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The Role of NDRG2 in the Chronic Pain Process

Yue Hao[1], Zhaoshuang Li[1], Xinya Wen[1], Jin Zheng[2,*]

[1]Shaanxi University of Chinese Medicine, Xianyang 712046, Shaanxi, China
[2]The Second Affiliated Hospital of Air Force Military Medical University, Xi'an 710000, Shaanxi, China
**Correspondence Author*

Abstract: ***Chronic pain is an important physiological response of the organism that involves a variety of molecules and cells, among which astrocytes play an important role in the development of chronic pain. NDRG2, a member of the N-myc downstream regulatory gene family, is expressed in astrocytes of the central nervous system and is involved in physiological functions affecting apoptosis, astrocyte activation, blood-brain barrier integrity and glutamate clearance regulation of glutamate clearance and other physiological functions. In this paper, we focus on the involvement of NDRG2 in the regulation of chronic pain by affecting the function of astrocytes, and discuss whether NDRG2 can be used as a potential target for pain treatment, taking into account the existing studies.***

Keywords: NDRG2, Astrocyte, Chronic pain**.**

1. Introduction

Pain is an unpleasant sensory and emotional-emotional experience associated with or similar to actual or potential tissue damage [1-2]. In physiologic situations, pain serves to protect the organism by warning it to avoid all types of injurious stimuli and to avoid them in the future. And when pain persists or recurs for more than 3 months it is defined as chronic pain (CP) [3]; chronic pain is characterized by spontaneous pain, nociceptive hypersensitivity, and nociceptive allergy, and its development involves a variety of factors including biological, psychological, and social. According to existing studies, chronic pain has been found to be a huge personal and economic burden, affecting more than 30% of the global population [4]. Therefore exploring to discover the mechanisms that generate and maintain chronic pain is of great significance.

It is well known that astrocytes play an important role in many key neural processes, which include chronic pain generation. It has been found that activated astrocytes induce the generation of chronic pain. When the human peripheral nervous system and central nervous system are injured, neuroinflammation is induced, which is manifested by alterations in astrocytes, release of neuroglial mediators and modulators, increased excitatory inputs and decreased inhibitory inputs, which ultimately leads to chronic pain [5-6]. N-Myc Downstream Regulated Gene 2 (NDRG2) is a member of the NDRG family, and numerous studies have shown that NDRG2 is both a tumor suppressor gene and a stress response gene; NDRG2 has been found to be highly expressed in astrocytes and to play a role in inflammation-associated pain, neuropathic pain, and so on. Therefore, this paper summarizes the research progress of NDRG2 in chronic pain by combing the existing studies and targeting NDRG2, and intends to explore the possibility and potential application value of NDRG2 as a target for pain treatment.

2. Astrocytes and Their Role in Pain Regulation

Central nervous system (CNS) cells include neuronal cells and glial cells, of which glial cells mainly include three types: astrocytes, microglia and oligodendrocytes. Astrocytes accounted for about 20-40% of the total number of the largest number of cell types in the CNS, not only play a supportive, protective and trophic role in neuronal cells, but also regulate glutamate and ionic balance, regulate cholesterol and sphingolipid metabolism, release gliotransmitters and regulate synaptic plasticity. They not only support, protect and nourish neuronal cells, but also regulate glutamate and ion homeostasis, cholesterol and sphingolipid metabolism, release gliotransmitters and synaptic plasticity, release neurotrophic factors and promote neurogenesis, and play important roles in many physiological and pathological processes in response to complex environmental factors [7,8]. In cases of tissue injury or disease, astrocytes, which are otherwise quiescent, are activated and, because of their anatomical close contact with neurons and synapses, play an important role in signaling and processing and are involved in a wide range of neuropathological changes, including chronic pain [10,11].

Astrocytes are activated by different stimuli and transformed into a reactive state characterized by morphological, molecular, and functional changes, and various reactive molecules induce pain hypersensitivity. This reactive state is known as astroglial reaction, which usually refers to the up-regulation of Glial fibrillary acidic protein (GFAP) expression, and is mostly accompanied by morphological changes of astrocytes, such as enlarged cytosol and thickened protrusions, etc. In this state, astrocytes are activated and transformed into a reactive state characterized by various morphological and molecular changes. Astrocytes in this state are activated astrocytes or reactive astrocytes [6]; of which GFAP is a marker of activated astrocytes [7]. Astrocyte activation can occur in the spinal cord and supraspinal centers of the central nervous system; peripheral nerve injuries and spinal cord injuries can also cause strong astrocyte activation in the spinal cord or medulla oblongata; astrocytes are activated by inflammation or peripheral nerve injuries and induce spinal cord neuronal hyperactivity by releasing various molecules, resulting in altered neuronal plasticity leading to chronic pain [12].

2.1 Intracellular Kinases

The mitogen-activated protein kinases (MAPK) signaling pathway, a highly conserved tertiary kinase pattern involved

in a variety of cellular physiological and pathological processes, consists of a cascade of sequentially activated protein kinases. MAPK is a downstream member of the signaling pathway, including three canonical protein kinases: extracellular signal regulated kinases (ERK), p38MAPK, and c-Jun N-terminal kinase (JNK) [13]. MAPK is activated in response to a variety of extracellular stimuli, and through a cascade reaction of phosphorylation and dephosphorylation at two phosphodiesterase sites, it converts the extracellular stimulus signals into a signal. MAPK is activated in response to various extracellular stimuli, and through a cascade of phosphorylation and dephosphorylation at two sites of the phosphodiesterase, MAPK converts extracellular stimulus signals to the nucleus, generating a biological effect and transducing signals from the cell surface to the nucleus. The MAPK family has been found to play an important role in the regulation of pain [6,14].

p38 MAPK can be activated in neuronal cells and glial cells in different pain states. activated p38 MAPK translocates to the nucleus and regulates the transcription of genes, and the protein products of these genes contribute to peripheral and central sensitization, thus playing an important role in the onset and/or maintenance of pain [15]. Crown ED et al. found that p38 MAPK activation was increased in spinal cord injury (SCI) and developed horizontal mechanical allodynia in rats; Crown ED et al. found that p38 MAPK activation was increased in spinal cord astrocytes, microglia, and dorsal horn neurons at the site of injury; and that inhibition of the enzymatic activity of p38 MAPK dose-dependently reversed the manifestation of horizontal mechanical allodynia in rats [16]. Pradal J et al. used the method of antigen-induced arthritis (Antigen Induced Arthritis (AIA) mouse model and injected the highly selective p38 MAPK inhibitor VX-745 into its joint cavity, the model mice did not show signs of knee inflammation such as swelling or joint stiffness [17]. Ni et al. found that chronic constriction nerve injury (CCI) was induced by sciatic nerve ligation in SD rats [18]. nerve injury (CCI) in SD rats and found that astrocytes were activated after CCI; whereas the behavioral responses of paw withdrawal threshold (PWT) and paw withdrawal latency (PWL) were attenuated after injection of astrocyte inhibitors; injection of phosphorylated p38 mitogen-activated protein kinase (p-p38 MAPK) inhibitor SB 203580 inhibited microglia activation [18]. Luo et al. gave chronic sciatic nerve ligation (chronic constriction injury (CCI)) model mice intrathecal injections of p38 MAPK isoform-specific antisense oligonucleotides (antisense oligonucleotides (ASOs) attenuated mechanical nociceptive hypersensitivity in CCI model mice; intrathecal injection of p38αASO also attenuated mechanical and cold nociceptive abnormalities after tibial fracture surgery in mice [19]. Choi et al. found that mechanical nociceptive hypersensitivity was observed in CCI model mice and that neurosteroid metabolizing enzyme cytochrome 17α-hydroxylase (P450c17) expression was increased; intrathecal administration of ketoconazole or the p38 MAPK inhibitor SB203580 significantly inhibited CCI-induced mechanical nociceptive hypersensitivity as well as the level of p38 phosphorylation; and ultimately concluded that the activation of P450c17 in spinal cord astrocytes via p38 phosphorylation in astrocytes ultimately led to the development of abnormal mechanical development of pain [20]. Thus, inhibition of the p38 MAPK signaling pathway is one of the mechanisms that exert analgesic effects.

ERK signaling plays an important role in pain perception and uses phosphorylated ERK (pERK) as a biomarker of activated cells [21]. ERK activation is involved in pain signaling by regulating gene expression, leading to central sensitization of the nervous system and pain hypersensitivity [22]. Previous studies have found that enhanced ERK activation in astrocytes occurs after spinal nerve ligation (SNL) [23]. ERK is activated in glial cells (e. g. microglia, astrocytes) and neurons, which are involved in various types of pain [24]. Thus ERK is also now recognized as an important molecule in pain signaling.

Phosphorylated JNK (pJNK) also occurs in astrocytes and neuronal cells in the dorsal root ganglia and is able to regulate neuronal function, immune response, and embryonic development. Hanwool Park et al. in streptozotocin (STZ)-induced diabetic neuropathic pain rats found elevated levels of pJNK protein expression in neurons and astrocytes, and immunohistochemical analysis showed increased pJNK immunoreactivity; curcumin is known to reduce postoperative nociceptive hypersensitivity and attenuate diabetic neuropathic pain, and curcumin 50 mg/kg administered continuously for 4 weeks reduces foot-licking and tail-shrinking responses and decreases the elevated expression of pJNK in STZ-induced model rats [25]. Blanton et al. used the JNK inhibitor SU3327 in a formalin-induced mouse pain model, which produced strong antinociceptive effects [26]. JNK is essential for chronic inflammatory pain and can be persistently activated in the spinal cord after nerve injury.

2.2 Transporters

Astrocyte glutamate transporter-1 (GLT-1) or excitatory amino acid transporter2 (EAAT2) is one of the high-affinity glutamate transporters/excitatory amino acid transporters (EAATs). glutamate transporters/excitatory amino acid transporters (EAATs). Glutamate is the predominant excitatory neurotransmitter in the central nervous system and is essential in maintaining normal nervous system function, and its neurotoxic accumulation induces synaptic dysfunction and is involved in the induction and maintenance of pain [27]. Glutamate transporters located in the astrocyte membrane are responsible for sequestering most of the glutamate released from synapses, removing and inactivating glutamate accumulated in synaptic gaps and extracellularly; under physiological conditions, extracellular glutamate is kept at a low level by uptake by neighboring astrocytes [28]. Five isoforms of high-affinity glutamate transporters have been identified, namely EAAT1, EAAT2, EAAT3, EAAT4, and EAAT5; of these, EAAT1 and EAAT2 are mainly located in astrocytes, and the clearance of extracellular glutamate is largely dependent on them. It has also been found that EAAT1 on the astrocyte cytosol is more active than glutamate transporters located on other neuronal cells [29]. Action potentials in presynaptic neurons stimulate the release of glutamate into the synaptic gap and the stimulation of pre- and postsynaptic glutamate receptors; glutamate diffuses from the synapse and is taken up by astrocytes via the EAATs. EAAT2 is responsible for most of the glutamate uptake in the CNS [30]. In the spinal cord of a rat model of spared nerve injury (SNI)-induced spinal cord, the expression of GLT-1 and

GLT-1 mRNA is down-regulated [31]. The down-regulation of GLT-1 often occurs prior to or concomitant with nociceptive sensitization; the administration of drugs to inhibit or knockdown spinal GLT-1 either induces or exacerbates the pain, and the enhancement of GLT-1 via viral gene transfer to enhance GLT-1 expression can alleviate chronic pain [32]. Xiong et al. explored the relationship between GLT-1 and pain behavior using a rat trigeminal neuralgia model. The results suggested that the application of gap junction protein 43 (connexin-43, Cx43) blocker Gap26 group rats compared with the model group rats, Cx43 expression was significantly reduced and accompanied by up-regulation of GLT-1 expression, and the pain behavior of the experimental animals was significantly reduced; the application of GLT-1 agonist ceftriaxone group rats compared with the model group rats, the expression of GLT-1 was significantly up-regulated, and the pain behavior was called other groups significantly reduced. behavior called other groups significantly reduced. This suggests that Cx43 can affect the expression of GLT-1 in rats and lead to corresponding behavioral changes, and it is that Cx43 is involved in pain behavioral changes in rats with trigeminal neuralgia by regulating the expression of GLT-1 [33]. Glutamate aspartate transporter (GLAST), also known as excitatory amino acid transporter-1 (EAAT1), similarly takes up glutamate from extracellular sources and aborts glutamatergic transmission. In an experiment exploring the effects of spinal electrical stimulation on a rat model of diabetic neuralgia, it was found that the mechanical foot-shrinking response threshold of the experimental animals was significantly elevated and their spinal glial cell GLT-1 and GLAST mRNA expression and protein content were significantly increased after administration of spinal electrical stimulation [34].

2.3 Release of Substances

Inflammatory mediators released by astrocytes are also involved in pain modulation, including macromolecular mediators such as: cytokines TNF-α, IL-1β, chemokines CCL2, CXCL1, growth factors, and proteases; and small molecule mediators such as: glutamate, ATP, D-glycine, and prostaglandin E2 These glial mediators are able to modulate neuronal and synaptic activity and pain sensitivity [35].

Cytokines and chemokines are secreted proteins that regulate the immune response and control the transport of immune cells, which play a role in the pathogenesis of chronic pain. Tumor Necrosis Factor alpha (TNF-α) is a pro-inflammatory cytokine involved in the body's inflammatory and immune responses and belongs to the ligand/receptor protein (TNF) superfamily, which is usually released by activated astrocytes and microglia when nerve injury occurs [36]. Zheng et al. induced mechanical allodynia by systemic injection of 2', 3'-dideoxycytidine (ddC, one of the nucleoside reverse transcriptase inhibitors) in male Sprague-Dawley rats, and found that ddC induced the overexpression of mRNAs and proteins of GFAP and TNF-α in the dorsal horn of the spinal cord; the ddC-induced mechanical allodynia could be blocked by inhibiting the TNF-α signaling. Subsequent intrathecal injection of neuroglial inhibitors also reversed the production of ddC-induced nociception [37]. This suggests that TNF-α is involved in the induction of pain. Interleukin-1beta (IL-1beta) is also expressed in microglia and astrocytes, and its expression is up-regulated in astrocytes in chronic pain. IL-1beta activates IL-1 receptors expressed by nociceptive neurons, activating the MAPK pathway and sensitizing the neurons [38]. IL -1 β has been implicated in the induction of nociception in different types of chronic pain such as inflammatory pain, neuropathic pain, and astrocyte expression is upregulated in bone cancer pain. In addition, activation of astrocytes with the help of optogenetics also leads to increased secretion of IL-1β and TNF-α [39]. Both IL-1β, TNF-α can cause phosphorylation of p38 MAPK in neurons and glial cells, which promotes the development of neuropathic and inflammatory pain [40].

Originally recognized as regulators of peripheral immune cell migration, chemokines are expressed in neurons and glial cells of the central nervous system. In recent years, a growing number of studies have revealed the expression, distribution, and function of chemokines in the spinal cord under chronic pain conditions. Neurological injury or inflammation can promote the expression of chemokines and their receptors by astrocytes, which bind to the receptors to activate primary sensory neurons and immune cells, inducing the release of inflammatory mediators, and thus participating in pain regulation [41]. In a rat model of bone cancer pain with persistent mechanical pain, the expression of phosphorylated NFκB, chemokine ligand 1 (C-X-C motif chemokine ligand 1 (CXCL1)), and its main receptor chemokine receptor 2 (CXCR2) was increased in the ventral cortex of the brain; microinjection of the NF-κB inhibitor BAY11-7082 reduced pain and decreased pain in rats in the model. rats and reduced the expression of CXCL1 in the spinal cord; microinjection of CXCL1-neutralizing antibody 6 to 9 days after the injection significantly reduced mechanical allodynia. In addition, injection of CXCL1 into the cortex of normal rats induced nociceptive hypersensitivity [42]. WANG et al. also demonstrated that CXCL1 is expressed in spinal cord astrocytes, and that CXCL1 and CXCR2 contribute to the development and maintenance of neuropathic pain in the spinal nervous system [43]. Further studies showed that CXCL1 is expressed in astrocytes and neurons in the dorsal horn of the spinal cord and is involved in the regulation of peripheral and central neuronal sensitization and neuropathic pain through protein kinase C (PKC) [44]. The chemokine CCL2/CCR2 axis plays a key role in nociceptive processing, neuroinflammation, and neuron-glia communication. Dansereau et al. investigated the role of the CCL2/CCR2 system in the dorsal root ganglion (DRG) in peripheral inflammatory nociceptive sensitization. A chronic inflammatory pain model was induced using complete Freund's adjuvant (CFA) and this was found to cause an increase in CCL2/CCR2 expression in the ipsilateral DRG, which was reduced by treatment with the CCR2 antagonist INCB3344; an increase in CCL2 release following peripheral inflammation was demonstrated in DRG explants; pharmacologic inhibition of CCR2 reversed the pronociceptive effects of CCL2 in formalin-injected rats [45].

Zhu et al. used tibial nerve compression in rats as a model of neuropathic pain, in which tibial nerve compression increased CCL2 expression in sensory ganglia, whereas micro sympathectomy decreased CCL2 levels and partially ameliorated mechanosensitization and guarding behaviors,

and the results suggest that neuropathic pain may be mediated to a large extent by influencing CCL2 expression [46]. CCL2 and CXCL1 are expressed in spinal astrocytes and act on CCR2 and CXCR2 in spinal neurons to increase excitatory synaptic transmission from astrocytes to neurons leading to central sensitization [47]. In addition, there are Enzymes Metalloproteases, MMPs, and channel proteins involved in the regulation of chronic pain.

3. Association of NDRG2 Gene with Astrocytes in Pain

3.1 Expression of NDRG2 Gene in Astrocytes

NDRG2 (N-myc down-stream regulated gene 2), a member of the N-myc down-stream regulated gene (NDRG) family, is a gene associated with cell proliferation and differentiation as well as with a variety of cellular stress responses, and is thought to play a role in neural differentiation, synapse formation and axon survival [48].

NDRG2 is specifically expressed in astrocytes of the CNS, widely distributed in their cytoplasm, and co-localized with the astrocyte-specific markers glial fibrillary acidic protein (GFAP) and S100 beta (S100 β), and is even more abundantly expressed than GFAP [49- 51].

3.2 Role of NDRG2 Gene in Astrocytes

The function of NDRG2 in the central nervous system is mainly related to astrocyte activity [49]. In astrocytes, NDRG2 is involved in regulation that affects apoptosis, astrocyte proliferation, blood-brain barrier integrity, and glutamate clearance; several preclinical studies have demonstrated that NDRG2 plays a role in the generation of many neurological disorders [49].

In a diabetic rat model of glucocorticoid-mediated mechanical nociceptive hypersensitivity, NDRG2 expression was found to be upregulated in activated astrocytes; intrathecal injection of the glucocorticoid receptor antagonist RU486 inhibited astrocyte activation as well as overexpression of NDRG2, thereby reversing astrocyte reactivity and diabetic tactile hypersensitivity [52]. Takarada- Iemata et al. suggested that NDRG2 plays a key role in reactive astrocyte proliferation. They found that after cortical stabbing, NDRG2 and GFAP expression was elevated in astrocytes around the area of injury; reduced levels of GFAP expression in astrocytes were found after cortical stabbing using NDRG2 knockout mice, suggesting that NDRG2 plays a key role in astrocyte activation [53]. Ma et al. found that continuous administration of a neuropathic pain model (spared nerve injury (SNI)) rats with elemene attenuated the mechanical nociception and anxiety state of the rats and down-regulated SNI-induced NDRG2 and GFAP expression in the dorsal horn of the spinal cord [54].

In a rat model of neuropathic pain (NP) induced by spinal nerve ligation (SNL), NDRG2 expression was increased in spinal astrocytes; inhibition of NDRG2 expression by intrathecal injection of NDRG2-RNAi-adenovirus significantly alleviated the SNL-induced mechanical and thermal hypersensitivity as well as upregulated the expression of glutamate transporter 1 (GLT-1) and downregulated proinflammatory cytokines in the dorsal horn of the spinal cord of rats at day 10 post-SNL. SNL-induced mechanical and thermal hypersensitivity, as well as up-regulating the expression of glutamate transporter 1 (GLT-1) and down-regulating the levels of pro-inflammatory cytokines in astrocytes in the dorsal horn of the rat spinal cord; intrathecal injection of AG490, an inhibitor of the JAK/STAT3 signaling pathway, significantly attenuated the mechanical and thermal hyperalgesia in rats with overexpression of the NDRG2 gene, inhibited the spinal cord dorsal horn-reactive astrocytes, and restored the normal expression level of GLT-1 in astrocytes. This experiment demonstrated that NDRG2 regulates GLT-1 expression through the JAK/STAT3 signaling pathway, thereby modulating astrocyte activation and inflammatory responses [48]. In cultured astrocytes, NDRG2 gene silencing significantly increased the number of 5-bromo-2'-deoxyuridine (BrdU)-binding cells and proliferating cell nuclear antigen (PCNA)-positive cells, and decreased the length of cell processes and the amount of F-actin. In contrast, adenovirus-mediated overexpression of NDRG2 significantly reduced the number of BrdU-positive and PCNA-positive cells and increased the amount of F-actin; these results suggest that Ndrg2 may regulate astrocyte activation by inhibiting cell proliferation and stabilizing cell morphology [55]. In a study on the expression and role of NDRG2 after stabbing injury to the cerebral cortex of wild-type mice, NDRG2 expression was elevated in astrocytes around the injured area, and knockdown of NDRG2 decreased the induction levels of reactive astrocyte and microglia markers in the injured cortex, and both IL-6 expression and STAT3 phosphorylation were significantly reduced. Wild-type astrocytes treated with forskolin upregulated both IL-6 and GFAP expression; adenoviral-mediated overexpression of NDRG2 reversed the reduction in IL-6 expression after forskolin stimulation [56]. NDRG2 knockout (Ndrg2-/-) mice exhibit symptoms of lacey ADHD such as hyperactivity, impulsivity, and impaired memory, and this deletion of the NDRG2 gene inhibits the astrocyte glutamate clearance, leading to elevated interstitial glutamate levels and increased excitatory transmission. If NDRG2 peptide is used, it reverses the reduced glutamate clearance from astrocytes and reduces excitatory conductance caused by NDRG2 deficiency and can alleviate ADHD-like symptoms in Ndrg2-/- mice [57].

4. Research Prospects

Based on the specific expression of NDRG2 in astrocytes and the important role played by astrocytes in chronic pain, it can be hypothesized that NDRG2 also plays an important role in the development of chronic pain. NDRG2 is involved in neuroinflammation and cellular stress response at this stage and regulates the activation of astrocytes, whereas whether NDRG2 is involved in regulation through the JAK/STAT signaling pathway still requires further study. involved in regulation still requires further studies. In addition to astrocytes, NDRG2 may also interact with other cells or molecules to regulate pain production and maintenance, which need to be investigated urgently.

It is believed that with the exploration of NDRG2, we will be able to find relevant evidence of its role as a therapeutic target

for pain.

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