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Targeting Epigenetic Mechanisms for Chronic Pain: A Valid Approach for the Development of Novel Therapeutics

[*]Casey O. Ligon, [*]Rachel D. Moloney, Beverley Greenwood-Van Meerveld

Oklahoma Center for Neuroscience, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA (C.O.L., R.D.M. and B.G.-V.M.) and the V.A. Medical Center, Oklahoma City, OK, USA (B.G.-V.M.)

Minireview

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[*] contributed equally to this manuscript.

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Address Correspondence to: Beverley Greenwood-Van Meerveld, Ph.D., FACG, AGAF, Oklahoma Center for Neuroscience, BRC 272, 975 N.E. 10th St., Oklahoma City, OK 73104, Tel: (405) 456-3547 E-Mail: Beverley-Greenwood@ouhsc.edu

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Abbreviations: 5-aza, 5'-aza-2'-deoxycytidine; 5-mC, 5-methylcytosine; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BDNF, brain-derived neurotrophic factor; CCI, chronic constriction injury; CeA, central nucleus of the amygdala; CFA, complete Freund's adjuvant; CORT, corticosterone; CRF, corticotrophin releasing factor; DNMT, DNA methyltransferase, DRG, dorsal root ganglia; E2, estradiol; GABA, gamma aminobutyric acid; GR, glucocorticoid receptor; H3, histone 3; H4, histone 4; HAT, histone acetyltransferase; HDAC, histone deacetylase; HMT, histone methyltransferase; Hypoesth, hyoesthesia; IBS, irritable bowel syndrome; lncRNA, long noncoding RNA; KMT, lysine methyltransferase; Mech, mechanical hypersensitivity; miRNA, microRNA; MS, maternal separation; ncRNA, noncoding RNA; NRM, nucleus raphe magnus; NSAID, non-steroidal anti-inflammatory drug; PFC, prefrontal cortex; PRMT, protein arginine N-methyltransferases; PSNL, partial sciatic nerve ligation; SAHA,

suberoylanilide hydroxamic acid; SC, spinal cord; SCN, sciatic nerve; shRNA, short hairpin RNA; siRNA, small interfering RNA; SNL, spinal nerve ligation; Ther, thermal hypersensitivity; TALEN, transcription activator-like effector nucleases, TRPV1, transient receptor potential cation channel subfamily V member 1; TSA, trichostatin A; WAS, water avoidance stress; VMR, visceromotor response

Abstract

Chronic pain is a multi-faceted and complex condition. Broadly classified into somatic, visceral or neuropathic pain, it is poorly managed despite its prevalence. Current drugs used for the treatment of chronic pain are limited by tolerance with long-term use, abuse potential, and multiple adverse side effects. The persistent nature of pain suggests that epigenetic machinery may be a critical factor driving chronic pain. In this review we discuss the latest insights into epigenetic processes, including DNA methylation, histone modifications and miRNAs, and describe their involvement in the pathophysiology of chronic pain and whether epigenetic modifications could be applied as future therapeutic targets for chronic pain. We provide evidence from experimental models and translational research in human tissue that have enhanced our understanding of epigenetic processes mediating nociception and then speculate on the potential future use of more specific and selective agents that target epigenetic mechanisms to attenuate pain.

Introduction

Acute pain signals real or potential tissue damage, making it an important protective sensation that influences behavior to prevent further injury and promote healing. Acute pain can be mild or severe, with a relatively short duration, and typically resolves with the treatment or healing of the damaged tissue. In contrast, chronic pain is a pathologic state that does not serve a protective function and is considered harmful to the organism. Defined as pain persisting for 6 months after the resolution of, or in the absence of, an injury, chronic pain affects approximately one fifth of adults in the United States and is associated with a significantly reduced quality of life and an increased risk of mental health disorders (Breivik, Collett et al. 2006, Tsang, Von Korff et al. 2008, Goldberg and McGee 2011). The economic effects of chronic pain are equally profound, with an estimated cost of $300 billion in the United States alone (IOM 2011).

The precise mechanisms underlying the transition from acute to chronic pain are not well understood, though adaptations in numerous structures within pain pathways (peripheral neurons, dorsal root ganglia (DRGs), spinal cord neurons, and the brain) are known to be involved (Woolf and Salter 2000, Kuner 2010). This plasticity, which is essential for the development of chronic pain, involves significant changes in neurotransmitters and other molecules, cells, and neural networks and is maintained, in part, by epigenetic processes (Graff, Kim et al. 2011, Buchheit, Van de Ven et al. 2012, Karpova 2014). Indeed, epigenetic processes, comprising DNA methylation, chromatin remodeling, and noncoding

RNA, have been increasingly implicated in the development of chronic pain in recent years, thus highlighting new potential targets for drug development and pain management.

While current therapeutics are effective at relieving acute pain, drugs used to manage chronic pain are typically less efficacious, with the development of novel pharmacologic therapies experiencing little progress in recent decades. Indeed, chronic pain is still predominantly treated with two classes of drugs: non-steroidal anti-inflammatory drugs (NSAIDs) and opioids. Both NSAIDs and opioids, along with the small number of other drugs that do not fall into these categories, produce a number of adverse side effects and, critically, often fail to provide adequate long-term relief in many chronic conditions (Johnson and Greenwood-Van Meerveld 2014). Given these limitations, the need for improved therapeutic options for the management of chronic pain is apparent.

This review briefly summarizes the pathways of chronic pain, categorized as somatic, visceral, or neuropathic, and the currently available therapies. We then describe the primary mechanisms of epigenetic regulation and review the recent findings in the field of epigenetics and chronic pain. Finally, we discuss the epigenetic drugs and their usefulness in the context of pain management and highlight the potential approaches for targeting epigenetic mechanisms in a specific and selective manner.

Chronic Pain

Chronic pain pathways involve stimuli originating from skin, skeletal muscle, joints, or visceral organs. These pathways and the mechanisms of their sensitization have been expertly described in several excellent reviews (Besson 1999, Almeida, Roizenblatt et al. 2004, Wilder-Smith 2011, Woolf 2011) Briefly, the classic acute pain pathway involves the activation of primary nociceptive afferents within the periphery, which then send the stimulus to the dorsal horn of the spinal cord (Almeida, Roizenblatt et al. 2004). After synapsing with the dorsal horn, the signal is then transmitted across the midline to the anterolateral tract of the spinal cord, where it ascends to the thalamus in the brain. From the thalamus, the signal is relayed to the somatosensory cortex for localization. Ascending nociceptive signals traveling via the spinoparabrachial and spinoreticular tract activate limbic structures, such as the amygdala, to produce an emotional response to the stimulus. The remaining constituent regions of the central pain matrix [prefrontal cortex (PFC), cingulate, and parietal cortex] define the magnitude and quality of the pain signal. Pathways comprising of motor cortex and brain stem areas are then activated, contributing to descending modulation of the pain signal. Although the exact mechanisms vary depending on site and pathology, sensitization of this acute pain pathway occurring at the level of the periphery, spinal cord, or central pain matrix leads to the development of chronic pain. In the periphery, following tissue damage or immune stimulation, primary afferent nociceptors are sensitized due to the release of a myriad of

algesic molecules [cytokines, histamine, prostaglandins, substance P, etc.] by local tissue or immune cells participating in the healing process. Additionally, activation of G protein coupled receptors on the primary afferent nociceptors induces second-messenger pathways that change intracellular calcium, modify existing receptors, and alter receptor expression, all of which lead to further sensitization of the nociceptor. The second site of sensitization occurs within the dorsal horn of the spinal cord, where the sensitized primary nociceptive afferent releases neurotransmitters (including glutamate, substance P, BDNF, and others) that stimulate the 2nd order neuron. Sensitization is promoted through changes in gene regulation that shift the balance of receptors, leading to an increased excitably of the neuron – through changes in AMPA, potassium, and mGlu receptors. The final hypothesized mechanism that produces chronic pain is supraspinal hypersensitivity. Central sensitization at the level of supraspinal sites, specifically regions encompassing the pain matrix discussed above (thalamus, PFC, amygdala) alter the descending control of pain responses and thus maladaptive, chronic nociceptive behaviors occur (Cervero 1995, Cervero 2000). Indeed, brain imaging studies have revealed not only gross structural changes (decreased grey matter, cortical thickness) (DaSilva, Becerra et al. 2008, Cauda, Palermo et al. 2014) but altered connectivity between regions in this matrix (Ellingson, Mayer et al. 2013, Mansour, Baliki et al. 2013, Irimia, Labus et al. 2015). Moreover, it has been proposed that long-term potentiation and thus plasticity occurs to maintain central sensitization (Reichling and Levine 2009, Luo, Kuner et al. 2014).

Epigenetic Mechanisms

The term ‘epigenetics’ describes phenotypic trait variations resulting from developmental or environmental cues rather than alterations to the genomic DNA sequence itself (Waddington 1942). The most thoroughly investigated mechanisms through which these variations arise involve modifications that regulate chromatin, which is made up of combinations of DNA, histones, and other proteins. These modifications, which are dynamic and reversible, lead to the structural remodeling of chromatin and result in the differential expression of genes. Epigenetic mechanisms include DNA methylation and the post-translational modification of histones, with the entirety of these different modifications referred to as the epigenome (Bernstein, Meissner et al. 2007). More recently, epigenetic mechanisms involving RNAs that do not encode protein or participate in translation have been identified. Only approximately 1.5% of the human genome encodes protein, while the non-coding elements of the genome serve to regulate the expression of the coding regions through various mechanisms such as long noncoding RNAs (lncRNAs) and microRNAs (miRNAs). MicroRNAs are a subset of small noncoding RNAs (ncRNAs) that inhibit gene expression by targeting specific mRNAs for degradation (Farh, Grimson et al. 2005, EPC 2012). Long noncoding RNAs are ncRNAs with a length greater than 200 nucleotides that can regulate transcription via a number of different mechanisms. Aside from directly targeting mRNA, lncRNAs have also been shown to interact transcription factors, chromatin-modifying complexes, and

various other molecules to alter gene transcription (Bonasio and Shiekhattar 2014). Although miRNAs and lncRNAs do not directly modify chromosomal regions themselves, they can confer altered activity states of genes in response to environmental cues and therefore are generally considered epigenetic regulators.

Histone modifications

Chromatin structure is significantly impacted by post-translational modifications at the freely accessible N-terminal tails of histones, particularly on histones H3 and H4. These modifications can take the form of lysine acetylation, serine/threonine phosphorylation, lysine/arginine methylation, ubiquitination and ADP-ribosylation. In general, the presence of acetyl or phospho groups promote active transcription states by decreasing the affinity between histones and DNA, resulting in greater availability of DNA to interact with transcriptional machinery (Geranton and Tochiki 2015). Histone methylation, however, can have both activating and silencing effects on gene expression depending on the histone residue modified. For instance, H3K4, K36, and K79 methylation is indicative of active transcriptional states while methylated H3K9 and H4K20 are signifiers of condensed and inactive chromatin (Ng, Yue et al. 2009). It is important to note that genes can demonstrate both active and repressive modifications, representing a poised state requiring additional external cues to promote either increased expression or gene silencing.

There a number of enzymes dedicated to modifying histones. Histone acetyltransferases (HATs) are responsible for transferring acetyl groups to histone tail lysine residues. Acetylation is reversed by histone deacetylases (HDACs). There are four known classes of HDACs-the zinc-dependent classes I, II, and IV, and class III (or sirtuins), which are (NAD)-dependent (Geranton and Tochiki 2015). Similarly, there are several known enzymes dedicated responsible for methylating histone residues. Histone methyltransferases (HMTs) generally fall into two classes: lysine methyltransferases (KMTs) and the less well understood protein arginine N-methyltransferases (PRMTs) (Izzo and Schneider 2010). Initially thought to be an irreversible modification, histone methylation has been shown to be a dynamic process with the recent discovery of a number histone demethylases (Cloos, Christensen et al. 2008).

DNA modifications

Another key epigenetic mechanism of chromatin regulation and, consequently, gene expression is methylation of cytosines in genomic DNA to produce 5-methylcytosine (5mC). Cytosine methylation occurs predominantly at CpG dinucleotides, particularly those within intergenic regions and repetitive sequences such as long and short interspersed nuclear elements (LINEs and SINEs) (Zamudio and Bourc'his 2010). Typically, methylation in the regulatory regions of promoters silences gene transcription by preventing transcription factor binding or by recruiting gene-silencing complexes. However, DNA methylation is not an exclusively repressive mark. As with histone modifications,

DNA methylation is a complex mechanism has been shown to promote both gene activation and silencing depending on the surrounding epigenome (Smith and Meissner 2013).

DNA methylation occurs via DNA methyltransferases (DNMTs). DNMT1 is responsible for the maintenance of methylation patterns as cells divide, while DNMT3A and DNMT3B are the enzymes that engage in *de novo* DNA methylation in response to environmental cues (Smith and Meissner 2013). DNA demethylation, however, is a much more complex process and much less understood. DNA methylation was long thought a static mechanism but it has recently been recognized as a more plastic process after the identification of demethylation-mediating proteins such as Gadd45a and the TET family proteins, among others (Chen and Riggs 2011).

Evidence for Epigenetic Changes in Preclinical Studies of Pain

In recent years a significant amount of research examining the involvement of epigenetic processes in human disease has been conducted, resulting in the development of a handful of novel therapeutic agents that target these processes. However, the science of pain epigenetics is still very much in its infancy, with understanding of the links between epigenetics and the transition to, and maintenance of, chronic pain only beginning to emerge. Consequently, it is not yet clear if targeting these epigenetic mechanisms is a practical method by which chronic pain can be managed.

Somatic Pain

Most studies investigating epigenetic mechanisms involved in chronic somatic pain have been performed in animal models of inflammatory pain. Generally, inflammation-induced histone modifications contribute to hypersensitivity via dysregulation of HDAC and HAT activity and expression, resulting in hypoacetylation at promoter regions of antinociceptive genes or genes critical to proper pain circuit functionality and the silencing of these genes (Zhang, Cai et al. 2011). In the case of DNA methylation, inflammation is capable of inducing hypermethylation at the promoter region of antinociceptive genes and consequently silencing these genes (Pan, Zhu et al. 2014). Other studies have observed hypomethylation of pronociceptive genes and, therefore, increased expression of those genes in models of inflammatory pain (Qi, Zhou et al. 2013). Alterations in the levels of epigenetic enzymes such as methylated CpG-binding proteins have also been reported in models of inflammatory pain (Zhang, Tao et al. 2014).

Epigenetic modifying enzymes themselves (i,e, HDACs, HATs) have also implicated in somatic pain modulation and can impact downstream expression of both pro and anti-nociceptive genes. Crow *et al*. showed that HDAC4 was essential for appropriate transcriptional responses after injury. Pro-nociceptive genes Calca and Trpv1 expression were consistently lower within the DRG sensory neurons in HDAC4 conditional knockout animals when compared to their littermate controls (Crow, Khovanov et al. 2015). Furthermore, this down-

regulation of HDAC4 reduced the sensitivity to capsaicin *in vitro* and reduced thermal hypersensitivity in the complete Freund's adjuvant (CFA) model of inflammatory pain (Crow, Khovanov et al. 2015). The CFA model has also revealed changes in specific miRNAs. CFA significantly reduced miRNA-219 expression in mice spinal neurons with bisulfite sequencing revealing CFA-induced hypermethylation of CpG islands in the miR-219 promoter (Pan, Zhu et al. 2014). Moreover, overexpression of spinal miR-219 prevented and reversed thermal hyperalgesia and mechanical allodynia (Pan, Zhu et al. 2014).

Other gene targets shown to be epigenetically regulated in the CFA model include BDNF, whereby histone H3 acetylation at the *bdnf* gene promoter was reduced significantly 3 days after CFA injection, with concomitant increases in bdnf mRNA levels and BDNF protein levels (Tao, Chen et al. 2014). Others have implicated cystathionine-β-synthetase in the pathophysiology of CFA-induced inflammatory pain where its promoter was differentially methylated in DRG samples from inflamed rats versus controls (Qi, Zhou et al. 2013). Further examination revealed significant up-regulation of methyl-binding domain protein 4 and growth arrest and DNA damage inducible protein 45α in inflamed rats (Qi, Zhou et al. 2013), further implicating DNA methylation as a key mechanism underlying CFA-induced inflammatory pain. A translational study of note that examines DNA methylation employed a rodent model of lower back pain and reported DNA methylation-induced down-regulation of SPARC, an extracellular matrix protein that has been linked to age-dependent disc degeneration (Tajerian, Alvarado et al. 2011).

The most commonly investigated drugs in the context of chronic somatic pain are HDAC inhibitors (HDACis). Studies indicate that HDAC inhibition or the promotion of histone acetylation in these models is typically analgesic. One study in 2011 by Zhang and coworkers showed that 3 days post-intraplantar CFA administration, animals exhibited decreased acetylated H3 at the *Gad2* promoter in the nucleus raphe magnus (NRM) of the brain resulting in the down-regulation of GAD65, an enzyme essential for normal GABAergic neuron functionality. Following repeated infusion of HDAC inhibitors such as trichostatin A (TSA) or suberoylanilide hydroxamic acid (SAHA) into the NRM, CFA-induced thermal hyperalgesia was attenuated and H3 acetylation at *Gad2* was restored (Zhang, Cai et al. 2011). A second study utilizing a mouse CFA pain model found that inflammation promoted up-regulation of class II HDACs and down-regulation of acetylated H3K9/K18 in the spinal cord. These epigenetic changes, along with inflammatory thermal hyperalgesia, could be prevented with a single intrathecal dose of a number of class II HDAC inhibitors 30 min prior to CFA administration or reversed with an intrathecal dose of these inhibitors at 1, 5, and 25 hrs post CFA (Bai, Wei et al. 2010). Treatment with demethylation agent 5'-aza-2'-deoxycytidine (5-aza) has also shown efficacy in the CFA model with marked reductions in pain behavior and spinal neuronal sensitization (Pan, Zhu et al. 2014), however further data on the therapeutic potential of DNA methylation is limited.

Visceral Pain

Few studies have addressed the epigenetics of chronic visceral pain. Similar to somatic pain, evidence suggests that the maintenance of chronic visceral pain is a combination of histone modifications and DNA methylation at various levels of the pain pathway. For example, studies investigating models of stress or pharmacologically induced visceral hypersensitivity have found changes in acetylated histone-promoter interactions in the brain and spinal cord and concomitant dysregulation of the related pro and antinociceptive genes in these tissues (Tran, Chaloner et al. 2013, Tran, Schulkin et al. 2014, Hong, Zheng et al. 2015). Similarly, stress-induced visceral pain has also been linked to alterations in DNA methylation patterns within the brain, leading to increased expression of pronociceptive neurotransmitters (Tran, Chaloner et al. 2013).

There have also been some recent insights into the role that miRNAs play in experimental visceral pain models of chronic cystitis and esophageal reflux disease, with several miRNAs identified in the development of chronic visceral pain (Sengupta, Pochiraju et al. 2013, Zhang and Banerjee 2015). However, evidence directly linking these miRNAs to visceral pain behaviors is currently lacking. In another study, Zhou et al. investigated the effects of miR-29 on increased intestinal permeability, which has previously been associated with visceral hypersensitivity (Zhou, Zhang et al. 2009, Zhou and Verne 2011, Camilleri, Madsen et al. 2012). After experimentally inducing colitis or exposing wildtype mice to a stressor they observed an up-regulation of miR-29a and miR-29b along with increased intestinal permeability in these animals. In *Mir29*$^{-/-}$ mice,

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however, this colitis or stress induced intestinal permeability was greatly diminished. Finally, in microarray and permeability experiments, they showed that miR-29a/b target Claudin-1 and nuclear factor-kB-repressing factor mRNA for degradation, thus increasing intestinal permeability (Zhou, Costinean et al. 2015).

Histone deacetylase inhibitors are the most common epigenetic drugs to be investigated as potential therapeutics for chronic visceral pain. The studies examining HDAC inhibitors report that these drugs significantly improve outcomes in these models. Intrathecal administration of SAHA to rats with 17β-estradiol (E2)-induced visceral hypersensitivity stimulated hyperacetylation of H3 and increased binding of H3K9ac to the promoter region of the metabotropic glutamate receptor 2 (mGluR2) gene *Grm2*. This increased association between H3K9ac and *Grm2*, in conjunction with binding of activated estrogen receptor alpha (ERα), leads to the up-regulation of mGluR2 in the spinal cord and the attenuation of visceral hypersensitivity (Cao, Bai et al. 2014). Tran et al. showed that visceral hypersensitivity in rats resulting from chronic water avoidance stress could be prevented by daily intracerebroventricular infusions of TSA during the course of the stress paradigm (Tran, Chaloner et al. 2013). In a second study, this group utilized a pharmacologically induced model of visceral hypersensitivity to examine histone deacetylation in the brain. This study showed prolonged exposure of the central nucleus of the amygdala (CeA) to corticosterone to produce visceral hypersensitivity is associated with deacetylation of H3K9 and its decreased association with the 1_7 region of the glucocorticoid receptor (GR)

promoter within the CeA, leading to a down-regulation of GR and, in turn, up-regulation of the pronociceptive corticotrophin releasing factor (CRF). Bilateral infusions of TSA and SAHA into the CeA reversed these changes to the epigenome and attenuated visceral hypersensitivity (Tran, Schulkin et al. 2014). Another study examining the role of epigenetic modulation of GR in visceral pain showed water avoidance stress increases methylation of the GR promoter and reduces its expression in a regional specific manner in DRG neurons. The authors also found stress up-regulated DNMT1-associated methylation of the cannabinoid receptor promoter, increased expression of the histone acetyltransferase EP300, increased histone acetylation at the TRPV1 promoter and increased expression of the TRPV1 receptor in DRG neurons. They also showed that knockdown of both DNMT1 and EP300 in L6-S2 DRG neurons reduced both DNA methylation and histone acetylation, respectively, which prevented stress-induced visceral pain (Hong, Zheng et al. 2015). Finally, chronic visceral hypersensitivity induced by early-life stress has been shown to induce histone deacetylation in the lumbosacral spinal cord, with maternally separated rats showing a decrease in H4K12 acetylation that correlated with visceral hypersensitivity in adulthood. Moreover, peripheral treatment with SAHA attenuated the stress-induced effects (Moloney, Stilling et al. 2015).

Neuropathic Pain

Many studies have investigated epigenetic modulation in models of neuropathic pain. To date, most have focused on histone modifications and

report changes at all levels of the pain pathway. Typically nerve injury induces alterations in acetylation status of H3 and H4 leading to the dysregulation of a myriad of genes responsible for proper neuronal function and pain perception. For example, the up-regulation of pro-nociceptive genes has been linked to histone hyperacetylation in the spinal cord following nerve injury (Zhu, Li et al. 2014). Other studies have shown injury causes neuroinflammation-induced neuropathic pain through marked histone modifications and increased expression of multiple pro-inflammatory genes in leukocytes infiltrating the injured tissue (Kiguchi, Kobayashi et al. 2012, Kiguchi, Kobayashi et al. 2013). Injury-induced neuropathic pain has also been linked to histone hypoacetylation in the DRG, which leads to decreased expression of genes essential to C-fiber functionality (Matsushita, Araki et al. 2013). In addition to changing histone acetylation patterns, nerve injury also alters histone methylation. Specifically, Laumet and colleagues showed that spinal nerve ligation (SNL) decreases the expression of several potassium channels and their related genes through increased expression of the histone methyltransferase G9a, resulting in neuropathic pain symptoms (Laumet, Garriga et al. 2015).

As with the previously outlined studies, HDAC inhibitors and HAT inhibitors are the most commonly utilized epigenetic drugs in neuropathic pain studies. Although the mechanisms underlying the various models of neuropathic pain are varied and complex, the administration of HDAC inhibitors has generally produced positive outcomes in regard to pain behaviors. It has been demonstrated that partial sciatic nerve ligation (PSNL)-induced hypoesthesia

could be prevented by administration of the HDAC inhibitors valproate (VPA) and TSA. The investigators report that PSNL down-regulates ion channels Nav1.8 and Kv4.3, which are extensively involved in pain perception, as well as *TRPV1*, *TRPM8,* and *CGRP* gene expression in DRGs. Pretreatment with HDAC inhibitors maintained H3 and H4 acetylation at these genes, thereby promoting their expression and normal C-fiber functionality (Matsushita, Araki et al. 2013). Another study showed that intrathecal administration of HDACi MS-275 prevented SNL-induced mechanical and thermal hyperalgesia by increasing acetylation of H3K9 and altering HDAC1 expression in the dorsal horn of the spinal cord (Denk, Huang et al. 2013). Finally, VPA attenuated SNL-induced mechanical hyperalgesia via the restoration of glutamate transporter GLT-1 in the spinal cord, though the authors did not specifically investigate epigenetic changes in this study (Yoshizumi, Eisenach et al. 2013). Histone acetyltransferase inhibitors have also proven to be particularly effective at improving outcomes in models of neuropathic pain. Following PSNL, the HATi anacardic acid reportedly decreased acetylation of H3K9 at the promoters of macrophage inflammatory protein 2 (MIP-2) and CXC chemokine receptor type 2 in macrophages and neutrophils infiltrating the injured tissue, thereby attenuating PSNL-induced thermal hyperalgesia (Kiguchi, Kobayashi et al. 2012). The same authors also demonstrated that CC-chemokine ligand 2 (CCL2) and CCL3, two other important contributors to peripheral sensitization following injury, could be down-regulated by anacardic acid through a similar epigenetic mechanism involving H3K9 and H3K4me3 (Kiguchi, Kobayashi et al. 2013).

In a separate study, the chronic constriction injury (CCI) rat model revealed that p300 (transcriptional co-activator and histone acetyltransferase E1A binding protein) expression was increased in the lumbar spinal cord on day 14 after CCI. The treatment with intrathecal p300 shRNA or C646, an inhibitor of p300 acetyltransferase reversed CCI-induced mechanical allodynia and thermal hyperalgesia (Zhu, Huang et al. 2012). This work was then expanded upon in a following study wherein curcumin, which has known HAT inhibitory properties, was shown to effectively attenuate neuropathic pain by silencing pronociceptive genes *Bdnf* and cyclooxygenase-2 (*COX-2*) by reducing the binding of p300/CBP, H3K9ac and H4K5ac to their promoters (Zhu, Li et al. 2014). Currently only one study has investigated the effects of targeting DNA methylation in the context of neuropathic pain models. This study primarily focused on the use of 5-aza to antagonize DNMTs following injury, and showed 5-aza attenuated mechanical and thermal hyperalgesia by reversing CCI-induced expression of methyl CpG binding protein 2 (MeCP2) and global DNA methylation (Wang, Liu et al. 2011).

Several studies have examined the effects of targeting specific ncRNAs in the treatment of neuropathic pain, though only a few have directly linked the expression of these RNAs to pain behavior. Sakai and colleagues observed a decrease in miR-7a in the DRGs of SNL and CCI rats (Sakai and Suzuki 2014). Using an adeno-associated virus vector to restore miR-7a in DRGs, they were able to attenuate mechanical and thermal hypersensitivity in both models and identified the *β*2 subunit of the voltage-gated sodium ion channel as a potential

target for miR-7a (Sakai and Suzuki in 2014). Another investigation showed miR-103 expression was decreased in the dorsal horn of SNL rats while the expression of Cav1.2-comprising L-type calcium channel (Cav1.2-LTC) was increased. The investigators then found that miR-103 regulates the expression of 3 subunits of Cav1.2-LTC and that knockdown of miR-103 using siRNA in naive rats results in mechanical hypersensitivity. Moreover, intrathecal administration of miR-103 significantly reduced SNL-induced hypersensitivity (Favereaux, Thoumine et al. 2011). Long noncoding RNAs have also been implicated as contributors to neuropathic pain. Specifically, the lncRNA identified as antisense RNA for voltage-dependent potassium channel *Kcna2* mRNA was increased in the DRGs of SNL rats. Mimicking this increase in Kcna2 antisense in naïve animals produced neuropathic pain symptoms, while blocking the increase attenuated the development of nerve injury-induced neuropathic pain (Zhao, Tang et al. 2013).

Evidence for Epigenetic Changes in Clinical Studies of Pain

The data from clinical studies to date is sparse, with few publications specifically demonstrating changes in the epigenetic signature of human subjects. Interestingly, epigenetic processes themselves, specifically DNA methylation, have been shown to occur after chronic opioid use in pain patients. This study showed increased DNA methylation in a cohort of opioid-treated versus non-opioid-treated pain patients (Doehring, Oertel et al. 2013).

In whole blood from 50 healthy identical twins with discordant thermal pain sensitivities, changes in DNA methylation were apparent when compared to 50

unrelated individuals *(Bell, Loomis et al. 2014).* The authors identified 9 differentially methylated regions in the twin cohort correlated with pain sensitivity. These included targets previously implicated in pain processing (TRP channels, GRIN1, DCLK1) but also new targets (ANK3). To advance these exciting findings, future studies could apply similar approaches in patients with various forms of chronic pain to further catalogue epigenetic marks. These studies would begin to investigate whether epigenetic changes can explain chronic pain in specific patient populations.

A limited body of evidence exists to support altered epigenetic processes in chronic visceral pain disorders. Gastrointestinal visceral pain such as that associated with IBS has also been associated with altered epigenetic processes linked to pro-nociceptive gene expression. Colonic biopsies from this patient population revealed reduced miR-199a/b expression in IBS-diarrhea patients compared with controls. Moreover, this correlated directly with both increased visceral pain scores and TRPV1 expression (Zhou, Yang et al. 2015). Other miRNAs have also been implicated in IBS patients including hsa-miR-150 and hsa-miR-342-3p, which were both found to be significantly increased in the whole blood of IBS patients versus healthy controls (Fourie, Peace et al. 2014). Both of these miRNAs are implicated in pain processes and inflammation. In another condition characterized by severe visceral pain, Wu *et al.,* proposed that endometriosis is a epigenetic disease, showing that the promoter region of progesterone receptor B (PR-B), but not PR-A, is hypermethylated (Wu, Strawn et al. 2006). Moreover, they have previously shown aberrant methylation of

HOXA10 in the eutopic endometrium of women with endometriosis (Wu, Halverson et al. 2005). Although these findings do not directly involve pain circuitry, they provide further evidence for altered epigenetic processes in chronic pain disorders.

In patients with lower back pain caused by disc degeneration, reduced SPARC (secreted protein, acidic, rich in cysteine) expression was apparent in the intervertebral discs and DNA methylation was altered (Tajerian, Alvarado et al. 2011). The authors show that within the promoter region of the SPARC gene there are significant increases in DNA methylation, which may underpin decreases in the SPARC gene expression. Following on from this *ex vivo* work, the authors perform *in vitro* experiments aimed to further tease apart the exact mechanism, showing that methylation of the of both the human and murine promoter silence SPARC promoter activity (Tajerian, Alvarado et al. 2011).

Current Approaches to Treat Chronic Pain

As alluded to earlier, the etiology of chronic pain is complex and thus treatment has many obstacles due to its persistent nature. Current therapeutic approaches have modest efficacy and poor side effect profiles, and often fall short in directly treating the underlying pathologic nociceptive signaling. The most common treatment for both acute pain and chronic pain are the non-steroidal anti-inflammatory drugs (NSAIDs). These compounds act through inhibition of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) activity, thus preventing the synthesis of prostaglandins and thromboxanes (Buvanendran and

Lipman 2010, Buvanendran 2013). Some of the most commonly used NSAIDs include aspirin, ibuprofen, ketoprofen, and naproxen. The most typical side effects of NSAIDs include heartburn and nausea. However in the context of chronic pain, frequent or long-term use of NSAIDs may lead to stomach ulcers or high blood pressure. Acetaminophen is a another widely used pain medication with a mode of action that involves inhibition of COX-1 and COX-2 (Graham, Davies et al. 2013). However, acetaminophen is not classified as an NSAID because it exhibits only modest anti-inflammatory activity. However, when administered in combination with opioid analgesics, acetaminophen is used in the management of more severe pain. Although well tolerated, acetaminophen can alter liver function when combined with alcohol, and acute overdoses of acetaminophen can cause potentially fatal liver damage (Jaeschke, Xie et al. 2014).

For moderate to severe chronic pain opioidergic compounds (de Leon-Casasola 2013), including morphine and oxycodone, are typically prescribed. Working through the mu-delta heterodimer, these compounds are generally effective (Pasternak and Pan 2011). However, common side effects including constipation, dizziness, lightheadedness, drowsiness, nausea and vomiting severely limit their usefulness. More importantly, the long-term daily use of opioid analgesics leads to physical dependence and even hyperalgesia (Bruehl, Apkarian et al. 2013).

Alternative approaches for chronic pain management include anti-depressants (serotonin-norepinephrine reuptake inhibitors), calcium channel

blockers, α_2-adrenergic receptor agonists and GABA receptor agonists (Eisenberg and Peterson 2010, Jackson and Argoff 2010, Murinson 2013, Nicholson 2013). A spectrum of side effects is associated with these compounds; the most common include sedation, dizziness, dry mouth, edema, withdrawal symptoms if discontinued and severe hypotension. Taken together, the current treatment options for chronic pain are unsatisfactory with low efficacy and are associated with a host of adverse side effects, thus the necessity for the development of novel therapeutics remains an urgent medical need.

Epigenetic Modifiers as Therapeutic Targets for Chronic Pain

Epigenetics offers the opportunity to remodel an aberrant signaling cascade, which may underpin the persistent experience of pain. Over the last 2 decades there has been rapid advancement of pain genetics and these studies have provided a solid foundation for the emergence of pain epigenetics. However, there are still many obstacles to overcome to develop this concept into an effective treatment approach. Here we will summarize the potential of epigenetic therapies but also the current limitations. The concept of using epigenetic therapies for the treatment of chronic pain remains unproven but evidence to date suggests that this approach may offer more benefit to patients over the current analgesic regiments. Identifying a specific epigenetic mark or landscape that is unique to the disease state is critical for the development of an effective therapy. The idea that an epigenetic therapeutic which could target a specific pathway to rectify the underlying cause of chronic pain as opposed to

simply treating the symptom, is very attractive. By identifying a discrete epigenetic signature, which is responsible for chronic pain in a patient population and designing compounds to directly target this signature, will undoubtedly be a huge advancement in the field. Epigenetic drugs offer the opportunity to rectify the underlying pathology of chronic pain and not just attenuate the symptom.

Currently the application and availability of epigenetic-based therapies used clinically for a variety of disorders and diseases is limited. Both HDAC inhibitors and DNMT inhibitors are being investigated as anti-cancer agents, however, therapies for the treatment of persistent pain non-life threatening states have yet to be developed. Therapies that manipulate single epigenetic marks that subsequently target a specific gene should be considered for development. Over the last decades, investigators focused on single gene expression differences or mutations that are involved in nociception. Future approaches should focus on the development of locally acting therapeutics targeting the epigenetic mechanism (s) that have been identified to contribute to chronic pain. For example identifying specific genes within the nociceptive circuitry will allow us to use tools such as siRNA to target in a discrete manner the aberrant gene. Moreover, the epigenome itself could be targeted with designer DNA-binding proteins developed from transcriptional activator-like effectors (TALENS) or zinc finger proteins (Day 2014). The use of micro-RNA based therapies is also of interest. Indeed a limited number of studies have identified specific micro-RNAs that are altered in chronic pain and are amenable to manipulation via mimics to

induce analgesic effects (Sun, Li et al. 2012, Willemen, Huo et al. 2012, Pan, Zhu et al. 2014, Lu, Cao et al. 2015).

The current epigenetic modifying compounds are nonspecific and non-selective, acting both centrally and peripherally as well as at many epigenetic sites. The changes in the epigenome during the development of persistent pain are also extraordinarily complex and dynamic. Moreover, the resulting epigenetic mark largely depends on the injury or insult sustained as well as the past experiences of each individual. With this in mind, the current epigenetic drugs in development and in use for the treatment of cancer (Copeland, Solomon et al. 2009, New, Olzscha et al. 2012) that may potentially be investigated in the context of pain are highly non-specific HDACis and DNMTis (summarized in Table 1) with numerous side effects, and seem unsuitable for long-term treatment of chronic pain.

Summary

Chronic pain represents a large unmet medical need and it is clear that alternative therapies are required with novel mechanisms of action. Having reviewed the primary mechanisms of epigenetic regulation and the recent findings in the field of epigenetics and chronic pain, it is apparent that new therapies targeting epigenetic marks represents a legitimate approach. However, this strategy for treating chronic pain is currently limited by the paucity of selective compounds with a side effect profile that is suitable to support chronic use by patients often with non-life threatening but severely debilitating chronic

pain. Thus continuing to build upon our knowledge of genetic and epigenetic mechanisms in pain pathways will undoubtedly aid in our understanding and development of novel therapeutics to treat chronic pain disorders.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Ligon, Moloney, Greenwood-Van Meerveld. Ligon and Moloney contributed equally to the manuscript.

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Footnote

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Figure Legends

Figure 1. Summary of epigenetic changes during various chronic pain conditions and the effects of pharmacological treatment. Abbreviations: CCI, chronic constriction injury; CeA, central nucleus of the amygdala; CFA, complete Freund's adjuvant; CORT, corticosterone; DRG, dorsal root ganglia; E2, estradiol; Hypoesth, hyoesthesia; Mech, mechanical hypersensitivity; MS, maternal separation; NRM, nucleus raphe magnus; PSNL, partial sciatic nerve ligation; SC, spinal cord; SCN, sciatic nerve; SNL, spinal nerve ligation; Ther, thermal hypersensitivity; WAS, water avoidance stress; VMR, visceromotor response; ↓, down-regulate or silence; ↑, up-regulate or induce. Modified public domain images (commons.wikimedia.org) of the sagittal brain and neuron are from users Nickbyrd, and Looxix respectively.

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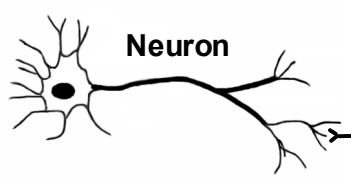
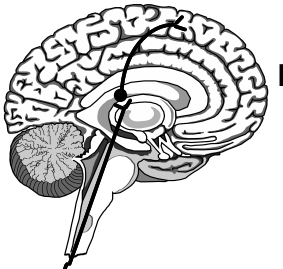
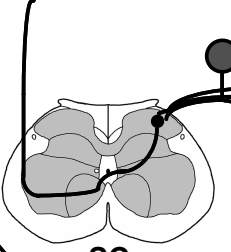
Tables

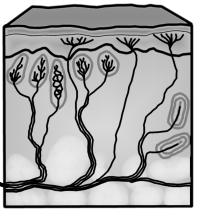
Table 1: Summary of HDAC and DNMT inhibitors

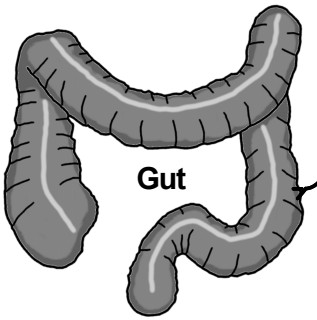
Epigenetic Modifier	Classification	Compound	Target
HDAC inhibitors	*Hydroxamates*	SAHA (vorinostat)	Pan inhibitor
		PXD101 (belinostat)	Pan inhibitor
		LBH589 (panobinostat)	Classes I and II
		ITF2357 (givinostat)	Pan inhibitor
		4SC-201 (resminostat)	Pan inhibitor
		PCI 24781 (abexinostat)	Classes I and II
	Cyclic peptides:	Depsipeptide/FK228	Class I
	Benzamides:	MS-275 (entinostat)	Class I
		MGCD0103 (mocetinostat)	Class I
		M344	Class I
	Aliphatic fatty acids:	Valproate	Classes I and IIa
		Butyrate	Classes I and IIa
	Unknown mechanism:	CI-994	HDAC1, HDAC2
		BML-210	HDAC 1-5 & 7
		NVP-LAQ824	Unknown
DNMT inhibitors		5-azacitidine	Pan inhibitor
		5-aza-2′-deoxycytidine	Pan inhibitor
		1-β-D-arabinofuranosyl-5-azacytosine	Pan inhibitor
		dihydro-5-azacytidine	Pan inhibitor
		MG98	DNMT-1











Neuropathic Pain

Model	Mark/Enzyme	Location	Expression	Behavior	Pharmacology	Reference
SNL	↓miR-103	SC	↑Cav1.2-LTC	Mech	AAV-miR-103 = ↓Mech, Cav1.2-LTC	*Favereaux et al., 2013*
SNL		SC		Ther Mech	it MS-275 = ↓Mech, ↑H3K9ac, HDAC1	*Denk et al., 2013*
SNL		SC	↓*GLT-1*, *GLAST*	Mech	po VPA = ↓Mech, ↑*GLT-1, GLAST*	*Yoshizumi et al., 2013*
SNL/ CCI	↓miR-7a	DRG	↑β2 subunit of Nav	Ther Mech	AAV-miR-7a = ↓Ther, Mech, β2 subunit	*Sakai et al., 2013*
CCI	↑H3K9ac, H4K5ac, p300/CBP at *Bdnf* and *COX2*	SC	↑*Bdnf, COX2*	Ther Mech	ip curcumin = ↓Ther, Mech, *Bdnf, COX2, H3K9ac, H4K5ac, p300/CBP at Bdnf and COX2*	*Zhu et al., 2014*
CCI	↑DNA methylation	SC	↑*MeCP2*	Ther Mech	it 5-aza = ↓Ther, Mech, DNA methylation, *MeCP2*	*Wang et al., 2011*
PSNL	↑H3K9ac at *CXCL2 and CXCR2*	SCN	↑*CXCL2, CXCR2*	Ther	ip AA = ↓Ther, H3K9ac at *CXCL2 and CXCR2*	*Kiguchi et al., 2012*
PSNL	↑H3K9ac and H3K4me3 at *CCL2 and CCL3*	SCN	↑*CCL2, CCL3*	Ther	ip AA = ↓Ther CCL2, CCL3	*Kiguchi et al., 2013*
PSNL	↓H3/H4ac at *Nav1.8*	DRG	↑*REST*, ↓*Nav1.8, TRPV1, TRPM8, CGRP*	Hypoesth	ipl SAHA =↓Hypoesth, ↑H3/H4ac at *Nav1.8*, *Nav1.8, TRPV1, TRPM8*	*Matsushita et al., 2013*

Somatic Pain

Model	Mark/Enzyme	Location	Expression	Behavior	Pharmacology	Reference
CFA	↓H3K9ac at *Gad2*	NRM	↓*GAD65*	Ther	Inf. TSA/SAHA to NRM = ↓Ther, ↑H3K9ac at *Gad2, GAD65*	*Zhang et al., 2011*
CFA	↓H3K9/K18ac, ↑class II HDACs	SC		Ther	it class II HDACis =↓Ther, ↑H3K9/K18ac	*Bai et al., 2010*
CFA	↑DNA methylation	SC	↓miR-219, ↑*CamKIIγ*	Mech	it 5-aza = ↓Mech, CamKIIγ, ↑miR-219	*Pan et al., 2014*

Visceral Pain

Model	Mark/Enzyme	Location	Expression	Behavior	Pharmacology	Reference
WAS				VMR	icv TSA = ↓VMR	*Tran et al., 2013*
CORT	↓H3K9ac at *GR*	CeA	↓*GR*, ↑*CRF*	VMR Mech	Inf. TSA/SAHA to CeA =↓VMR, Mech, *CRF* ↑H3K9ac at *GR, GR*	*Tran et al., 2014*
E2	↓H3ac	SC	↓*Grm2*	VMR	it SAHA = ↓VMR, mGluR2 ↑H3K9ac to *Grm2*	*Cao et al., 2014*
MS	↓H4K12ac	SC		VMR	ip SAHA = ↓VMR, ↑H4K12ac	*Moloney et al., 2015*

Figure 1