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Epigenetics and the Transition from Acute to Chronic Pain

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Abstract

Objective. The objective of this study was to review the epigenetic modifications involved in the transition from acute to chronic pain and to identify potential targets for the development of novel, individualized pain therapeutics.

Background. Epigenetics is the study of heritable modifications in gene expression and phenotype that do not require a change in genetic sequence to manifest their effects. Environmental toxins, medications, diet, and psychological stresses can alter epigenetic processes such as DNA methylation, histone acetylation, and RNA interference. As epigenetic modifications potentially play an important role in inflammatory cytokine metabolism, steroid responsiveness, and opioid sensitivity, they are likely key factors in the development of chronic pain. Although our knowledge of the human genetic code and disease-associated polymorphisms has grown significantly in the past decade, we have not yet been able to elucidate the mechanisms that lead to the development of persistent pain after nerve injury or surgery.

Design. This is a focused literature review of epigenetic science and its relationship to chronic pain.

Results. Significant laboratory and clinical data support the notion that epigenetic modifications are affected by the environment and lead to differential gene expression. Similar to mechanisms involved in the development of cancer, neurodegenerative disease, and inflammatory disorders, the literature endorses an important potential role for epigenetics in chronic pain.

Conclusions. Epigenetic analysis may identify mechanisms critical to the development of chronic pain after injury, and may provide new pathways and target mechanisms for future drug development and individualized medicine.

Key Words. Epigenetics; Pain; DNA Methylation; Histone Deacetylase Inhibitors; RNA Interference

Introduction

In recent years, we have developed a better understanding of the cellular mechanisms that link inflammation, peripheral sensitization, and pain [1]. In addition, we have learned more about the human genetic code [2] and mutations (particularly single nucleotide polymorphisms [SNPs] and copy number variations) that are associated with specific chronic pain syndromes [3,4]. These physiologic and genetic advances, however, do not fully explain why one patient develops chronic pain following an injury, and another patient does not. Despite recent improvements in techniques for acute pain management, 30–50% of patients still develop chronic pain following surgeries such as amputation, thoracotomy, hernia repair, and mastectomy [5].

It is also notable that monozygotic twins may exhibit significantly different inflammatory and chronic pain phenotypes [6–8], indicating that the etiological basis of these

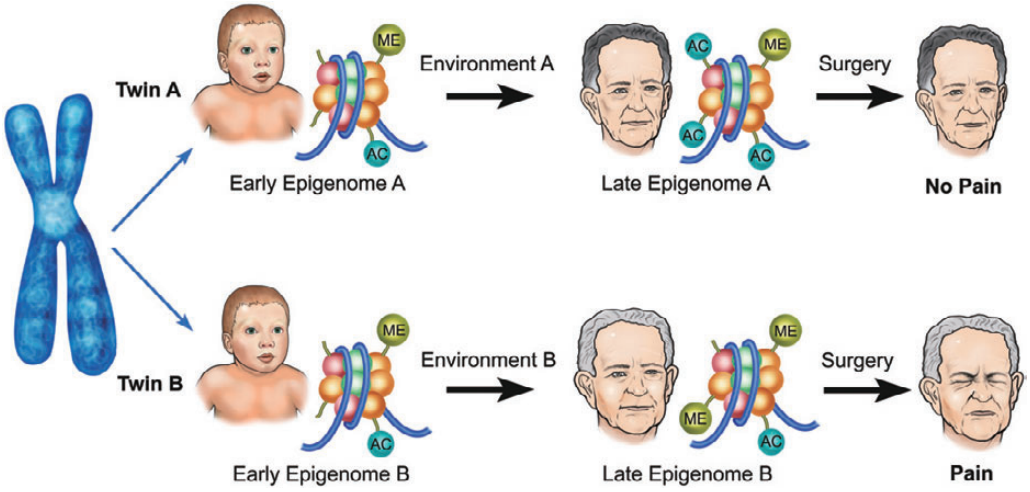

Figure 1 Epigenome and chronic pain. Twin A and Twin B demonstrate similar "epigenomes" at birth with few (if any) differences in methylation and acetylation patterns. Environmental factors throughout development affect histone acetylation patterns and cytosine methylation patterns, resulting in phenotypic differences by adulthood. With surgery or nerve injury, these epigenetic differences may result in differing risks of chronic pain.

disorders is not due simply to differences in genetic sequence. We now appreciate that response to injury is determined by complex interactions between the genome and the environment. These alterations might well be epigenetic in nature, i.e., heritable modifications that are not intrinsic to the genetic code, but that affect gene expression in a tissue-specific manner, resulting in an observable phenotype (Figure 1) [9].

Epigenetic processes are responsible for cellular differentiation during embryogenesis and are critical for normal development [10]. These processes also play an important role in memory formation, as correlations between hippocampal activity, DNA methylation, and histone phosphorylation in the brain have been found [11,12]. The spinal cord sensitization seen in painful conditions shares common mechanisms with the neural plasticity of memory formation [13], and it is likely that similar epigenetic mechanisms regulate both of these neural processes.

Multiple examples of the importance of epigenetic influences in development are found throughout nature. One of the best-described cases of environmental influence on gene expression involves the control of bee development by ingesting royal jelly. This nutritive substance induces changes in juvenile bee DNA methylation patterns and leads to development of the bee's phenotype to become a queen rather than a worker [14]. The concepts of epigenetic heritability and stability have also been described in plants [15] and mammals [16]. For instance, high-fat diets fed to paternal rats induce functional changes in β-islet cells of female offspring [16]. Similar modifications in DNA methylation were noted in the fathers and offspring, suggesting the nongenetic heritability of this metabolic disorder.

Nondevelopmental epigenetic modifications are also triggered by environment, nutrition, and stress [17–19], and may play a role in the onset of chronic pain following nerve injury [20,21]. We have long appreciated the importance of the psychosocial environment to the incidence and severity of chronic pain [22–27], and mounting evidence suggests that epigenetic mechanisms supply the link between disease expression and environment [18,28]. Nongenetic factors are important in the development of cancer [29,30], neurologic disorders [31], and painful disorders such as bladder pain syndromes [7], myofascial pain [32], and temporomandibular joint pain [8]. Twin disease models of neurodegenerative conditions [33], inflammatory periodontal disease [34], and autoimmune disease [35] demonstrate variable disease expression depending on the DNA methylation pattern [6].

Environmental factors alter gene expression and phenotype for painful disorders by inducing epigenetic modifications such as histone acetylation, DNA methylation, and RNA interference (RNAi) [36–38]. Following injury, expression of transcription factors such as nuclear factor-kappa B (NF-κB) is increased [39], sodium channels in the injured axon are upregulated [40], μ-opioid receptors in the dorsal root ganglion are downregulated [41,42], substance P expression is altered [43], and the dorsal horn of the spinal cord is structurally reorganized through axonal sprouting [44]. As with DNA variation, epigenetic modifications may be inherited and may be propagated over multiple cell divisions; however, they are flexible enough to respond to

modifying influences. This concept may in part explain how we interact with our environment at the (epi)genomic level, and is potentially of great importance in understanding the relationship between gene expression and complex diseases such as chronic pain.

Genetics, Epigenetics, and Pain

Over the past several decades, much has been written about the association of genetic polymorphisms and the development of chronic pain [45,46]. It was believed that, through knowledge of genetic variation, we could develop the foundation for individualized medicine that optimizes therapy for each patient based on one's specific genetic sequence [47]. Expectations for personalized medicine were high after completion of the human genome project [2], but thus far, our ability to use the genetic code to prevent or improve chronic pain has been somewhat limited [48]. It is the heretofore unquantifiable environmental effect that has been one of the limitations of genetic studies [45].

Multiple candidate gene association studies have been used for the investigation of pain, but have been limited by their focus on genomic regions where the pathophysiology is thought to be reasonably well understood. They are not designed to analyze painful conditions that result from interactions of multiple genes [49]. A few candidate gene polymorphisms have been linked to pain susceptibility, including catechol-O-methyltranferase (COMT). This gene modulates nociceptive and inflammatory pain and has been linked to temporomandibular joint pain syndromes [50]. Even studies of COMT, however, have demonstrated inconsistencies. Some investigators have found an association between a COMT SNP val158met [4,50] with increasing pain responses, while others failed to replicate these findings [51,52].

The SCN9A gene has also been studied as a marker for pain sensitivity. Mutations in this gene, which codes for the alpha-subunit of a voltage-gated sodium channel ($Na_v1.7$), are known to result in alterations of pain perception [53], and have been noted in rare pain disorders such as erythromelalgia and paroxysmal extreme pain disorder [54,55]. SCN9A polymorphisms have also been described in individuals who are insensitive to pain [3,56]. Although the implications of the SCN9A gene polymorphism are clear, clinical applications of this knowledge remain limited [47].

Genome-wide association studies (GWAS) have been used in an attempt to overcome some of the limitations of candidate gene analysis. These studies tell us where the genetic variation exists, but do not always fully explain the underlying biology. Furthermore, although GWAS have identified thousands of genetic variations in complex diseases, most of the variants confer only a modest risk with an odds ratio for disease of <1.5. These genetic variants, therefore, account for only a small fraction of the population attributable risk for heritable complex traits [57,58], implying a strong nongenetic predisposition to disease. GWAS directed toward painful conditions remain limited in number [45].

Specific Epigenetic Modifications

Histone Modifications

Histones octamers and their surrounding DNA form a nucleosome, the fundamental building block of chromatin (Figure 2A). The N-terminal histone tails may be modified by more than 100 different posttranslational processes including acetylation, phosphorylation, and methylation (Figure 2B). Most of the histone complex is inaccessible, but the N-terminal tail protrudes from the nucleosome and is therefore subject to additions that change the three-dimensional chromatin structure and subsequent gene expression [59,60]. One of the more common modifications involves acetylation. Histone acetyl transferases add acetyl groups, altering the histone protein structure. This change prevents the chromatin from becoming more compact, allowing transcription factors to bind more easily. This state of increased acetylation and "permissive chromatin" generally increases transcription activity and RNA production from that genetic sequence, especially when located in gene promoter regions [61,62]. Conversely, histone deacetylases (HDACs) remove acetyl groups from histones, generally suppressing gene expression. In concert, these activities serve important regulatory functions.

DNA Methylation

Another ubiquitous epigenetic modification involves methylation of DNA cytosine nucleotides. In this process, DNA methyltransferase enzymes (DNMT1, DNMT3A, and DNMT3B) add a methyl group to the 5-carbon of the cytosine pyrimidine ring, converting it to 5-methylcytosine. This methylation generally silences gene expression either by preventing the binding of transcription factors [63,64], or by attracting methylated DNA-binding proteins such as MeCP2 that themselves repress transcription (Figure 2C) [65,66]. The methylation process is vital for normal embryonic development and growth [67], and these methylation patterns are propagated during cell division.

The degree of cytosine methylation tends to mirror the degree of tissue specialization. For instance, DNA in neurologic tissue is highly methylated, while sperm DNA is relatively unmethylated [68]. More recent research has focused on the regulatory importance of cytosine methylation in promoter regions where methylation may silence a previously active gene sequence in the process of tissue specialization [69]. In addition to the cytosine nucleotides dispersed throughout the genome, there are regions particularly rich in cytosine-phosphate-guanine (CpG) linear sequences, described as "CpG islands" [70]. These "CpG islands" are found in promoter regions or first exons of approximately 60% of human genes, and are often unmethylated during development, allowing a transcriptionally active state [71]. Although promoter site methylation may

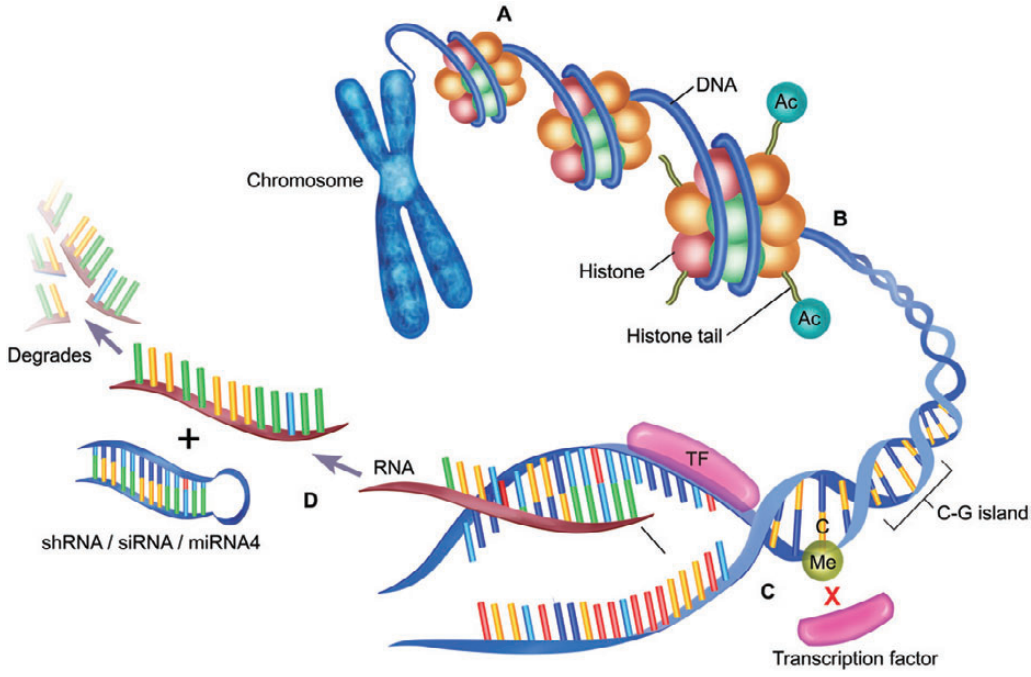

Figure 2 Epigenetic mechanisms. (A) DNA wraps around histone octamers to form a nucleosome, the fundamental building block of chromatin. (B) Histone proteins may be modified through several processes, including acetylation. The addition of an acetyl group to histone tails generally opens the chromatin structure and facilitates transcription factor binding, enhancing gene expression. (C) Methylation of cytosine nucleotides in C-G rich sequences ("CG islands") prevents the binding of transcription factors and generally silences gene expression. These CG islands are often found near promoter regions and serve a significant role in gene regulation. (D) Posttranscriptional regulatory mechanisms include short hairpin RNA (shRNA), small interfering RNA (siRNA), and micro RNA (miRNA) that bind RNA and induce their degradation.

silence gene expression during development, genes may still be reactivated even in specialized neurologic tissues [72,73]. This potentially modifiable plasticity of neural tissue methylation may hold promise for reversing the neurologic molecular remodeling that occurs during the transition from acute to chronic pain.

Several disease states, including cancer, schizophrenia, and opioid addiction, are associated with DNA methylation abnormalities [30,74–76]. In cancer, these altered methylation patterns may lead to tumor growth by downregulating tumor suppressor genes [30]. Methylated gene domains demonstrate not only stability, but also heritability [70]. The epigenetic influence across generations is demonstrated in rodent studies in which spermatogenesis is suppressed, and methylation patterns are altered for several generations after using the antiandrogenic compound vinclozolin during embryonic development [77].

Noncoding RNA

Gene expression can also be controlled by RNAi that involves endogenous molecules such as small interfering RNA (siRNA), microRNA (miRNA), and short hairpin RNA (shRNA). These small noncoding RNA molecules can silence gene expression by binding to mRNA and inducing subsequent degradation of the direct gene product (Figure 2D) [78]. These molecules can self-propagate through cell division and epigenetically transmit regulatory information across generations [79]. Interfering RNAs carry great therapeutic promise and have been used in animal trials for chronic neuropathic pain [80] and neurodegenerative disease [81], as well as in human clinical trials for cancer [82].

Our understanding of epigenetic processes has increased dramatically over the past decade. Efforts are currently underway, through such groups as the International Human Epigenome Consortium, to sequence and create maps of cell-specific DNA methylation and histone modifications [83].

Techniques of Epigenetic Analysis

There are many challenges in defining the specific epigenetic changes that lead to a particular disease state. Many earlier epigenomic studies have been limited by either inadequate genome survey or small sample size, and the

relationship in many diseases between phenotypic expression and epigenomic variation remains unclear [84]. It is unlikely that single gene epigenetic modification will explain the complex pain phenotypes seen after injury or surgery. Epigenome-wide association studies have been proposed as a possible solution to improve our understanding of the links between disease state and epigenetic modifications. Comprehensive epigenomic maps are currently being developed with promising future applications [84].

Another challenge with epigenetic studies and disease variation is the need for enhanced comprehension of the distinction between cause and consequence [84]. To fully understand if a particular biomarker represents the cause *of* a disease or the effect *from* a disease, we will need to perform analyses at multiple time points before and after the development of a disease. This initiative has already begun with the establishment of the U.S. National Institutes of Health Roadmap Epigenomics Mapping Consortium [85].

Regardless of the relationship between biomarkers and causation, however, epigenetic modifications throughout the course of a chronic disease can be used as biomarkers. In particular, DNA methylation is well suited as a potential predictive biomarker secondary to its relative chemical stability. Reliable biomarkers are critical if we are to develop personalized epigenetic interventions. Candidate markers would need to be found in an accessible space (blood), but still reflect the neurobiological process occurring at the proximal tissue (spinal cord/brain). Whether the circulating leukocyte epigenome can report on more inaccessible tissues (such as central nervous system [CNS]) is uncertain, but there is growing evidence that methylation patterns tend to be similar between proximal tissue and more easily accessible circulating blood cells. For example, it was recently shown that the pattern of CpG island methylation in the promoter region of the prodynorphin gene in both human brain tissue collected postmortem and matched peripheral blood mononuclear cells is virtually identical [86].

The burgeoning field of epigenetics is using novel technologies to measure these heritable, yet modifiable, patterns of transcriptional regulation. DNA methylation is analyzed through bisulfite sequencing that allows the epigenetic information present in the form of cytosine methylation to be retained during amplification (Figure 3B). Traditional molecular analysis of specific gene loci relies on the ability to amplify the DNA of interest using cloning and polymerase chain reaction (PCR) techniques. If this amplification is done, however, without somehow immortalizing the methylation status of a particular cytosine, that information will be lost after the first PCR cycle. To solve this problem, unmethylated cytosines can be modified through the bisulfite reaction, deaminating them to uracil. Methylated cytosines, however, are not deaminated by bisulfite, remaining unchanged during subsequent amplification. Probes can then be designed to determine whether a specific promoter region has retained a particular cytosine (previously methylated) or whether this cytosine has been converted to uracil (previously unmethylated). The methylation status of the promoter can then be determined using the cytosine/uracil ratio.

Histone protein modifications have also been studied since 1988 through a process of chromatin immunoprecipitation (ChIP) (Figure 3A) [87]. This process involves fragmentation of the chromatin and immunoprecipitation using an antibody to the protein or modification of interest. For example, an antibody to a specific acetylation site on histone H3 is used to precipitate all DNA associated with that particular acetylated histone. Following immunoprecipitation, the DNA fragments are then typically identified through microarray hybridization. More recently, "next generation sequencing" (NGS) technologies have been combined with ChIP, providing a high resolution, genome-wide analysis of histone modification. Whereas microarray techniques analyze regions of the genome previously identified, NGS carries the possibility of capturing all the DNA fragments isolated by immunoprecipitation [71]. These NGS technologies will continue to expand our understanding of epigenetic changes and the chromatin regulatory state throughout the genome.

The Role of Epigenetic Modification in the Transition from Acute to Chronic Pain

Prevention of chronic pain after injury has been the focus of numerous previous trials involving interventions such as multimodal analgesics and catheter-based local anesthetic infusions [88–90]. Although these techniques are successful in reducing the burden of acute pain [91], they have not succeeded in dramatically reducing the incidence of chronic post-injury or post-surgical pain [92–94]. The shortcomings of our preventive strategies are most pronounced following surgeries that have a higher risk for developing chronic pain such as amputation, thoracotomy, hernia repair, coronary artery bypass, and mastectomy [5,95,96].

Our therapeutic limitations may be partially due to our inability to prevent the epigenetic changes that occur following injury and surgery. A patient's gene expression profile changes rapidly in the post-injury period [97], with over 1,000 genes activated in the dorsal root ganglion alone after nerve injury [98]. There is significant evidence for epigenetic control of this gene activation in the transition from acute to chronic pain. First, immunologic response and inflammatory cytokine expression are under epigenetic control [99,100]. Second, glucocorticoid receptor (GR) function, which affects pain sensitivity, inflammation, and the development of autoimmune disease, is modulated both through posttranslational mechanisms and DNA methylation [101–103]. Third, genes such as glutamic acid decarboxylase 65 that code for pain regulatory enzymes in the CNS are known to be hypoacetylated and downregulated in inflammatory and nerve injury pain states [104]. Finally, epigenetic modifications are involved in opioid receptor regulation and function, with implications for endogenous pain modulation systems and pain severity [63,76].

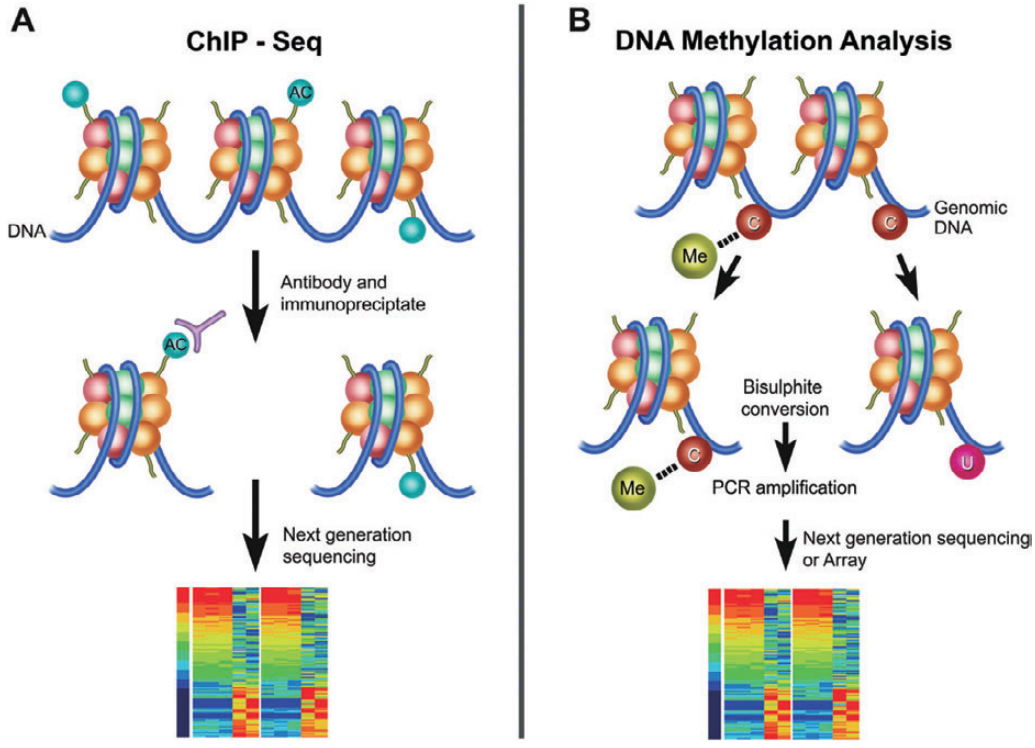

Figure 3 Laboratory techniques in epigenetics. (A) In ChIP-seq analysis, an antibody is used on chromatin to immunoprecipitate and select for acetylation and other histone modifications. The results may then be analyzed through several techniques including genome-wide next generation sequencing. In this manner, the histone acetylation patterns of a particular tissue may be determined. (B) The analysis of DNA methylation employs bisulfite sequencing to convert unmethylated cytosines to uracil. This process does not affect the methylated cytosines. The methylation patterns can be calculated by comparing the ratio of cytosine to uracil.

The important link between epigenetic regulation and pain is also supported by studies involving intervertebral disc degeneration and chronic low back pain. Tajerian et al. found that DNA methylation of an extracellular matrix protein, secreted protein, acidic, rich in cysteine, is linked to accelerated disc degeneration both in humans and in animal models of this disease [38]. The correlation between pain and epigenetics is additionally observed in a study of DNA methylation in human cancer where endothelin receptor type B (EDNRB) is heavily methylated and downregulated in painful squamous cell carcinoma (SCC) lesions [105]. The investigators noted similar findings in their mouse model of SCC, and were able to improve mechanical allodynia when EDNRB transcription was virally augmented [105]. These human and animal studies strongly support a role for gene methylation in regulating the pain experience.

Cytokines

Injury and autoimmune disease are characterized by excessive cytokine production, and anti-cytokine therapies have been successfully used to treat painful conditions such as ankylosing spondylitis [106,107] and neuropathy [108,109]. The link between cytokine expression and pain is supported by the demonstration of T-cell infiltration and inflammatory interleukin (IL) release in animal models of neuropathic pain [110]. Furthermore, interventions that modify the immune response to injury also reduce pain. Such modifications include depletion of mast cells [111], reduction of peripheral macrophages using clodronate [112], and impairment of complement activation and neutrophil chemotaxis [113].

One of the inflammatory master switches, nuclear factor-κB (NF-κB), induces multiple cytokines [114] and cyclooxygenase [115]. NF-κB is epigenetically regulated by acetylation and remodeling of chromatin [114,116,117]. When activated, this transcription factor demethylates and induces cytokines such as Tumor necrosis factor-alpha (TNF-α), IL-1, IL-2, and IL-6 [118,119]. Activation of NF-κB is associated with autoimmune and neurodegenerative disease [120]. Conversely, inhibition of NF-κB reduces pain behavior after peripheral nerve injury [121].

The link between epigenetically induced cytokine production and pain intensity has been noted in multiple disease models such as migraine headache [122], diabetes [114], and osteoarthritis [99]. In osteoarthritis, DNA demethylation at specific CpG sites in human chrondrocytes produces aberrant expression of inflammatory cytokines (IL-1β) and metalloproteinases [99]. Thus, cytokine-induced painful joint damage appears to be epigenetically modulated.

GRs

Glucocorticoids are important endogenous regulators that appear to protect against excessive inflammatory response following injury. Stress-induced glucocorticoid production suppresses immune cell release of IL-6, TNF-α, and other inflammatory cytokines [123]. Exogenous glucocorticoids also have potent anti-inflammatory actions and are used extensively in the treatment of autoimmune disease and painful conditions. However, not all patients respond equally to their clinical effects, and it is believed that glucocorticoid resistance is a likely mechanism in the development of autoimmune disease and chronic pain [124].

The GR is controlled by a system of complex regulatory mechanisms, and clinical response to glucocorticoids correlates with the number of intracellular GRs [125]. Normally, individuals demonstrate variable GR promoter methylation [103] and variable response to glucocorticoid therapy [126]. Diverse methylation patterns are believed to lead to the use of alternative promoter sites and subsequent alteration in GR sensitivity [103].

GR expression is also modified by maternal care, grooming, diet [127,128], and early-life stresses [129,130]. Human studies have demonstrated epigenetic alterations in GRs of patients who previously suffered abuse [131]. The style of maternal care appears to specifically affect methylation patterns of exon 1_7 of the GR promoter, epigenetically linking receptor function and early-life experience [132]. Abnormalities in GR-mediated immune cell function may lead to the development of inflammatory adult phenotypes [133] and autoimmune disorders such as rheumatoid arthritis [101,134]. GR dysfunction may also play a role in fatigue, chronic pain states, and fibromyalgia [102,135]. These maternally influenced expression patterns, however, are not necessarily permanent and have been reversed in cross-fostering parent studies [136]. The GR appears to provide a potential link between injury, environmental stresses, and the severity of chronic pain.

Opioid Receptors

Both demethylating agents and HDAC inhibitors increase expression of the μ-opioid receptor [137], indicating that the endogenous opioid system is under significant epigenetic control. Consistent with these laboratory findings, increased CpG methylation has been noted in the promoter regions of the μ-opioid receptors of heroin users, consistent with receptor downregulation [76], Likewise, DNA methylation of the proenkephalin gene promoter inhibits transcription and gene expression of this opioid peptide [63].

Beyond the direct role of methylation in the regulation of opioid peptide expression, spinal opioid receptor activity also appears to be partially modulated by central GRs [138]. This association is of particular importance given the synergy between the increased central expression of GR following peripheral nerve injury [139] and direct epigenetic manipulation of the endogenous opioid system [63,137]. The interaction between modifications of the GR and the opioid receptor demonstrates the complex role that epigenetic alterations play in controlling the inflammatory and pain-modulating pathways.

"Epigenetic Intervention" to Prevent Chronic Pain

Genetic studies have taught us that variability in pain sensitivity results from multiple genetic and environmental factors. Environmental influences upon pain severity have been previously described and linked to early-life stress [47,140–143]. Although precise mechanisms have yet to be elucidated, epigenetic modifications are increasingly appreciated as a likely factor in this linkage [36,104,122].

Our need for targeted therapies has never been greater. Multiple analgesic drugs are now in use; however, most of these share a common function with opioids or anti-inflammatory medications. These medications have improved symptoms in some patients, but have created the additional morbidities of systemic toxicity, opioid tolerance, and addiction. Our options for safe and effective treatments for chronic pain remain limited with few recent "breakthroughs."

Since the sequencing of the human genome, there have been increasing calls for "personalized medicine" that tailors drug therapy to a patient's pain phenotype [47,144]. Although such therapies have demonstrated some efficacy as cancer treatments [145–147], we have not yet had great success with targeted pain therapies. We will now review some of the potential targets for "personalized epigenetic intervention" (Table 1).

Intervention: HDAC Inhibition

Given the association between histone deacetylation and cancer, neurodegenerative disease, and pain, histone deacetylase inhibitors (HDACis) have been evaluated as therapeutic agents for these diseases [30,36,148]. Thus far, HDACis are primarily used in cancer therapy. In these patients, HDACis alter the balance of acetylation/deacetylation and activate genes that suppress tumor growth and invasion [30,149–152]. In neurodegenerative disease, HDACis have been evaluated secondary to their ability to induce neural growth and to improve memory [153]. HDACis have also demonstrated evidence for

Table 1 Epigenetically active drugs and their mechanisms

Epigenetics Mechanism	Drug	Action	Clinical Use	Comments
Histone deacetylase inhibitor	Valproic acid	Inhibits classes I and II HDAC	Seizures, pain	Effective for migraine prophylaxis
	Givinostat	Inhibits classes I and II HDAC	Juvenile idiopathic arthritis	Effective in human arthritis trial
	Tricostatin A (TSA)	Inhibits classes I and II HDAC	Laboratory only	Produces analgesia in animal models. Enhances μ-opioid receptor transcription
	Suberoylanilide hydroxamic acid (SAHA)	Inhibits classes I HDAC	Laboratory only	Produces analgesia in animal models
DNA methylation	Glucosamine	Prevents demethylation of IL-1β gene promoter	Arthritis pain	Common clinical use; effect on IL-1β reduces inflammatory cytokine production
	Valproic acid	Induces demethylation of reelin promoter	Seizures, pain	Reelin modulates NMDA function and pain processing
	L-methionine	Induces methylation at glucocorticoid receptor promoter gene	Dietary supplement	Alters experimental stress response; used as dietary supplement for arthritis
RNA interference	siRNA targeted to NMDA receptor subunits	Gene silencing of NR1 and NR2 subunits of NMDA	Experimental	Produces analgesia in animal models
	siRNA to P2X3	Gene silencing of P2X3	Experimental	Produces analgesia in animal models; no observed neurotoxicity with intrathecal use
	siRNA to TNF-α	Gene silencing of TNF-α	Experimental	Produces analgesia in animal models

analgesia in both inflammatory and neuropathic pain [151,154,155]. The clinical effect of many of these drugs is thought to be partially attributed to reduced production of inflammatory cytokines such as TNF-α and IL-1β [156].

HDACis are organized into several different structural groups. Trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA) are hydroxamate-based HDACis. TSA inhibits both class 1 (ubiquitously expressed) and class 2 (selectively expressed) HDACs, whereas SAHA exhibits greater selectivity for class 1 HDAC. TSA produces analgesia in animal models with an associated decrease in expression of transient receptor potential type-1 cation channel (TRPV1) and protein kinase Cε [157]. SAHA reduces the nociceptive response of animals during the second phase of the formalin test [154]. These drugs increase acetylation of the transcription factor p65/Re1A, which enhances gene expression of the metabotrobic glutamate receptors (mGlu2) in dorsal root ganglia neurons. Activation of these mGlu2 receptors inhibits primary afferent neurotransmitter release in the dorsal horn of the spinal cord and provides analgesia in animal models of neuropathic pain [158]. TSA also enhances μ-opioid receptor transcription [159], indicating partial HDAC modulation of the endogenous opioid system.

Another HDACi, Givinostat, has not only demonstrated evidence of analgesia in animal models, but also efficacy in a human trial for juvenile idiopathic arthritis. Although randomized studies have not yet been performed, its use for this autoimmune inflammatory disease is especially encouraging given its relative lack of systemic toxicity [160].

The most commonly used HDACi, valproic acid (VPA), is part of the aliphatic-based drug class that inhibits classes I and II HDACs [151,161], and is effective following systemic or intrathecal administration [162,163]. VPA is of particular interest because it has been successful with long-term clinical use [164]. Although it is now used predominantly to treat chronic painful conditions [163–165], its inhibition of HDAC and potential to prevent specific epigenetic alterations may lead to preemptive use in the acute setting. It is not yet clear whether VPA-induced analgesia results from HDAC inhibition or its ability to potentiate gamma amino butyric acid (GABA) in the CNS.

Although therapies based on HDAC inhibition have been effective in treating pain and oncologic disease, nonspecific HDACis such as TSA affect the regulation of multiple

genes, which increases the possibility of side effects with this therapy [166,167]. The success of future drug development will likely depend upon our ability to target specific subclasses of HDACs that selectively alter pain processing without the toxicities of nonselective agents. The importance of this selectivity concept has been demonstrated in a mouse model in which a full knockout of the HDAC4 gene (a class IIa HDAC) is lethal, whereas a conditional knockout of this gene provides analgesia [168]. Further investigations of HDAC subclass function are needed in order to identify novel drug targets.

Intervention: DNA Methylation

DNA methylation is another key epigenetic mechanism. Methylation patterns, although generally stable throughout the genome, are responsive to pharmacologic intervention. One common medication that appears to act through epigenetic mechanisms is glucosamine [169]. In arthritis models, it has been demonstrated that glucosamine prevents demethylation of the IL-1β gene promoter, thereby decreasing expression of this cytokine. Decreased IL-1β subsequently reduces NF-κB expression and downstream inflammatory cytokine production [119,170].

In addition to its function as an HDAC inhibitor, VPA induces demethylation of multiple genes [171]. One of these important genes encodes for reelin, a glycoprotein synthesized by GABAergic neurons of the CNS [172,173]. Reelin modulates N-methyl-D-aspartate (NMDA) receptor function [174], and is important for sensory processing [175]. Mutations of this gene cause alterations in mechanical and thermal hypersensitivity [173], which indicates the potential significance of VPA regulation of reelin in the development of chronic pain.

L-methionine administration has also been tested as a potential drug for epigenetic intervention. This amino acid appears to increase methylation patterns of the GR gene, thereby altering the hypothalamic-pituitary-adrenal response to stress [176]. In addition, dietary methyl supplementation in an animal model improves the health and longevity of offspring [177]. Both of these findings suggest that nutritional status partially controls the activity of the GR and its role in inflammatory disease.

The combined action of pharmacologic DNA demethylation and HDAC inhibition increases activity at the proximal promoter site of the μ-opioid receptor gene, increasing μ-opioid receptor expression [137]. Carried out in concert, these processes may represent an important balance that allows less stable histone modifications to lead to more stable changes in DNA methylation, thus facilitating longer-term modifications in the endogenous opioid receptor system.

Intervention: RNAi

Epigenetic therapies based on RNAi also hold promise for preventing and treating chronic pain. These methods target specific disease pathways.

RNAi is an endogenous mechanism for gene silencing in plants [178] and mammals [179], and involves subgroups such as siRNA, miRNA, and shRNA. Given their ability to silence undesirable gene products in malignancy, these small RNA molecules have been used for cancer therapy [82]. They have also been shown to improve chronic neuropathic pain [80].

siRNA targeted for the NR2 subunit of NMDA receptors abolishes formalin-induced pain behavior in rats [180]. Likewise, injection of siRNA aimed at the NR1 subunit of the NMDA receptor alleviates experimentally induced allodynia in mice [181]. Successful RNAi studies have targeted TRPV1 channels [182], brain-derived neurotrophic factor [183], cytokines such as TNF-α [184], and pain-related cation channels ($P2X_3$) [80]. Importantly, direct intrathecal administration of siRNA targeting $P2X_3$ in animals has not demonstrated significant toxicity [80], indicating that this intervention may be applicable to humans in coming years.

Conclusions

The transition from acute to chronic pain is a complex process involving local inflammation and nociceptor activation that may resolve in some patients and may lead to the development of chronic pain in others. As we learn more about the various ways that injury and environment change gene expression, we can begin to elucidate disease mechanisms and gain insight into potential therapies. Epigenetic alterations such as DNA methylation, histone acetylation, and RNAi are necessary for normal tissue specialization and neurologic development. However, these same modifications play a significant role in the induction of the chronic pain phenotype following neurologic injury.

In contrast to the genetic determinism inherent in genomic studies, the field of epigenetics strives to understand the environmental control over gene expression. Such knowledge will open up opportunities for developing novel analgesics. Future personalized therapies will likely be based on epigenetic interventions that alter the transcriptional expression that occurs in chronic pain states. Given the strong mechanistic implications of epigenetic modifications in the development of chronic pain, and our current treatment limitations, we possess both the promise of epigenetic tools and the imperative to prevent the transition from acute to chronic pain.

Authors' Contribution

TB, TV, and AS conceived, wrote, and performed the final editing of this manuscript. Medical illustrations were created in collaboration with Stan Coffman from Medmedia Solutions, Durham, NC. We also wish to thank Kathy Gage, BS, Duke University Department of Anesthesiology, for her editorial assistance in the preparation of this work.

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