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Critical Review

Epigenetics: A Promising Paradigm for Better Understanding and Managing Pain

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Abstract: **Epigenetic regulation of gene expression is a rapidly growing area of research. Considering the longevity and plasticity of neurons, the studies on epigenetic pathways in the nervous system should be of special interest for both epigeneticists and neuroscientists. Activation or inactivation of different epigenetic pathways becomes more pronounced when the cells experience rapid changes in their environment, and such changes can be easily caused by injury and inflammation, resulting in pain perception or distortion of pain perception (eg, hyperalgesia). Therefore, in this regard, the field of pain is at an advantage to study the epigenetic pathways. More importantly, understanding pain from an epigenetics point of view would provide a new paradigm for developing drugs or strategies for pain management. In this review, we introduce basic concepts of epigenetics, including chromatin dynamics, histone modifications, DNA methylation, and RNA-induced gene silencing. In addition, we provide evidence from published studies suggesting wide implication of different epigenetic pathways within pain pathways.**

Perspective: ***This article provides a brief overview of epigenetic pathways for gene regulation and highlights their involvement in pain. Our goal is to expose the readers to these concepts so that pain-related phenotypes can be investigated from the epigenetic point of view.***

Key words: *Pain, epigenetic, chromatin, histone modification, transcription factor, DNA methylation, opioid receptors.*

The regulation of gene expression through chromatin modifications and remodeling underlies the phenomenon of epigenetics. Epigenetic mechanisms confer pluripotent progenitor cells that possess identical genomic DNA with the ability to differentiate into morphologically and functionally distinct populations, such as neurons, astrocytes, and Schwann cells. This wide range of differentiation originates from modulating genome expression in different manners that are inheritable with each somatic cell division.

Beyond determination of cell fate, epigenetic regulation is of special interest in the nervous system due to the longevity of neurons. Unlike other organs in the body, the regeneration rate of neurons in both the central and peripheral nervous systems is remarkably low, suggesting that neurons are designed to survive for decades, possibly even the perpetuity of the organism.

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In order to survive such a long period of time, neurons must possess the greatest capacity for adaptation to changes in the environment, including physical and psychological stress. Given the inherent stability of DNA, such flexibility is most likely the result of epigenetic regulation of genes.

Easily detectable phenotypes that reflect the wide range of epigenetic mechanisms in the neurons are observed in extreme conditions such as development, where the cells experience rapid environmental changes and chemical stress. Likewise, injury and inflammation cause such environmental changes to the innervating neurons that perceive pain. So far, the focus of pain-related research has been mainly on genomics and pharmacogenomics.[38,44,45] Yet, recently, an increasing number of investigators have started to realize the importance of epigenetic mechanisms that link the phenotype and genotype related to pain.[71] Those studies on epigenetics of pain are reviewed in this manuscript as initial evidence showing participation of epigenetic mechanisms in pain state.

Understanding the mechanism of how injury and inflammation induce changes in gene expression leading to changes in pain perception (eg, analgesia or allodynia) would allow us to develop better therapeutic strategies. Our goal in this review is to introduce the basic concepts of epigenetics and to review direct evidence of epigenetic mechanisms involved in pain signal. Epigenetic drugs that are already available for other diseases will be discussed briefly as well. In this review we will 1) briefly discuss the achievement and limitation of pain genetics; 2) discuss basic aspects of molecular mechanisms that are important for understanding gene regulation; 3) examine existing evidence of epigenetic mechanisms' contribution to pain pathways by reviewing transcriptional regulation of opioid receptors; and 4) mention some epigenetic drugs that may be used for intervention.

Genetics and Pharmacogenomics in Pain and Analgesia

Pain has an impact on the patient's life at several levels. In addition to the suffering, pain, especially chronic pain, can impair the individual's ability, quality of life, mood, and sleep, which can result in difficulties in personal and social life. Even though objective assessment of pain has been challenging, many self-report scales have been developed in order to standardize the measurements and have been used successfully in both clinical practice and research.[25] The interindividual variability in pain response and analgesic response to pharmacological agents, and variability in analgesic response patterns in different strains of mice, have indicated genetic basis of pain perception.[11,59,60] Following such observation, significant efforts have been made to identify genes and polymorphisms that are involved in specific pain phenotype and to develop tailored therapy for each patient.[14,75]

Numerous genes and single nucleotide polymorphisms (SNPs) have been identified to affect pain perception and response to analgesic drugs. However, accumulating data have been showing some controversial results on the genetic associations with pain-related phenotype.[74] For example, the most prevalent SNP on human μ-opioid receptor (*ORPM1*A118G, asn40asp, rs1799971) was initially reported to show increased binding affinity for β-endorphin and to cause subsequent increase in G-protein coupled potassium channel activation, compared to the wild type.[2] Yet, more recent independent study found no significant difference in ligand-binding affinity, including that of β-endorphin, and in the activation of intracellular signaling cascade, between cells expressing the mutant and the wild-type ORPM1.[1] Similarly, catechol-O-methyltransferase (COMT) val158met SNP (rs4680) was reported to be associated with higher pain sensitivity by some groups, while others found the same SNP to have no association with pain perception.[39,89] Such inconsistencies from genetic studies suggest possible existence of additional, DNA sequence independent, factors that play a role in pain phenotypes. Such pathways, proteins, and mechanisms are essential topics in the field of epigenetics.

Fundamental Concepts of Epigenetics

Chromatin Dynamics Forms the Basis of Epigenetics

Chromatin is the structural conformation of DNA in association with assembly proteins. The nucleosome is the fundamental repeating unit of chromatin, consisting of about 140 base pairs of DNA wrapped around a histone octamer (consisting of 2 copies of H2A, H2B, H3, and H4). Chromatin is dynamic, often switching between 2 higher order chromatin structures: euchromatin and heterochromatin.

Euchromatin is decondensed chromatin and consists largely of coding sequences that are "poised" for transcription.[63] The transcriptional activity of the genes in euchromatin is determined by recruitment of transcription factors and other nucleosome-remodeling complexes, which may be either repressors or activators. On the other hand, heterochromatin is a highly compacted chromatin structure where genes are silenced. Heterochromatin is known to define centromeres, facilitating chromosome segregation during mitosis and meiosis. Also, heterochromatin is formed at telomeres, ensuring stability of genome. Histone tail deacetylation and methylation of specific lysine residues recruit heterochromatin-associated proteins (eg, HP1) that establish DNA methylation and the formation of heterochromatin.[61,66]

The nucleosome is also dynamic with posttranslational histone modifications, subunit variation, DNA methylation, and small noncoding RNA interaction. All these modifications on nucleosomes are interrelated and regulate euchromatin and heterochromatin formation in an orchestrated manner.[32] In other words, the complexity achieved by combinations of all the different modifications mentioned above provides multidimensional layers to the readout of DNA and results in different patterns of gene

expression or silencing from the same genome. Therefore, in order to gain a complete understanding of the epigenetic regulation of gene networks, all the basic paradigms must be incorporated into an integrated picture.

The Histone Code and Subcode Hypothesis: Codifying Gene Activation and/or Silencing

Histones are small, basic proteins that are extremely conserved throughout evolution. The first 20 or so amino acids of histones, known as the histone tail, are very highly conserved, and they structurally extend from the nucleosomal disk, which allows access of posttranslational modification enzymes.[54] In the histone tail, serine (S), threonine (T), and tyrosine (Y) residues can undergo phosphorylation, and other residues, such as lysine (K) and arginine (R), can be methylated, acetylated, ubiquitinated, and sumoylated.[76] Furthermore, lysine residues have potential to be mono-, di-, or tri-methylated.[42] These histone modifications are known as "marks" and provide specific docking sites for many chromatin-associated proteins. The Histone Code hypothesis predicts that the type, location, and combination of histone marks determine recruitment of a specific chromatin-associated protein or transcription factor, and subsequently determine whether the gene would be expressed or silenced under a particular set of circumstances.[32]

The histone marks can be created, recognized, and reversed by different proteins: writers, readers, and erasers respectively.[84] The writers include histone acetyltransferases (HATs), histone kinases, and histone methyltransferases.[56,73] Marks imprinted by writers are recognized by the reader proteins containing domains engineered to recognize a particular mark, ie, chromodomain for methylation and bromodomain for acetylation.[34,43] The modifications can also be reversed by eraser proteins, such as histone deacetylases (HDACs), phosphatases, lysine specific demethylases (LSDs), and Jumonji domain-containing proteins. The histone subcode hypothesis predicts that some of these chromatin-associated proteins may also be posttranslationally modified to create an additional layer of the chromatin code, a subcode, that determines transcriptional activity of the target genes.[51] This hypothesis was supported by changes in localization of pan-nuclear HP1γ, which becomes exclusively euchromatic with phosphorylation of Ser 83 residue, suggesting that p-Ser 83-HP1γ may adopt different modes for silencing the target gene compared to nonphosphorylated HP1γ.[51] Together, the histone code and histone subcode hypotheses show how posttranslational modifications of different nuclear proteins precipitate differential protein complex recruitment, gene expression, and chromatin dynamics.

Nucleosome Remodeling Machines and Histone Variants

In addition to histone modifications, chromatin structures can be altered by recruitment of nucleosome remodeling complexes. Nucleosome remodeling machines, such as SWI/SNF, nucleosome remodeling and deacetylation (NuRD), and chromatin accessibility complex (CHRAC), can move nucleosomes along DNA in ATP-dependent manner.[47,77,88] Several of these molecular machines have been found to be conserved from yeast to human.[49,82] The SWI/SNF family of nucleosome remodeling complexes transiently alters the structure of nucleosome, exposing DNA.[67] Similarly, facilitates chromatin transcription (FACT) initiates nucleosome release from DNA and allows transcriptional elongation by RNA Pol II.[64] These nucleosome remodeling complexes work in concert with histone modification complexes such as Spt-Ada-Gcn5-acetyltransferase (SAGA) to regulate gene transcription.[19]

Other nucleosome remodeling complexes, such as the SWR1 complex, are involved in exchange of histones and histone variants.[57] There are a number of histone variants that may occupy a nucleosome to yield an effect on transcriptional activity.[22] For instance, histone H3 has 3 variants in mammals, H3.1, H3.2, and H3.3, which differ from each other by 1 to 4 amino acids in the tail.[52] These small differences allow the variants to have unique patterns of modifications and different affinities to the binding factors. Together, the combinatorial effect between histone variants and their participation in the Histone Code, known as the histone "barcode," introduces another level of complexity to transcriptional regulation.[22] The mechanisms and timing of histone variant exchange and synthesis, and interrelationship between the chromatin dynamics and nucleosome remodeling is an active area of research.

Sequence-Specific Recruitment of Chromatin-Associated Protein Complexes

In order for the nucleosome remodeling complexes and histone modification complexes to function properly in regulating gene transcription, they must be recruited to the correct location at the correct time. In this regard, DNA sequences, especially the promoter sequences, play a significant role. Promoter footprinting and electrophoretic mobility shift assays were utilized to identify sequence-specific transcription factors.[31] Subsequent investigations on these sequence-specific transcription factors revealed DNA-binding domains and transcriptional regulatory domains, indicating that these proteins serve as adaptor proteins between DNA and chromatin-associated protein complexes.[50] For example, the Sp1/KLF (Kruppel-like factor) family was established based on their similarities in the DNA-binding domain, which is composed of 3 zinc fingers. Some of the members of this transcription factor family were able to compete with each other for the same promoter, and due to the variations in their regulatory domain, promoter occupancy of a specific family member resulted in distinct levels of transcription.[50] Proteins that interact with transcription factors to promote transcription are called coactivators, while proteins that repress transcription are called corepressors. These nonhistone chromatin proteins function as either coactivators or corepressors via mediating histone modifications such as acetylation, methylation, and ubiquitination.

DNA Methylation

In addition to the basis of sequence-specific recruitment of transcription factors, DNA can also function as a template for modifications. Unlike histone modifications, which can occur in great variability, from methylation to ubiquitination, DNA modification is limited to methylation of cytosine residues. DNA methylation was the first type of epigenetic changes to be studied as a mechanism for the inactivation of tumor suppressors. DNA methylation occurs on di-nucleotide CpGs by DNA methyltransferases (DNMTs), and the methylation is enriched at noncoding regions.[12] DNA methylation is closely related to heterochromatin formation and chromatin silencing. DNMTs interact with HP1 and the SUV39H1 histone methyltransferase, which functions to establish tri-methylation of histone H3-K9 and induce heterochromatin formation and gene silencing.[16,72] DNA methylation-mediated gene silencing has physiological significance. First, hypermethylation of 1 of the 2 parental alleles of a gene is a process of genomic imprinting that ensures monoallelic expression of genes.[5] Second, hypermethylation of repetitive genomic sequences prevent chromosomal instability and translocations.[35]

DNA methylation has been classified into 2 types, namely de-novo and maintenance methylation.[12] Human cells utilize 3 DNA methylases: DNMT1, DNMT3a, and DNMT3b. DNMT3a and 3b function to establish embryonic methylation patterns, whereas DNMT1 works at the replication forks during DNA replication to maintain the methylation patterns in semiconservative way. However, DNMT1 appears to be inefficient at maintaining the methylation of many CpG dense regions. Therefore, the de-novo activities of DNMT3a and DNMT3b are also necessary in somatic cells in order to reestablish the methylation patterns.[6] The mechanism of DNA methylation inheritance is more obvious than many other epigenetic regulators and the mark appears to be very stable. However, the presence of de-novo DNMTs suggests that certain amount of flexibility to this mechanism may also exist.

RNA-Directed Gene Silencing

Beside DNA and histone modifications, RNA interference (RNAi) machinery has been linked to posttranscriptional gene silencing and heterochromatin formation. RNAi machinery, including proteins like Dicer, Argonaute, and RNA dependent RNA polymerase (RdRP), breaks down double stranded RNA into small RNA molecules, named microRNA (miRNA), to interact and degrade the mRNA of homologous genes and inhibit translation.[13] Studies in *Schizosaccharomyces pombe* have shown that mutations of any proteins in the RNAi machinery lead to defects in chromosome segregation due to unstable centromeric heterochromatin.[23,83] This evidence suggests the role of miRNA in heterochromatin formation, in addition to posttranscriptional gene silencing. Similar evidence has been found in mammals. For example, mouse embryonic stem cells with depletion in a component of RNAi machinary, Dicer, showed significant decrease in the formation of centromeric heterochromatin and failed to differentiate.[33] In human-chicken hybrid cell line, loss of Dicer resulted in abnormal localization of heterochromatin proteins, depletion of H3-K9 methylation, accumulation of abnormal mitotic cells with premature sister chromatid separation, and ultimately cell death.[15] Therefore, these studies indicate an active participation of RNAs in chromatin remodeling and gene silencing. In summary, there are multiple layers of regulatory mechanisms that are functionally interrelated for target gene silencing or activation.

Pain Research: Opening the Door to Epigenetics

The field of pain is at a great advantage to study the epigenetic pathways. The wealth of information gained from previous molecular studies, microarray studies, and pain genetics provide a robust ground for further epigenetic investigations to come.[38,45,58,81] First, molecular studies have shown that injury and inflammation induce release of steroid hormones and proteins such as glucocorticoid, nerve growth factor, and substance P.[9,69,86] These steroid hormones and proteins bind to specific receptors to activate intracellular signal pathways and intranuclear transcription regulatory pathways for gene networks. Second, microarray data provide an extensive list of target genes to be studied. In combination with the molecular studies, this information allow us to concentrate on exploring epigenetic mechanisms, answering questions such as which complexes appear under which circumstances, how do different complexes or pathways interact with each other, and what are the target gene networks for each mechanism. Third, the data from pain genetics provide an additional list of genes to be studied, and some SNP information can be incorporated into DNA methylation studies. Among pain-related genes, opioid receptors are among a few examples of genes in which a substantial amount of investigation on transcriptional regulation has been done. Therefore, this opens up a great opportunity for investigators to study the effect of pain on gene transcription. In this section, we will use transcriptional regulation of an opioid receptor (ORPM1) as a model to illustrate how different aspects of epigenetic mechanisms we have discussed in the previous section can come together to regulated transcription of single gene.

Opioid receptors are G protein-coupled receptors, activated in response to opioids. They play critical roles in regulation of the pain experience, stress response, and action of analgesic opioid drugs. Therefore, understanding the expression patterns of these genes during pain state is important for generating strategies for pain management. There are 4 subtypes of opioid receptors, which are initially determined by their ligand-binding characteristics: μ-opioid receptors (OPRMs), δ-opioid receptors (OPRDs), κ-opioid receptors (OPRKs), and nociceptin receptors (ORLs).[85] Among these receptors, OPRMs have been identified to play a critical role in morphine response, tolerance development, and physical dependence.[37] Mechanisms of

transcriptional regulation of OPRMs have been described in a number of recent studies. As expected, there appears to be several layers of mechanisms that work together (ie, DNA methylation, histone modification, and miRNA induced repression).[28,85]

A number of sequence-specific transcription factors, including Sox18, Sp1, poly(rC)-binding protein (PCBP), cAMP response element-binding protein (CREB), spleen focus-forming virus proviral integration 1 (PU.1), activator protein 1 (AP1), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), have been identified to bind promoter region of *ORPM1* and to alter the transcription activity.[3,26,27,30,40,41,48] PU.1 was identified to be transcriptional repressor while others were activators. Most of these transcription factors are known to bind to specific DNA sequence and recruit chromatin-associated protein complexes to induce histone modifications and/or DNA modifications. For example, Sp1 and CREB have been shown to interact with p300 and/or CREB-binding protein (CBP), which are HATs, usually resulting in transcriptional activation of the target gene.[65,80] Questions addressing how these transcription factors activities are regulated, the existence of competition or cooperativity between transcription factors, and in what circumstances these transcription factors gain access to the target gene promoters remain to be investigated. More importantly, identities of epigenetic regulatory complexes (ie, histone mark writers, readers, and erasers) that are recruited specifically to the *OPRM1* promoter by these transcription factors are crucial information to further our understanding of epigenetic gene regulations in pain pathways.

Some of the chromatin-associated proteins on the *OPRM1* promoter have been identified by chromatin immunoprecipitation (ChIP) assays.[28] In this study, the authors identified transcription factor Sp1 binding, recruitment of nucleosome remodeling proteins Brg1 and BAF155, dissociation of transcriptional corepressors HDAC1, HDAC3, mSin3A, and MeCP2 on this promoter, and subsequent activation of transcription during neuronal cell differentiation. Brg1 is the ATPase subunit and BAF155 (Brg1-associated factor, 155kD) is a ubiquitous component of the nucleosome remodeling SWI/SNF complex. Other studies have indicated direct interaction between Brg1 and Sp1 to activate target gene expression.[55] Similar mechanisms of SWI/SNF complex-induced recruitment of RNA polII may apply to *OPRM1* regulation. The mSin3A is known to form a complex with HDAC proteins to induce histone deacetylation, which usually results in transcriptional repression.[20] Concordantly, the loss of mSin3A, HDAC1, and HDAC3 from the promoter region resulted in decrease of H3 and H4 acetylation.[28] Additional changes in histone modification, such as increase in H3K4me2 and decrease in H3K9me2, have been observed with transcriptional activation.[28] This result indicates involvement of more chromatin-associated proteins that have not been identified yet (ie, MLL complex for H3K4 methylation, G9a and Suv39 H3K9 methyltransferases, and JmjC-domain containing H3K9 demethylases).

In addition to histone modifications, nucleosome remodeling, and transcription factor interactions mentioned above, DNA methylation also plays a critical role in OPRM1 transcription. First, when the gene was activated by neuronal cell differentiation, the promoter region became demethylated and MeCP2 (methyl CpG-binding protein 2) level decreased.[28] Further study showed that disruption of MeCP2 expression or addition of an artificial demethylation agent, 5-Aza-C, resulted in activation of *OPRM1* gene, suggesting the DNA methylation on the promoter region was actively repressing the gene.[29] Second, an SNP in *OPRM1* (A118G, rs1799971) was shown to introduce a new CpG-methylation site, resulting in enhanced methylation of *OPRM1* DNA, which leads to reduced expression of the protein.[2,62] This result is a good example showing how genetic studies can be reevaluated from the epigenetics point of view. Many of the proteins introduced so far in relation to *OPRM1* transcription are rather large and work in multicomponent complexes. How all these complexes and proteins can come together in restricted space around the promoter region (~800 bp) is an intriguing question to be investigated.

Two miRNA species have been identified for regulation of *Oprm* transcription. First, miRNA23b, cloned from mouse brain cortex and hippocampus, was found to interact with 3′UTR of *Oprm* mRNA to decrease the polysome-mRNA association rate, and to ultimately result in decreased translation.[10,46,87] Second, let-7 family miRNAs, which includes 9 distinct, mature 22-nucleotide sequences with only 1 to 4 nucleotide differences from the canonical let-7a, were also found to downregulate translation by interacting with 3′UTR of *Oprm* mRNA.[24] Questions such as under what circumstances these miRNAs become active, do these miRNAs interact with any other mRNAs, and is there interaction between these two miRNA mechanisms remain to be investigated.

As we have reviewed here, evidence for epigenetic regulation of pain pathways is emerging from all directions. Transcription of the *OPRM1* is regulated by histone modifications, nucleosome remodeling, sequence-specific transcription factor binding, DNA methylation, and miRNA interactions, all at the same time. So far, the studies presented here have investigated each individual aspect of epigenetic mechanisms in isolation. With all the information we gained from individual studies, the importance of understanding how all of the mechanisms are orchestrated in a cell in response to injury or pain is apparent.

Epigenetic Mechanisms and Interventions in Pain Management

An important advantage for bringing epigenetic drugs into therapeutics is in the reversible nature of epigenetic pathways.[78] Unlike genetic mutations, if a pain phenotype is caused by epigenetic phenomena, such as DNA methylation or histone modifications, they can be chemically reversed. Such involvements of epigenetic mechanisms in pain have been already implicated and accordingly some therapeutic strategies for pain management have been suggested.[4,7,18] However, as

implicated above with the opioid receptor, transcription is regulated by complex multilayered pathways that involve proteins with multiple target genes and substrates. Therefore, understanding the epigenetic pathway is critical to applying epigenetic drugs for intervention. In this section, we will briefly review some of the available epigenetic drugs and how they may be applicable for pain management.

Histone deacetylase inhibitors (HDACis) have been evaluated as therapeutic agents in several conditions including cancer, autism, and neuropathic and inflammatory pain.[8,68,70] HDACis induce an increase in acetylated histones by blocking histone deacetylase activity of HDAC proteins. In general, histone acetylation has been associated with transcriptional activation. Recent study has investigated the potential of HDACis as analgesic drugs. Based on studies reporting that the upregulation of mGlu2 in dorsal root ganglia can induce analgesia and that transcription of mGlu2 is regulated by histone acetylation, the authors tested 2 HDACis, MS-275 (benzamine derivative) and SAHA (suberoylanilide hydroamic acid), in a mouse model of persistent inflammatory pain.[7] With 5-day treatment, both HDACis showed analgesic effect on the mice and increased mGlu2 expression in dorsal root ganglia without changing the expression of mGlu1a, mGlu4, or mGlu5 receptors. Another study showed that treatment with an HDACi, Trichostatin A, in a mouse model of endometriosis induced transient decrease in the capsaicin-activated type-1 cation channel (TRPV1) and significant improvement in response to a noxious thermal stimulus, suggesting its role as an analgesic drug.[53]

While these examples appear promising, with current knowledge, clinical use of these agents is still limited. As we have previously discussed, HDAC proteins have multiple target genes. Therefore, it is evident that HDACis will inhibit deacetylation not only of desired chromatins, but also of other chromatins, which may result in severe side effects. Drugs that affect DNA methyltransferase activities, such as glucosamine and valproic acid, would present similar specificity problem. Thus, in order to understand the mechanism and regard possible application of these drugs, changes in transcription of all genes, rather than a single target gene, need to be considered. Development of more specific HDACis for each HDAC family member, and perhaps, combined with gene therapy, development of HDACIs that can target only the histones associated with a specific target gene, will be extremely beneficial for therapeutics.

The siRNAs, as possible epigenetic drugs, stand on a slightly different ground from other epigenetic interventions. Unlike HDACis, siRNA sequence can be designed for a specific target gene. A number of siRNA treatments, targeting NR1 and NR2 subunits of *N*-methyl-D-aspartate (NMDA) receptors, transient receptor potential vanilloid 1 (RTPV1) channels, bone-derived neurotrophic factor (BDNF), and other pain-related genes, in animal models have been successful for alleviating pain.[17,21,36,79] Some of these may become one of the first epigenetic drugs that become applicable for clinical trials in pain management.

Concluding Remarks

Epigenetic regulation of gene expression is a rapidly growing area of research. As epigenetic mechanisms, including DNA methylation, histone modifications, RNAi, and chromatin dynamics, are theoretically reversible, there exists considerable confidence as to therapeutic possibilities. The fundamental concepts and general principles described in this review are only a brief summary of what is known today. Our goal is to expose the reader to these concepts so that pain-related phenotypes can be investigated from the epigenetic point of view.

Many fundamental questions connecting the concepts of epigenetics and pain remain to be answered. For example, in the innervating nociceptive neurons, which epigenetic pathways are activated or repressed during injury and inflammation? What is the epigenetic basis of peripheral sensitization and pain centralization (eg, hyperalgesia and allodynia)? How does inflammation at the end of a peripheral neuron affect gene expression in the connected neurons of the central nervous system? What is the epigenetic basis of neuropathic pain? How do the experience, stress, and memory of pain affect patterns of gene expression in the brain, and how can they be reversed? Do emotional pain and physical pain differ or coincide in terms of changes in gene expression? Does pain relief follow the same epigenetic pathways that generate pain? The questions regarding the epigenetics of pain range from basic molecular biology to psychology and even to philosophy. We anticipate that investigations in this field of research will reveal new paradigms for understanding the pathology of pain, and for developing better pain management strategies.

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