Epigenetic Modification of Nociceptive Mediators: Implications for the Etiology of Neural Hypersensitivity (Part I)

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SUMMARY Many factors have been identified contributing to the pathogenesis of pain, whereas we still cannot conquer the pain based on these findings suggesting that further studies are needed and other more potent mediators should be investigated. Epigenetics, in contrast to genetics, refers to the functionally relevant modifications to the genome that do not cause changes in underlying DNA sequence. These kinds of changes in gene expression or cellular phenotype regarded as landmarks of epigenetics are regulated by different types of modifications including gene methylation, histone acetylation, phosphorylation, imprinting and reprogramming etc. We, in this part (Part I), will review the general epigenetic modifications on molecular mediators on biological processes as the preface of the second part of the whole article (Part II will be available in the June issue of the journal). This general understanding of the epigenetic modification on the modulating factors that influence individual differences from pain sensitivity and responsiveness to analgesics possesses crucial clinical implications.


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OVER HUNDREDS of molecules so far were identified being involved in the regulation of the pathogenesis of pain, and they were classified into four categories: neurotransmitters (amino acids, monoamines, peptides and gaseous transmitters), cytokines (pro- and anti-inflammatory), endocrine and immune-mediators (hormones and human leukocyte antigen system), and second messengers and nuclear mediators (calcium, cAMP, NF-κB, menin etc.). Rare clinic-suitable drugs were
found to control over the pain when different pharmacological activators or inhibitors were administered through focusing on these various kinds of molecules (1, 2). Consequently, studies began to concentrate on the genetic control on pain and increasing evidence appeared showing that genes are the primary signature of pain with individual difference and also the determinant of the development of chronic pain (3). Nevertheless, we cannot change the gene sequence that has mutated before the pain appearance, and also we do not know what will be happened next to the already-happened pain because it is really difficult to predict the genetic changes under the present knowledge of science. Unlike genetics, epigenetics is the study of changes in gene expression and cellular phenotype otherwise the underlying DNA sequence (4). The development of epigenetics promises patients hope for controlling the pain through modifying the gene expression of pain-related molecules that finally determines the fate of the patient’s outcome.

**Nociceptive Transduction and Mechanisms**

Before the central nervous system (CNS) feels pain, the injury first needs to be sensed by nociceptors located at the terminals of the peripheral nerve fibers through forming action potentials (APs), and then these APs will be transmitted into the first delay station – dorsal root ganglia (DRG) followed by complicated modulation at the dorsal horn of the spinal cord. After modeling by the dorsal cell groups, the signal will be transduced upward into specific areas of the brain where the pain signals are remodeled and perceived. This is the so-called ascending facilitation. However, it is not ended after the pain was felt. The CNS activates its self-control mechanisms, i.e. the descending inhibition, through which the original pain signal was diminished. This is the macro-description of the pain transduction, but a more complex neural network is formed underlying this “ascending-descending” balance.

**Facilitation of Nociception**

Excitatory neurotransmitters are considered as the major compositions of the ascending facilitation-associated molecules for pain transduction. Glutamate, the principal excitatory neurotransmitter in the CNS, takes role by binding two types of receptors: ionotropic and metabotropic receptors (5). The ionotropic receptors include N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazol leptionic acid (AMPA) and kainate receptors (KAR), which are ion channels; and the metabotropic receptors belong to G-protein coupled receptors named mGluRs. Abundant evidence showed that NMDARs are critically involved in synaptic plasticity and pain facilitation in the CNS that has long been considered a target of pain management (6), and the AMPAR subunit trafficking in the dorsal horn contributes to the hypersensitivity that underlies persistent pain (7), and KAR plays integral signaling role at multiple levels of the pain neuraxis (8). Focusing on these types of receptors potentiates novel therapeutics via inhibiting their activities with pharmacologically or genetically developed probes. To the mGluRs, the final effect of their activation depends on their anatomic location and the signaling cascades to which they couple, from which both of the pro- and anti-nociceptive effects can be developed (9). Although increasing reports presented evidence supporting glutamate’s involvement in the regulation of the pathogenesis of different types of pain, we still cannot find ideal interventional means targeting on its multi-functional receptors. Furthermore, the precise relationship between glutamate and descending inhibition is still unknown.

Pro-inflammatory cytokines are another major kind of contributors to the ascending facilitation of pain (10). The localized inflammatory “soup” either at peripheral injury site or at the CNS facilitates sub-threshold stimuli to APs leading to hyperalgesia (11). Over a hundred of cytokines were identified and evaluated for their involvement in the modulation of pain. Tumor necrosis factor- alpha (TNF-α) is one of the key contributing factors to pain (12), and Etanercept, the monoclonal antibody of TNF-α, is the first drug available in clinics for controlling inflammatory responses from which the pain was alleviated (13). Interleukin- 1 beta (IL-1β) is an essential cytokine with broad-spectrum expression property (from peripheral to central) in the context of pain (14), and blockade of IL-1β is a potential target of pain therapy. Macrophage migration inhibitory factor (MIF), the earliest-discovered cytokine named on its inhibitory effect on T-lymphocyte’s random migration, has been identified as a pro-inflammatory cytokine contributing to the pathogenesis of pain by activating corresponding receptor CD74 (15), and the inhibitor focusing on its tautomerase activity was a promise means in controlling pain (16).

Cyclo-oxygenase (COX) is a kind of enzyme responsible for the formation of prostanooids, an important mediator of inflammation (17), and pharmacological inhibition of COX can provide relief from the symptoms of inflammation and pain (18). The expression of all these mentioned molecules after pain displayed different levels of upregulation, and blockade of them is considered a critical means of analgesia. Whether these inhibitions can produce as expected effect or not still needs to be investigated further.

Ion channels are a group of pore-forming membrane proteins functioning establishing resting membrane potential and shaping APs through gating the flow of ions across the cell membrane. Sodium channel, one of the major basal channels forming APs, was extensively studied especially the voltage-gated sodium channel on its role in pain and found that the creation of aberrant sodium channel clusters served as sites of ectopic sensitivity or spontaneous activity that is strongly associated with the development of...
pain (19). Potassium channel, the most diverse class of ion channels, exerts function through shaping APs and setting the resting membrane potential. Although a complex distribution of the voltage-gated potassium channels among sensory neurons exists, the physiological importance of potassium channels in nociceptive neurons was well documented (20). Voltage-gated calcium channels have been recognized as potential targets for analgesic development, but the availability of the related analgesics is rare (21). Chloride channel, a poorly understood ion channel, has also been found being involved in the modulation of nociceptive transmission (22). In most studies, chloride channels were activated indirectly as the sequence of other ion channels’ activation (23, 24), and also the calcium-activated chloride channels are involved in the delta-opioid receptor-mediated central antinociception (25). What is the precise interrelationship among these ion channels and their contributions to facilitating pain are still not elusive and further studies are needed on this topic.

Inhibition of Nociception

Opium, the first opioid discovered for pain relief, is the most widely-used medicine around the world and its history can be traced back to the Neolithic Age (26). Until 1804, morphine was first isolated by Dr. Friedrich Sertürner and it then became the most abundant opiate (27). Till today, morphine is still the most widely used analgesic by clinicians, whereas overwhelming evidence also criticized its side effects and the potential addiction. Even different types of opioids were synthesized, but the tolerance to opioid analgesics reduced the expectation of this kind of drug and promotes researchers to find suitable alternatives; in contrast to the tolerance, opioid-induced hyperalgesia is another challenge for health care-givers (28, 29). Beside exogenous opioids, the endogenous opioidergic system is a crucial part of the descending inhibition, which includes endorphins, enkephalins, dynorphins and endo-morphins (30). The activation of different types of receptors (mu, MOP; delta, DOP; kappa, KOP and nociceptin, NOP) is the basis of opioid analgesia. During chronic pain conditions, the endogenous opioidergic system encountered significant changes leading to over-balance of the self-adjusting ability, after then the pain was worsened (31). Therefore, we can conclude that the opioidergic system has duel effect, i.e. pro- and anti-nociception, and the final effect is based on the balance between these two facets. Additionally, evidence appeared showing that the opioidergic system is combined with other systems like the adrenergic (32) and the GABAergic (33) suggesting that in-depth experiments are necessary to clarify their precise relationship.

Gamma-aminobutyric acid (GABA), the key inhibitory neurotransmitters in the CNS, is a major component of inhibitory neuroactivities including descending inhibition. Experimental evidence showed that impaired GABAergic transmission was an important cause resulting in pain (34). The death of GABA-containing interneurons after nerve injury and altered storage or/and release of GABA are possible underlying mechanisms of the loss of GABAergic inhibition (35). Both types of GABA receptors (types A and B) all are involved in the process of pain-related central sensitization (33, 35). How to reach the ideal level of GABA and how to keep the GABA receptors at the optimum function are two problems that need to be solved when GABAergic system was considered as the target of pain management.

Monoamine neurotransmitters including noradrenalin, serotonin and dopamine form a complex interaction each other to exert inhibitory function on pain (36). Intrathecal clonidine, a centrally acting α2 adrenergic agonist, produced dose-dependent analgesia (37) suggesting that spinal adrenergic system is involved in the process of pain-coding. More recent clinical evidence showed that intrathecal (38), topical (39) and intravenous (40) administration of clonidine produced effective analgesia. Given the strong association among the three monoamines, the currently available drugs – antidepressants which have combined effect on two or three of them have been studied at length and now they are approved for the use in pain control (36). Furthermore, different types of subunits of the monoamines’ receptors determine the complex final effect if one drug has mixed role in activating or deactivating two or more subtypes of these receptors, and undoubtedly the side effects would be consequently resulted from (41). As thus how can we realize our pain-relief purpose through fine-tuning the monoaminergic systems? It is herein hard to reach this goal.

Anti-inflammatory cytokines, in contrast to the pro-inflammatory cytokines, were regarded theoretically as one part of the descending inhibition (10, 42). Although we describe the pro- and anti-inflammatory cytokines separately as ascending facilitation and descending inhibition, respectively, they actually exert functions simultaneously when the injury occurred peripherally or centrally. IL-10 is the major anti-inflammatory cytokine found to be involved in the modulation of pain and hematopoietic stem cell transplantation was considered a potential method to treat IL-10 and IL-10 receptor deficiency (43). The transforming growth factor-β (TGF-β) superfamily is a multifunctional, contextually acting family of cytokines, and found TGF-β was a relevant mediator of nociception and has protective effects against the development of chronic pain by inhibiting the neuroimmune responses of neurons and glia and promoting the activation of the endogenous opioidergic system in the CNS (44). Interferon-gamma (IFN-γ), an essential macrophage-activating factor, was found to be an important regulator of pain even though most studies on this cytokine were focused on it immunostimulatory and immunomodulatory effects (45).
So it is still necessary to find easy-to-use methods to relieve pain by concentrating on above-mentioned molecules through modifying their corresponding genes, a prospectively promise for conquering pain, especially for the refractory chronic pain.

**General Epigenetic Modifications**

The word “epigenetics” was coined by Dr. Waddington, C.H. in 1942 as a portmanteau of the words epigenesis and genetics (46), and its original meaning was the heritable changes in gene function that were not explainable by changes in the DNA sequence. The more precise definition of epigenetics is largely based on the recent understanding on its underlying mechanisms as it is the structural adaption of chromosomal regions in order to register, signal or perpetuate altered bioactivity states (47). As the basic thought considered that why the same gene yet performs distinct functions, the answer is that it is the epigenetic achieve the goal by switching on and switching off specific gene expression. The major reason for epigenetics became popular and be a hot topic for research is that it is irreversible and therefore have the potential to be manipulated therapeutically (48). Epigenetic control on gene expression is reached by methylating DNA or/and histone or/and acetylation histone, and these processes take place generally in combination each other.

**DNA Methylation**

DNA methylation, unlike the histone modification, occurs with limited variability through adding a methyl group to the 5 position of the cytosine pyrimidine ring or the number 6 nitrogen of the adenine purine ring (49). As the biochemical process of gene expression forwarded, a number of molecules function as the writers (attachment) or erasers (removal) or readers (binding) of the modifications to DNA to a specifically epigenetically modified site, and then take role in the regulation of gene expression (50). As the first studied epigenetic type, DNA methylation can stably change the expression of genes, and permanently and unidirectionally transduced. CpG islands are the genomic regions containing a high frequency of CpG sites where they are enriched in promoters in vicinity to transcriptional start sites and the methylation on them has been associated with long-term gene silencing including X-chromosome inactivation (49). DNA methylation typically occurs in a CpG dinucleotide context, but non-CpG methylation is prevalent in embryonic stem cells. In general, DNA methylation occurs mainly at the C5 position of CpG di-nucleotides and is carried out by two general classes of enzymatic activities – maintenance methylation and _de novo_ methylation (51, 52). Maintenance methylation is a necessary requirement to keep the gene being methylated after every cellular DNA replication cycle, and _de novo_ methylation is the subsequent alterations to the changes of environments and is more flexible the maintenance methylation. DNA methyltransferases (DNMTs) are enzymes regulating the methylation of DNA, and they are composed of three subtypes: DNMT1, DNMT3a and DNMT3b (53). It is thought that both DNMT3a and DNMT3b are the _de novo_ methyltransferases functioning to set up DNA methylation patterns early in development, but DNMT1 works to maintain the methylation in the semi-conservative way. However, these three types of DNMTs appear to maintain and reestablish the methylation patterns (54).

**Histone Methylation**

Histones, the chief protein components of chromatin, are located in eukaryotic cell nuclei to structure the DNA into nucleosomes. Histones are classified into five major families: H1, H2 (subtype A and B), H3, H4 and H5. H2A, H2B, H3 and H4 are considered as the core histones, but H1 and H5 are regarded as the linker histones (55). The methyl groups are transferred to amino acids of histone proteins of chromosomes is named histone methylation, which mainly occurs at lysine and arginine residues of histones H3 and H4 which can be mono-, di- and tri-methylated (56). Methylation and demethylation of histones, in most cases, switch the specific genes "off" and "on", respectively. Histone methylation occurs by loosening but demethylation by encompassing the tails which results in respective permission and blockade of the transcription factors to access the DNA (57). Lysine can be mono-, di-, or trimethylated, but arginine can only be mono- or di-methylated. Different degrees of residue methylation generally resulted in different functions (56). Histone methylation needs the involvement of the histone methyltransferases via transferring the methyl group from S-Adenosyl methionine onto the lysine or arginine of the H3 and H4 histones. In addition, there are proteins possess histone demethylation activities like the lysine-specific demethylase family and the Jumonji C family (58, 59). The balance between histone methylation and demethylation requires accurate interaction between histone methyltransferases and demethylases.

**Histone Acetylation**

Histone acetylation leads to activation of transcriptional activity by decondensating the chromatin, but histone deacetylation on the contrary reduces transcriptional activity by condensating the chomatin (60). Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are the major enzymes catalyzing above two activities. In consideration of the role of these two enzymes through neutralizing and restoring the positive charges in lysine residues, they are regarded as transcriptional activators and repressors, respectively (61). Based on these, HATs and HDACs became the focuses of studies using different types of pharmaceutical interventions.
HATs are classified into two different categories on their subcellular location: type A localized in the nucleus and type B localized in cytoplasm. Type A HATs are responsible for the regulation of gene expression by acetylating nucleosomal histones, but type B HATs are involved in the process of acetylation of newly synthesized histones before they assembled into nucleosomes (60, 62). To HDACs, four different classes exist: class I (HDAC 1-3 and 8), class II (HDAC 4-6, 7-10), class III (sirtuin, SIRT 1-7) and Class IV (HDAC 11) (63). Classes I and II are the typical HDACs whose activities can be inhibited by trichostatin A (TSA), class III belongs to the family of nicotinamide adenine dinucleotide-dependent proteins not affected by TSA, and class IV is an atypical category. HDAC inhibitors have long been used to treat epilepsy and to stabilize mood (e.g. valproic acid), and the recently approved vorinostat (SAHA) and romidepsin (FK228) for cutaneous T cell lymphoma (64).

Other Epigenetic Modificatons

In except of above-mentioned three major epigenetic modifications, histone ubiquitination and sumoylation are two other epigenetic means largely producing covalent modification of histones (65, 66), and histone phosphorylation stimulates histone acetyltransferases to promote histone acetylation and subsequently enhance transcription (67). Ubiquitination is considered as a signaling module from which the signal transmitted mainly on the nature of the modification, such as mono- or poly-ubiquitin or the lysine residues onto which the ubiquitin binds (68). Even the ubiquitinated H2A and H2B so far have been reported, but their precise roles are still not elucidated. Histone sumoylation regulated gene silencing by recruiting histone deacetylase and heterochromatin protein 1 (69). Besides, histone ADP-ribosylation, another epigenetic modification, facilitates gene transcription by directly remodeling nucleosomes (70). Although these different types of epigenetic modifications exist to control and mediate the chromatin remodeling processes sophisticatedly, it is really difficult to figure out the intricate crosstalk that occurs amongst them each other.

In sum, from peripheral to central sensitization, from ascending facilitation to descending inhibition, from neurotransmitters to cytokines, from DNA to histone modification to protein expression, all have great potential of finding novel therapeutic targets for the treatment of pain. The combined investigation between pain research and other disciplines like computational science and bioinformatics would provide in-depth insights in clarifying their interactions and finding potential more specific therapeutics.

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