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Current Perspective

HMGB1 as a Potential Therapeutic Target for Neuropathic Pain

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Abstract. Neuropathic pain, which is intolerable and persistent, arises as a direct consequence of a lesion or disease affecting the somatosensory system and can be debilitating for the affected patients. Accumulating evidence from animal studies has revealed the potential molecular basis for neuropathic pain, resulting in many promising therapeutic targets. While efforts at drug discovery have been made, conventional pharmacotherapy, including the use of opioid analgesics, is still insufficient for the relief of neuropathic pain. Therefore, novel target molecules that may lead to the development of promising analgesics are eagerly anticipated for improved treatment of neuropathic pain. In various insults such as sepsis and ischemia, high-mobility group box 1 (HMGB1) is released extracellularly to induce inflammation. HMGB1 was originally identified as a ubiquitous nuclear protein, but emerging evidence has suggested that HMGB1 also plays a role in neuroinflammation as a pro-inflammatory mediator. These findings suggest that HMGB1 may be involved in the pathology of neuropathic pain. In fact, some reports demonstrate an involvement of HMGB1 in the development and maintenance of neuropathic pain in experimental animals. Here, we overview the characteristics of HMGB1 as a pro-inflammatory mediator and show the promise of HMGB1 as a therapeutic target for neuropathic pain.

Keywords: HMGB1, neuropathic pain, neuroinflammation, pro-inflammatory cytokine, nucleokine

1. Introduction

Pain is physiologically significant in that it allows us to detect potentially damaging stimuli, providing an essential early warning system. Peripheral tissue injury or inflammation can produce reversible adaptive changes in the sensory nervous system, such as hyperalgesia, that play a protective role against further nociceptive stimuli, resulting in the promotion of wound healing and the subsidence of inflammation. In contrast, neuropathic pain includes nervous system injury and persistent alterations in pain sensitivity; pain may occur spontaneously and its threshold may fall dramatically, such that innocuous stimuli produce pain and the duration and amplitude of the response to noxious stimuli are amplified. Neuropathic pain is refractory, and many existing analgesics, such as opioids, are less efficacious at relieving neuropathic pain than nociceptive or inflammatory pain. Therefore, neuropathic pain severely compromises quality of life in affected patients. The pathophysiological mechanisms underlying the development of neuropathic pain have been studied intensively. Neuroinflammation, based on alterations in the crosstalk between the nervous and immune systems, has been an intimate focus of attention as a pathological mechanism involved in the development of neuropathic pain (1, 2). A great deal of effort has been made to develop medicine that targets molecules involved in neuroinflammation, and promising novel therapeutic targets have been identified. Nevertheless, there exist only a limited number of alternative analgesics leading to effective treatments for neuropathic pain. The identification of novel molecular machinery involved in neuropathic pain may lead to the development of promising analgesics, and is therefore eagerly anticipated. A decade ago,

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Invited article

high-mobility group box 1 (HMGB1) was identified as a potent inflammatory mediator in various diseases caused by an abnormally enhanced inflammatory immune response (3). Growing recent evidence has led to the hypothesis that HMGB1 plays an important role in inducing neuropathic pain in experimental models. Here, we review the progress in the field of HMGB1 biology and the promise of HMGB1 as a therapeutic target for neuropathic pain.

2. HMGB1 and inflammation

HMGB1, a non-histone DNA-binding molecule, was first discovered as a nuclear protein that stabilizes nucleosomes and allows the bending of DNA to facilitate gene transcription. Since the discovery of its alternative function as a delayed lethal mediator in sepsis (4), emerging evidence has shown that HMGB1 is a potent pro-inflammatory mediator when extracellularly present in various organs (3). There are two major pathways of HMGB1 secretion. Passive release involves instantaneous secretion from cells infected by pathogenic organisms or necrotic cells (5). In contrast, active secretion of HMGB1, initiated by cellular signal transduction through the interaction of plasma membrane receptors with extracellular products, occurs more slowly (6). The machinery underling active release is intriguing because the primary amino acid sequence of HMGB1 does not include a signal peptide sequence. This fact suggests that active release does not appear to work in the manner of the classical ER-Golgi secretory pathway, although its alternate mechanism remains poorly understood. One possibility is that acetylation of lysine residues within the nuclear localization sequence of HMGB1 leads to sequestration of HMGB1 within cytoplasmic vesicles and its subsequent release (6, 7). Active release is modulated by inflammatory mediators. Both early pro-inflammatory cytokines (e.g., tumor necrosis factor, TNF) and late pro-inflammatory mediators (e.g., inducible nitric oxide synthase, iNOS) may contribute to HMGB1 release (8, 9). These facts are intriguing in regard to a pathological role of HMGB1, since they suggest that HMGB1 could work at both early and late stages of inflammatory disease.

HMGB1 acts as an alarmin to orchestrate inflammatory responses (10) including the stimulation of cell migration, activation of various innate immune cells, and the suppression of phagocytosis of apoptotic cells during inflammatory events. In this way, HMGB1 functions to recruit, alert, and activate innate immune cells, thereby sustaining a potentially injurious inflammatory response during injury. The kinetics of systemic HMGB1 levels vary in a variety of diseases. In experimental sepsis, HMGB1 is a late mediator; early pro-inflammatory cytokines reach plateau levels in the circulation in a few hours, with HMGB1 reaching peak levels only subsequently, which parallels septic lethality (4). In contrast, ischemic injury elicits a rapid release of HMGB1 from injured cells, which accumulates in the circulation within a few hours and subsequently triggers a potentially injurious innate immune response (11). The revealed pathological role of HMGB1 indicates that cross-talk between pro-inflammatory cytokines and HMGB1 may prime and prolong inflammatory disease. These facts also indicate that HMGB1 may prolong persistent pain states in the pathology of chronic pain due to neuroinflammation.

3. HMGB1 and neuropathic pain

More than 10 years have passed since the discovery of the pro-inflammatory properties of HMGB1, yet there are fewer reports on the relationship between neuropathic pain and HMGB1 than might be expected (Table 1). The initial report showed that exogenously administered HMGB1 induces neuropathic pain-like behavior in rodents. Chacur et al. demonstrated that HMGB1 focally injected around a single sciatic nerve created ipsilateral mechanical allodynia, but no apparent thermal hyperalgesia, over the 24 h after injection in rats (12). Intriguingly, a higher dose of HMGB1 induced bilateral hind paw allodynia, as indicated by the presence of "mirror" pain (13), although its induction mechanism remains unclear.

It is of clinical significance that HMGB1 could be

Table 1. Protective effects of HMGB1-inhibiting agents in animal models of pain

Agents	Animal model of pain	Inhibited pain	References
Anti-HMGB1 antibody	Spinal nerve injury	Mechanical allodynia	(14)
Anti-HMGB1 antibody	Disc herniation	Mechanical hyperalgesia	(15)
Anti-HMGB1 antibody	Type 2 diabetes	Mechanical allodynia	(16)
Anti-HMGB1 antibody	Bone cancer	Mechanical allodynia	(17)
Glycyrrhizin	Tibial nerve injury	Mechanical hyperalgesia	(18)

involved in pathophysiological rather than physiological pain. Shibasaki et al. showed that HMGB1 was induced in the peripheral nerve in response to nerve injury and suggested that it contributed to the development of pain hypersensitivity, as revealed by anti-HMGB1 antibody treatment in the neuropathic pain model (14). In addition, immunohistochemical studies demonstrated that HMGB1 levels were upregulated in satellite cells and neurons of the dorsal root ganglia (DRG). Thus, HMGB1 may be released from neurons and satellite cells after nerve injury.

Neuropathic pain occurs in various painful syndromes. Lumbar disc herniation is one of the most common causes of lower back pain and sciatica, and it is characterized by neuropathic pain. The application of autologous nucleus pulposus onto nerve roots also induces neuropathic pain-like behavior in rodents and is used as a model for lumbar disc herniation. In this model, even without upregulation of HMGB1 in DRG autografted with nucleus pulposus, application of an anti-neutralizing antibody reduced the upregulation of TNF-α expression in the DRG and improved mechanical hyperalgesia (15).

Diabetic neuropathy, the primary cause of peripheral neuropathy, is one of the most common chronic complications of type 2 diabetes and may in some cases include neuropathic pain. In a study on the involvement of HMGB1 in mechanical allodynia in db/db mice, a model of type 2 diabetes (16), the development of mechanical allodynia in db/db mice was associated with the upregulation of HMGB1 protein in the spinal cord. An intrathecal injection of neutralizing antibody against HMGB1 inhibited mechanical allodynia. mRNAs of inflammatory mediators, such as TNF-α, IL-1β, IL-6, and monocyte chemoattractant protein-1 (MCP-1) were upregulated together with GFAP protein in the spinal dorsal horn of db/db mice, effects that were attenuated by intrathecal injection of the neutralizing antibodies. These results led to the hypothesis that a positive feedback circuit exists between HMGB1 and pro-inflammatory cytokines, which may enhance the effect of HMGB1 on diabetic pain and make treatment by current clinical therapies more difficult. In addition, the analgesic effect of blocking HMGB1 in both dose- and time-dependent manners may provide a novel treatment for diabetic pain.

Understanding the pathology of bone cancer pain is more complicated than for peripheral nerve injury–induced pain because it can include nociceptive, inflammatory, and neuropathic pain. Bone cancer pain is the most common source of pain in patients with bone sarcomas and bone metastasis, and causes excruciating and substantially life-altering pain. Interestingly, it is possible that HMGB1 has a universal relationship with various modes of pain and not just with neuropathic pain. A previous study investigated whether HMGB1 contributes to mechanical allodynia in bone cancer pain. In this work, Tong et al. used rats receiving an inoculation of carcinoma cells to the tibia in order to examine the involvement of HMGB1 in the expression of bone cancer pain (17). HMGB1 was increased in the spinal dorsal horn of bone cancer rats, and this was found to correlate with mechanical allodynia. Blocking spinal HMGB1 reversed bone cancer pain via downregulation of the tumor inoculation-enhanced expression of IL-1β. These results indicate that upregulated HMGB1 enhances spinal IL-1β expression and thereby modulates spinal excitatory synaptic transmission and pain responses.

More recently, a variety of approaches including ex vivo and in vitro techniques have been used to study the mechanisms underlying the development of neuropathic pain. Feldman et al. used a tibial nerve injury (TNI) model to reveal the mechanism by which HMGB1 contributes to neuropathic pain (18). TNI induced redistribution of HMGB1 from the nucleus to cytoplasm of sensory neurons in rat DRG and cell lines, without changing total protein content, a finding that is consistent with the release mechanism of HMGB1 proposed in other cell types (19). We also found increases of extracellular HMGB1 content within a few hours of in vivo partial ligation, dissection, and subsequent incubation in culture medium of the mouse sciatic nerve (T. Maeda et al., unpublished data). Although not yet known, the mechanism of HMGB1 release in injured sensory neurons could involve the phosphorylation of cytoplasmic HMGB1 by a serine/threonine protein kinase, as occurs in other cell types (20). In vitro experiments revealed that HMGB1 was released in a cell excitability-dependent manner and that application of HMGB1 increased the number of action potentials and intracellular calcium concentration in DRG neurons dissociated from TNI animals. These results strengthen the possibility of HMGB1 involvement in the development of chronic pain, as suggested by the in vivo experimental reports described above. Furthermore, these findings include the mechanism underlying HMGB1-induced or HMGB1-enhanced pain: HMGB1 may be released from sensory nerve tissue in response to injury and may sensitize the sensory nerve to nociceptive stimuli.

4. HMGB1 receptor and neuropathic pain

Toll-like receptors (TLRs) are germ-line encoded pattern recognition receptors that initiate innate immune responses via recognition of the molecular motifs of pathogens, known as pathogen-associated molecular patterns. TLR family members have been identified in spinal microglia and primary sensory neurons. Engage-

ment of TLRs initiates intracellular signaling pathways, leading to the synthesis and secretion of various inflammatory cytokines and chemokines. Therefore, emerging evidence indicates that TLRs and their associated signaling components contribute to pain hypersensitivity, and blockade of TLR signaling has been shown to reduce pathological pain (21). Because some members of the TLR subfamily are believed to function as receptors for HMGB1, HMGB1 could affect neuropathic pain through amplifying and maintaining the inflammatory response via the TLR pathway. In fact, nerve injury–induced neuropathic pain is impaired after deletion or inhibition of TLR2 and TLR4 (22 – 25), while application of a TLR9 antagonist blocked tumor-induced thermal hyperalgesia (26). There are currently no reports on how neuropathic pain is affected by HMGB1-induced activation of TLRs or by blockade of the HMGB1-TLR interaction. More studies are required to confirm whether the HMGB1–TLR interaction is directly involved in the pathogenesis of neuropathic pain.

RAGE, the receptor for advanced glycation end products, is an integral membrane protein of the immunoglobulin superfamily and is expressed in a wide range of tissues including the peripheral sensory pathway. AGEs, advanced glycation end products, are well-studied ligands for RAGE, and HMGB1 is believed to be one such ligand. RAGE is reportedly present in the DRG and associated with the activation of the immune system in primary afferents following nerve injury (27) or diabetic neuropathy (28). Downstream events following RAGE activation in the DRG neurons are involved in oxidative stress and caspase activation (29), while reactive oxygen species and caspase activation are considered to be important components in the development of chronic pain. A study on the relationship between RAGE and pain is that the RAGE-NF-κB axis operates in the development of functional sensory deficits in diabetic neuropathy (30). This study suggests that AGE–RAGE interaction induces oxidative stress to increase the thermal nociceptive threshold in a diabetic model, but makes no mention of its importance or otherwise to the development of neuropathic pain. Because RAGE is involved in various age-related diseases, such as Alzheimer's disease, cancer, cardiovascular disease, and diabetes, RAGE is an interesting subject for future studies on the relationship between HMGB1 and pain.

5. Perspectives

Seemingly unrelated conditions such as sepsis, ischemic injury, and neuropathic pain may converge on inflammation that is orchestrated by various inflammatory mediators. All the studies mentioned above have consistently indicated that HMGB1 is a pro-inflammatory mediator of the development of chronic pain, including neuropathic pain. Despite recent progress and growing interest in understanding the critical roles of other pro-inflammatory cytokines signaling in pain, there are still only a few reports on in vivo experiments for HMGB1, and even fewer on the pathological mechanism evaluated by either ex vivo or in vitro tests. Many questions thus remain unanswered. First, which receptors are stimulated by HMGB1 released upon injury or inflammation of nerve tissues? Moreover, what are the specific contributions of the receptors to neuroinflammation in persistent pain? These questions are important for a therapeutic approach to pain relief based on blocking the receptor for HMGB1. Second, what is the molecular and cellular mechanism underlying HMGB1-induced sensitization and allodynia in a pain modality-specific manner? It is especially important to identify which types of cells have the HMGB1-activated receptor required for the pathogenesis of chronic pain and what the pathological role of HMGB1-expressing cells is in the development of chronic pain. Given the important role that identified HMGB1 receptors and the cells expressing those receptors have in pain, targeting them may offer new treatments for debilitating and refractory pain.

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