Review

CellPress

Epigenetic mechanisms of chronic pain

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Neuropathic and inflammatory pain promote a large number of persisting adaptations at the cellular and molecular level, allowing even transient tissue or nerve damage to elicit changes in cells that contribute to the development of chronic pain and associated symptoms. There is evidence that injury-induced changes in chromatin structure drive stable changes in gene expression and neural function, which may cause several symptoms, including allodynia, hyperalgesia, anxiety, and depression. Recent findings on epigenetic changes in the spinal cord and brain during chronic pain may guide fundamental advances in new treatments. Here, we provide a brief overview of epigenetic regulation in the nervous system and then discuss the still-limited literature that directly implicates epigenetic modifications in chronic pain syndromes.

Chronic pain: a major clinical and socioeconomic problem

Chronic pain is characterized by persistent nociceptive hypersensitivity [1,2], experienced by patients as a marked reduction in thresholds required to induce pain, such that innocuous stimuli cause pain (allodynia), and an amplification of responses to noxious stimuli at the site of injury (primary hyperalgesia) and surrounding tissues (secondary hyperalgesia) [3]. Chronic pain is often a result of peripheral tissue damage and persistent inflammation (inflammatory pain), or of pathological adaptations in the peripheral or central nervous system (neuropathic pain). In the USA, the number of adults with chronic pain is estimated to be 100 million, with an economic annual cost of up to US$635 billion [4]. Unfortunately, current pain management interventions are insufficient, providing inadequate pain relief and multiple health and societal adverse effects. In a 2006 survey of patients with chronic pain undergoing treatment, over half reported having little or no control over their pain [4]. The ineffectiveness of current therapeutic strategies is at least partly due to an incomplete understanding of the mechanisms involved in chronic pain conditions. The development and maintenance of chronic pain involve long-term changes in multiple areas of the central nervous system (CNS), which are characterized by adaptations at the cellular and molecular levels. Here, we focus on recent studies that highlight the involvement of epigenetic mechanisms in the CNS in chronic pain conditions.

Rodent models of chronic pain

Several animal models have been used to investigate the molecular and cellular adaptations of nociceptive pathways. Inflammatory pain models use subcutaneous injections of inflammatory agents, such as formalin, capsaicin, or complete Freund's adjuvant (CFA), usually into the hind or forepaw, whereas neuropathic pain models typically involve surgical injury of a spinal or peripheral nerve [3,5]. These models result in reliable nociceptive sensitization, mimicking key components of the chronic pain experience in humans (Box 1). In the spinal cord, for example, neuropathic injuries induce long-lasting abnormal neural activity along primary afferent pathways [6], and *in vitro* recordings of dorsal horn (DH) neurons have shown potentiated excitatory responses and decreased firing thresholds in animals with neuropathic pain [7,8]. Moreover, evidence from multiple studies suggests that chronic pain development is correlated with changes in gene expression in the spinal cord [2,9,10] and cerebral cortex [11,12], and activity-dependent changes in gene expression have long been understood to be important for long-term alterations in neural activity [13–15].

Notably, chronic and intense pain can have effects at the level of gene expression in spinal and supraspinal areas located far from the initial lesion and may include brain areas that are not directly associated with the processing of sensory information. A growing number of studies directly implicate alterations in gene expression with the generation of certain chronic pain conditions, such as neuropathic or rheumatoid arthritis pain. However, until recently, the mechanisms by which acute tissue injury and pain induce changes in gene expression for a prolonged period of time remained poorly understood. Epigenetic mechanisms provide a plausible process through which stable changes in CNS activity may manifest in response to peripheral injuries (Box 2).

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Keywords: histone modifications; inflammatory pain; neuropathic pain; HDAC; miRNA; methylation; animal models.

0166-2236/
 http://dx.doi.org/10.1016/j.tins.2015.02.001

Box 1. Chronic pain behaviors

Chronic pain resulting from peripheral tissue damage and inflammation (inflammatory pain), or from pathological adaptations in the peripheral or CNS (neuropathic pain) is characterized by persistent nociceptive hypersensitivity [2,67]. Inflammatory pain models use subcutaneous injections of inflammatory agents, such as formalin or CFA, into the hind paw, fore paw, or masseter muscles, resulting in tissue injury. Neuropathic pain models usually involve surgical injury of a peripheral nerve [3]. These models have yielded much insight into the molecular mechanisms of chronic pain conditions. Chronic inflammatory and neuropathic pain can lead to changes in behavioral responses to tactile and thermal stimuli that are easily detectable in animal models. Experimenters can reliably detect and measure two major forms of sensitized behavioral responses: allodynia, (the sensation of pain to previously innocuous stimuli) and hyperalgesia (the enhancement of painful perception to noxious stimuli) [99,100]. Mechanical allodynia is reliably assessed in animal models and easily detected, because stimuli that would otherwise be innocuous will evoke noxious nociceptive behavior [101]. Mechanical thresholds can be assessed by monitoring the responsiveness of the affected paws to the application of von Frey filaments. This method allows investigators to apply an accurate and constant force to specific areas of the skin. In this manner, von Frey filaments that do not elicit a nociceptive response in naïve mice will elicit nociceptive responses in animals with mechanical allodynia [67,102]. Thermal hyperalgesia can be measured through enhanced behavioral responses to thermal stimuli. The Hargreaves test is a common assay, where a high-intensity heat lamp is directed to the mid-plantar hind paw of the injured limb [103]. Thermal allodynia can also be observed in the cold plate test, where animals with chronic pain will react to cold stimuli that are below the nociceptive threshold [104]. These represent the most commonly used tests; however, several other assays are also used to assess nociceptive behavior or sensory deficits. Typical behavioral responses to noxious stimuli include licking, flicking, or quick withdrawal of the affected paw. These phenomena reflect similar sensitization of pain seen in humans [1], and may correspond to changes of synaptic transmission in the spinal cord DH [8,40,54] or pain-related brain areas [67,95,98].

Epigenetic mechanisms drive long-lasting cellular and behavioral changes

Epigenetic mechanisms enhance or suppress gene expression without alterations of the primary DNA sequence. Epigenetic mechanisms have been shown to be involved in synaptic plasticity, learning, and memory [16,17], as well as in several neuropsychiatric disorders, including depression and drug addiction [18–20]. These mechanisms include DNA methylation, several types of histone modification (e.g., acetylation, methylation, phosphorylation, and ADP ribosylation), and expression of miRNAs. Epigenetic mechanisms can be dynamic and responsive to changes in experience, thus representing a complex interplay between an organism and its environment [21,22]. Acetylation of most histone subunits, at any of several Lysine (Lys) residues, typically promotes gene transcription, while histone methylation can either repress or activate gene transcription depending on the amino acid residue undergoing methylation. For example, methylation of Lys9 or Lys27 of histone H3 is usually associated with gene repression, whereas methylation of Lys4, Lys36, or Lys79 of H3 is usually associated with gene activation [23]. Likewise, 5-methylation of cytosine nucleotides in DNA (which occurs in part at CpG dinucleotides) generally mediates gene repression, while 5-hydroxymethylation of cytosine produces the opposite effect. However, chromatin modifications do not occur in isolation, and we know from work in single cell systems that the activation or repression of a gene typically involves numerous histone and DNA modifications, and the recruitment of perhaps hundreds of chromatin regulatory proteins to the regulated gene.

DNA and histone methylation in spinal systems and their involvement in chronic pain

A widely recognized mechanism through which DNA methylation controls transcription, is through suppression of transcription factor binding at promoter regions by a complex comprising a DNA methylation-dependent DNA binding protein, such as methyl-CpG-binding protein 2 (MeCP2), or methyl DNA-binding domain (MBD) proteins. MeCP2 is best known as a transcriptional repressor that has been implicated in the modulation of activity-dependent gene expression [24]. Specifically, MeCP2 inhibits transcription of specific genes by binding to methyl-CpG sites in the DNA [25]. Phosphorylation of MeCP2 results in its removal from methylated CpG promoter regions and, thus, can lead to the enhancement of gene expression

Box 2. How epigenetics can mediate chronic pain

Epigenetics refers to the long-lasting changes in gene expression that, although do not alter the actual DNA sequence, can result in functional alterations of cellular activity [16,22,105]. Neurons within the CNS undergo epigenetic modifications in response to an organism's experience with the external environment [106]. DNA is tightly wound around histones, forming a complex called chromatin. Gene expression is dependent on transcription factors accessing promoter regions on DNA sequences, a process that is regulated by epigenetic modifications to the chromatin structure. Epigenetic mechanisms include DNA methylation, several types of histone modifications (e.g., acetylation, methylation, phosphorylation, ubiquitination, and ADP ribosylation), and expression of miRNAs [22]. These adaptations can modify neuronal morphology and activity to produce changes in behavior [106]. Indeed, epigenetic mechanisms have been shown to be involved in synaptic plasticity, learning, and memory [16,17], as well as in the pathophysiology of several neuropsychiatric disorders, including depression and drug addiction [18–20]. Moreover, recent studies have found multiple epigenetic modifications in spinal and brain regions in correspondence with chronic pain conditions. Mounting evidence indicates that epigenetic modifications contribute substantially to sensitized behavioral responses to mechanical and thermal stimuli [30,86,95,107]. These modifications may occur in primary afferent, DH, or spinothalamic tract neurons, and in any of several brain regions of the pain matrix. For example, epigenetic mechanisms could alter receptor expression levels at neuronal synapses in the superficial DH [43], and this effect could augment primary afferent activation of spinal systems, facilitating the development of hyperalgesia. Similarly, epigenetic changes in top-down regulatory brain regions (such as the ACC, PFC, PAG, and RVM) [82] could lead to facilitation of stimuli induced activation, perhaps paving the way for the development of allodynia or other sensory deficits. Epigenetic changes may also occur in interneurons [86], causing disinhibition of critical nociceptive pathways that may potentiate allodynia, or even promote hyperalgesia. Glial cells, namely astrocytes in the spinal cord [30] and brain [94], can also undergo injury-induced changes in gene expression that are mediated by epigenetic mechanisms and potentially contribute to pain syndromes. Thus, epigenetic mechanisms present dynamic processes for controlling changes in neuronal activity and behavior that may be responsible for the persistent manifestation of a chronic pain state.