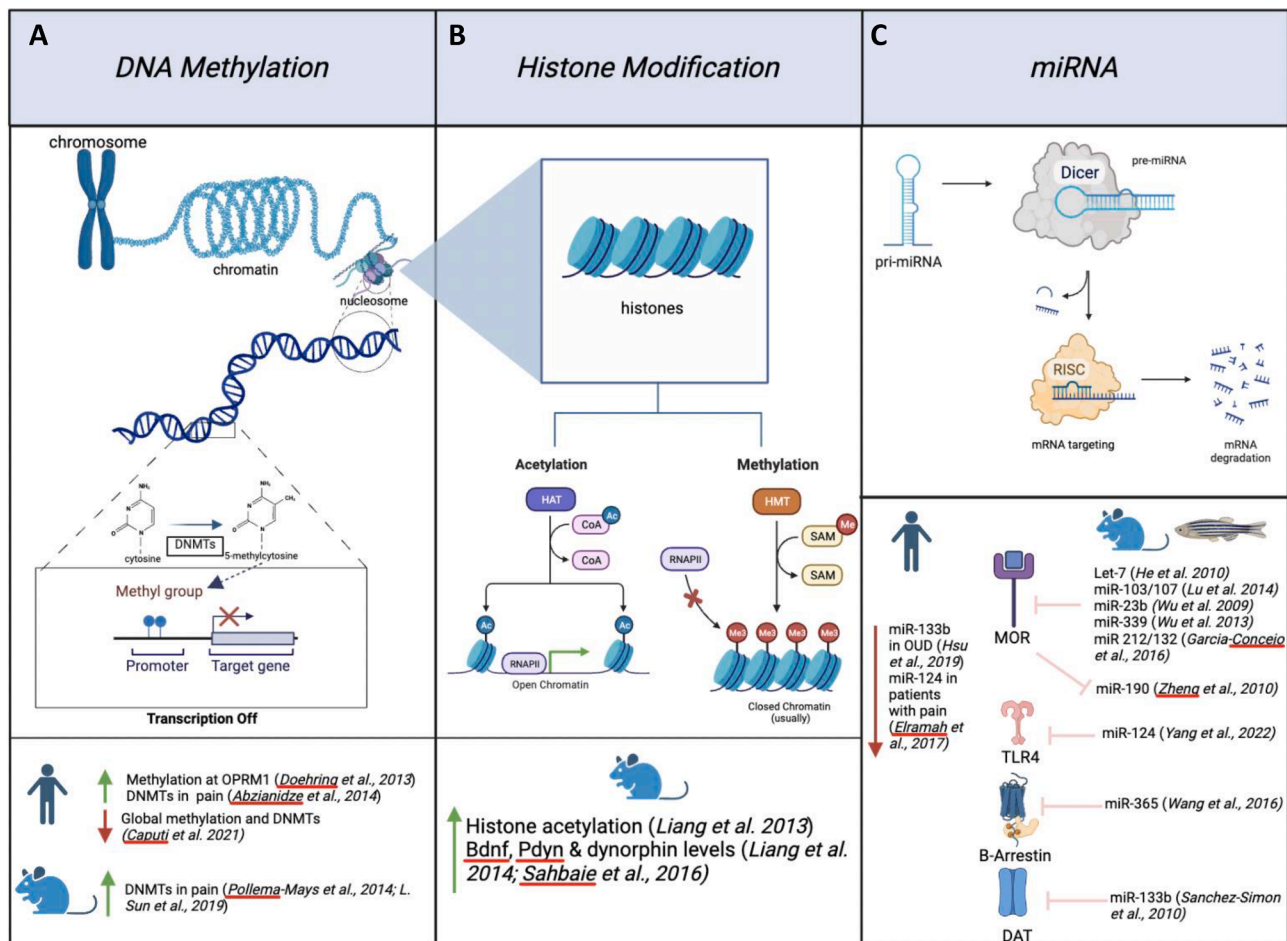
Fig. 2. Epigenetic changes associated with opioid induced hyperalgesia. 2A Increase in DNA methylation by DNMTs mostly repress transcription. 2B Histone modifications can influence accessibility of the DNA in the nucleosome for transcription and acetylation and methylation are shown as two examples. 2C miRNAs target mRNA and repress translation. Some of the key studies discussed in the context of OIH for all three epigenetic modifications are listed with their corresponding organisms.

mechanistic level, primarily based on studies of OIH in animal models. Yet there remains a crucial gap in knowledge, including how multiple epigenetic modulators contribute to OIH. There is still no documented way to avoid OIH clinically as well as no concrete underlying mechanism. Further, there are not many studies on the epigenetic effects of OIH even though many pain pathologies have epigenetic underpinnings, especially involving miRNAs. Several underlying mechanisms of OIH interact with each other, highlighting the complexity and interconnected nature of human physiology. Additional studies of the epigenetic mechanisms of OIH will provide insights on what drives the disorder and the contributions of specific pathways.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Funding

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