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Abstract: Chronic pain is among the most disabling and costly disorders, with prevalence ranging from 10% to 55%. However, current therapeutic strategies for chronic pain are unsatisfactory due to our poor understanding of its mechanisms. Thus, novel therapeutic targets need to be found in order to improve these patients' quality of life. PI3K and its downstream Akt are widely expressed in the spinal cord, particularly in the laminae I-IV of the dorsal horn, where nociceptive C and Aδ fibers of primary afferents principally terminate. Recent studies have demonstrated their critical roles in the development and maintenance of chronic pain. In this review, we summarized the roles and mechanisms of PI3K/Akt pathway in the progression of chronic pain through sciatic nerve injury, diabetic neuropathy, spinal cord injury, bone cancer, opioid tolerance, or opioid-induced hyperalgesia.

Keywords: PI3K; Akt; chronic pain

Introduction:

Although physiological pain serves an important protective function, chronic pain can profoundly compromise the quality of life [1-3]. Chronic pain is divided into neuropathic pain, inflammatory pain and cancer pain[4]. Several studies have demonstrated that many pathological processes are characterized by chronic pain, such as sciatic nerve injury, human immunodeficiency virus-associated sensory neuropathy, diabetic neuropathy, spinal cord injury, bone cancer, opioid tolerance, and opioid-induced hyperalgesia. There are many features in chronic pain, including pain in response to normally innocuous stimuli (allodynia), an increased responsiveness to noxious stimuli

(hyperalgesia) and pain experienced in the absence of any obvious peripheral stimulus (spontaneous pain)[5, 6]. The mechanism of chronic pain is extremely complicated including central sensitization and peripheral sensitization [2, 7-10].

Phosphoinositide 3-kinase (PI3K) has been demonstrated to be essential in the development and maintenance of chronic pain[11, 12]. It is a lipid kinase that phosphorylates the D3 position of the inositol ring of phosphoinositide and thereby generates intracellular signaling molecules such as phosphorylation of Akt (pAkt) at Thr308 and Ser473 [13]. The mammalian PI3K signaling family is categorized into three classes (I, II, III) according to their structure and substrate specificity [14, 15]. Class I isoforms have been most extensively studied. Class IA, which consists of PI3Kα, PI3Kβ and PI3Kδ isoforms, is often activated by hormones, cytokines, intergrin's, and growth factors via the tyrosine kinase receptors; while PI3Kγ (the only member of class IB) is activated by G-protein-coupled receptors (GPCRs)[16-18]. It was demonstrated that the PI3Kβ also can been coupled to GPCR [19]. PI3Kδ and γ are mainly expressed in the hematopoietic system and mediate immune responses, whereas PI3Kα and β are ubiquitously expressed and regulate functions such as proliferation and survival. It is known that PI3Kγ can also been found in endothelium[18], heart[17, 20] and brain[21]. Several lines of evidence have shown that activation of PI3K signaling is involved in the modulation of nociceptive information and central sensitization produced by intense noxious stimuli [22-24]. Importantly, PI3K has been reported to mediate central sensitization and hyperalgesia induced by activation of the central RTK system NGF/TrkA, BDNF/TrkB and G-CSF/G-CSFR signaling[25]. Our previous studies revealed that PI3K mediates pain behaviors induced by the activation of peripheral ephrinBs/EphBs signaling in mice [26]. Besides, Akt plays an important role in diverse biological processes through phosphorylation on Thr308 and Ser473, as a key downstream substrate in the PI3K pathway[24]. It is also worth noting that systematic or spinal blocking PI3K with wortmannin or LY294002 prevents the mechanical and thermal hyperalgesia in a dose-dependent manner [27].

PI3K/Akt Pathway and Bone Cancer Pain

Bone cancer pain (BCP) is one of the most severe type of chronic pain [28-30]. It was reported that up to 85% of patients with advanced prostate, breast, and lung cancer had bone metastasis [31-33]. Moreover, one-third of these patients experienced unbearable pain, which severely affected their quality of life. It is now considered that BCP is mechanistically unique compared with neuropathic and inflammatory states [34-36]. In a rat model of BCP, we found that PI3K and its downstream target pAkt were up-regulated in a time-dependent manner and were required for the development and maintenance of BCP [37]. Additionally, PI3K is distributed mainly in the superficial layers of the spinal dorsal horn neurons, astrocytes and a minority of microglia [13, 37, 38]. PI3K pathway is activated by signals at the cell plasma membrane, such as IL-3, nerve growth factor, and insulin-like growth factor[39]. In the model of BCP, the MCP-1 activated the PLC-β2, PI3K, ERK, p38, and Akt by binding to CCR2 [40]. In the central nervous system and peripheral nervous system, the activation of PI3K/Akt signaling pathway can mediate the mechanical and thermal hyperalgesia induced by nerve injury, incision, or inflammation[13, 41, 42]. There is growing evidence that PI3K/Akt signaling pathway plays a critical role in regulating other signaling pathways, such as Raf/MEK/ERK pathway[43, 44], ephrin Bs/EphBs pathway[37], MAPK pathway[45], and MCP-1/CCR2 pathway[46], which contribute to tumor genesis and cancer pain. For example, ERK signaling has been reported to contribute to synaptic and neuronal plasticity, and is involved in the

modulation of peripheral and central sensitization induced by noxious stimuli and nociceptive information[47, 48]. It has been reported that activation of ephrinB2/EphB4 receptor signaling mediates micro vascular endothelial cell and retinal endothelial cell migration and proliferation via the PI3K/Akt pathway [49, 50]. Then the microglia were activated, which contributed to the development of pain [46]. In a subset of prostate cancer cells, it has been demonstrated that Akt can positively regulate the Raf/MEK/ERK pathway at the level of B-Raf [44]. We have found that the activation of spinal chemokine receptor CXCR3 mediates bone cancer pain through an Akt-ERK crosstalk pathway [27]. A large body of evidence suggests that inhibition of PI3K attenuates the mechanical allodynia in BCP rats and can suppress BCP-associated behaviors [27, 51]. In the neural stem cells (NSCs), it has been demonstrated that fluoxetine can modulate the neuroprotection through up regulating expression of the phosphorylated-Akt and ERK1/2. Besides, expression of phosphorylated-Akt and phosphorylated-ERK1/2 in fluoxetine-treated NSCs was effectively blocked by both PI3K inhibitor (LY294002) and MEK inhibitor (PD98059) [43]. In the rats model of BCP, we have found that spinal or peripheral ephrinB1/EphB1 receptor signaling activated PI3K/Akt pathway, accompanied with thermal hyperalgesia and mechanical allodynia [26]. It has been demonstrated that the expression level of p-Akt co-expressed with OX-42 is increased, and decreased after inhibition by the PI3K inhibitor LY294002 in microglia. MCP-1 has been found to stimulate the PI3K/Akt pathway in some kinds of cells (monocytes, HEK-293, COS-7 and PC-3)[52]. The PI3K/Akt signaling expressed in microglia could be activated by MCP-1, which lead to microglial activation and pain [46]. PI3K/Akt signaling pathway not only affect cancer pain, its dysregulation has also been involved in multiple pathological processes of tumor, including tumor genesis, invasion, proliferation, cell cycle progression, apoptosis and metastasis[53].

PI3K/Akt Pathway and Neuropathic Pain

Neuropathic pain is broadly defined as chronic pain that is initiated or caused by a primary lesion or dysfunction in the nervous system and may arise from a spectrum of traumatic insults to the nervous system [54-56]. An increasing number of studies have demonstrated that many factors can contribute to the development of neuropathic pain, such as inflammation [57], and changes in neurotransmission [58, 59]. Neuropathic pain appears in many anomalous situations, including spinal cord injury[60], diabetes, peripheral nerve injury[61, 62] and in some inflammatory conditions[24, 41, 63, 64]. A growing body of evidence has shown that activation of the PI3K/Akt pathway in the spinal cord contributes to hyperalgesia in many neuropathic models [46].

PI3K/Akt Pathway and Sciatic Nerve Injury

According to the previous studies, the rat model of partial sciatic nerve ligation (PSL) was designed to investigate peripheral neuropathic pain65, 66]. In this model, one group of rats were anesthetized and received a unilateral L5 sciatic nerve ligation; but in sham-operated rats, the left L5 spinal nerve was isolated, but without ligation [67].

Studies showed that the activation of PKA, PKC and MAPK signal pathway after peripheral nerve injury plays an important role in regulating the expression of sodium channel subtypes, and neuropeptides in DRG and contributes to the generation of pain-related behaviors[68, 69]. Furthermore, evidence shows that PI3K and Akt are crucial mediators that lead to the activation of the transcription factor nuclear factor κB (NF-κB) induced by interleukin-1(IL-1) and tumor necrosis factor-α (TNF-α)[70, 71] which play a central role in the development of neuropathic

pain[72]. It has been strained that neuroprotection is mediated via a TNFR2-PI3K-Akt-NF-κB pathway in which the duration of NF-κB activation is the critical determinant in mounting resistance toward excitotoxic insults. In accordance with the important role of NF-κB in neuroprotection, it has been demonstrated that upon in vitro glutamate exposure of TNF-treated cortical neurons, a PI3K-dependent Akt phosphorylation was ensued by NF-κB activation [71]. Several lines of evidence indicate that PI3K activation is the upstream of growth factor-induced Akt[73, 74] which is involved in pain hypersensitivity induced by intradermal injection of capsaicin in rats[75], so that PI3K can mediate pain behavior through the Akt signal pathway. In the PSL model, immunohistochemistry work shows that the p-Akt IR-positive neurons in ipsilateral L5 DRG and spinal cord significantly increased, but the significant change was not detected in the contralateral L5 spinal dorsal horn[64, 67]. To investigate the role of PI3K and Akt actvation in the development of neuropathic pain induced by L5 PSL, the PI3K inhibitor wortmannin or LY294002 as well as Akt specific inhibitor Akt inhibitor IV or (-)-Deguelin were injected intrathecally 30 min before surgery and once daily thereafter until the 7th day after L5 PSL. Compared with the control group, in which rats received vehicle injection as above, wortmannin, LY294002, Akt inhibitor IV and (-)-Deguelin treatment significantly reduced mechanical allodynia and thermal hyperalgesia after L5 PSL on the 1st day and 3rd day, but not on the 7th day [67]. In the model of paclitaxel-induced painful peripheral neuropathy, Akt inhibitor Mk-2206 at various doses (1, 10 and 50 nmol) was intrathecally injected 30 min prior paclitaxel treatment for 10 consecutive days. Blocking of Akt1 activation with different inhibitor (MK-2206 or LY294002) attenuated mechanical allodynia and thermal hyperalgesia induced by paclitaxel [76]. The p-Akt is usually referred to as the marker of PI3K activation, which suggests that the PI3K and PI3K-Akt signal pathway might contribute to the development of neuropathic pain at an early stage.

PI3K/Akt Pathway and Diabetic Neuropathy

Diabetic neuropathy occurs in 25% of diabetic patients, and its mechanism remains largely unknown[77]. Diabetic neuropathy are characterized by a progressive loss of nerve fibers affecting both the autonomic and somatic divisions of the nervous system and only a minority are associated with pain[78]. Deficits in nerve growth factor (NGF) production and/or NGF transport in the target tissues of NGF-responsive neurons have been implicated in the pathogenesis of diabetic neuropathy[79, 80]. Previous work showed that a reduced retrograde axonal transport of NGF and neurotrophin-3 (NT-3) in the vagus nerve of diabetic rats occurred in the presence of normal production of neurotrophins and neurotrophin receptors[81]. Several lines of evidence have shown that the interaction between neurotrophins and the tyrosine kinase (Trk) receptor can activate the PI3K/Akt signal pathway which mediates neuron survival, differentiation, axon growth, and protects nerve regeneration[82, 83]. The PI3K/Akt signal pathway located in the distal axon of neurons has a unique role in the retrograde transport of NGF and brain-derived neurotrophic factor (BDNF) in sympathetic, sensory neurons and Moto neurons [84, 85]. Inhibition of PI3K in the distal axons attenuates the retrograde transport of NGF and also induces neuron apoptosis [86]. It has been reported that diabetes decreases the activity of the PI3K and Akt in the vagus nerve, without affecting the protein expression of the p85 subunit of PI3K, Akt and phosphorylation of Akt, but increases the phosphorylation of p70s6 kinase[87]. A growing body of evidence have demonstrated that peripheral noxious insults caused by intraplantar carrageenan or bone cancer lead to increases in phosphorylation of mTOR (p-mTOR) and S6K1 (p-S6K1) in rat spinal dorsal horn but not in

DRGs [88, 89]. In the chronic inflammatory pain and L5 spinal nerve ligation-induced neuropathic pain, western blot analysis showed significantly increased levels of p-mTOR and p-S6K1 [90]. These findings indicate that the impaired PI3K/Akt signal pathway contributes to diabetic neuropathy.

PI3K/Akt Pathway and Spinal Cord Injury

Chronic neuropathic pain and sensory abnormalities are common secondary consequences of spinal cord injury (SCI), affecting 60% of patients with traumatic or ischemic injury [91-94]. SCI pain and associated dysesthesias manifest as at- and below-level neuropathic symptoms that are defined as either spontaneous(pain independent of peripheral stimuli) or evoked (occurring in responses to a noxious or non-noxious stimuli)[95]. It has been found that injury to the spinal cord results in enhanced intrinsic growth and hyper excitability of adjacent peripheral afferents that may contribute to the development of at-level pain syndromes[94, 96, 97].

Recent evidence shows that injury induced upregulation of chemical signals including NGF and Wnts leads to activation of PI3K and the subsequent inhibition of glycogen synthase kinase-3β (GSK-3β) that may positively promote axonal elongation that contribute to the development of SCI pain[98-100]. In the excitotoxic SCI rats model using intraspinal quisqualic acid (QUIS), GSK-3β is inhibited by phosphorylation of the Sre-9 residue and directly promotes neurite outgrowth [101, 102]. Biochemical and immunohistochemical approaches shows a significantly increased level of GSK-3β expression [99]. In addition, QUIS animals treated with LY294002 revealed manifest reductions in neurite formation and elongation compared to the sham-vehicle animals[103]. To demonstrate if alterations in GSK-3β were evident early (3 days), and if application of a GSK-3β activator could reverse these spinal injury induced changes, LY294002 was intrathecally delivered once daily for 3 consecutive days starting on the day of surgery. Results show that short term administration GSK-3β activator (LY294002) prevents the development of at-level spontaneous dysesthesias and reduces DRG outgrowth[103]. Therefore, this indicates that PI3K can mediate the development of neuropathic pain after SCI.

PI3K/Akt Pathway and Inflammatory Pain

Tissue injury is normally associated with inflammation and inflammatory pain. Inflammatory pain is induced by inflammatory mediators released in injured tissue, such as prostaglandin E2, NGF, and bradykinin [104, 105]. It is also well documented that peripheral tissue inflammation or injury causes two changes in the nociceptive system, peripheral sensitization and central sensitization[106]. Furthermore, enhanced synaptic transmission is considered to be essential for central sensitization after inflammatory stimuli [107]. Research demonstrates that PI3K is a key player in the establishment of central sensitization, the spinal cord phenomenon associated with persistent afferent inputs and contributes to chronic pain states in painful inflammatory conditions[41, 108]. Notably phosphorylation of the downstream kinase Akt at threonine 308 (pAkt-T) or at serine 473 (pAkt-S) is used as a marker of PI3K activation [109, 110]. In addition, PI3K regulates secondary messengers that activate various effectors such as Akt and ERK via the generation of PIP3[111]. These results led to the conclusion that PI3K signaling is involved in the modulation of nociceptive information, central sensitization, and synaptic plasticity in the central nervous system [41, 112].

In the inflammatory heat hyperalgesia rat model induced by intradermal injection of capsaicin and NGF, the levels of pAkt-T and pAkt-S significantly increased compared with sham group.

LY294002, a PI3K inhibitor, blocked the increase in pAkt-T and pAkt-S levels in a dose-dependent manner [113]. Furthermore, when LY294002 or wortmannin is injected intradermally before the capsaicin injection, spontaneous pain behaviors, such as lifting and licking the affected paw, were suppressed in a dose-dependent manner. Several lines of evidence suggest an involvement of PI3K-linked cascades in the regulation of synaptic plasticity in CNS [114, 115]. Intraplantar injection of carrageenan produced a persistent thermal and tactile hyperpathia [116]. Our study found that spinal PI3K/Akt mediates pain behavior induced by plantar incision [13]. In addition to peripheral sensitization, evidence shows that spinal mechanisms also play a major role in this model [117, 118]. It has been observed that wortmannin dose-dependently attenuated carrageenan-induced thermal and tactile hyperalgesia, and reversed an established thermal hyperalgesia when given as a post-treatment [89]. Moreover, LY294002 is reported to attenuate the phase I response which represents acute nociceptive processing [41].

PI3K/Akt Pathway and Opioid Tolerance and hyperalgesia

Despite a plethora of available potential treatment options for chronic pain, opioids are still the gold standard for its pharmacological management in the clinical setting. However, long-term use of these drugs is often limited due to the development of opioid tolerance or opioid-induced hyperalgesia(OIH), characterized as progressive loss of analgesic potency after continuous morphine exposure that necessitates dose escalation to achieve equal pain relief [119-123]. μ-opioid receptors (MOR) is a GPCR existing in the superficial dorsal horn of the spinal cord. As mentioned above, PI3Kγ can be regulated GPCR. Recent investigations have revealed that PI3K and Akt can be additional signaling mediators of MOR in sensory neurons. It was identified as a signaling pathway of MOR that involves PI3Kγ, with subsequent stimulation of Akt and neuronal NOS (nNOS) [124]. Additional evidence for a prominent role of PI3Kγ in opioid signal transduction has been obtained, plus the essential function of PI3Kγ in the development of long-term MOR desensitization and tolerance in the DRG [125]. Recent studies reveal that PI3Kγ is an essential element of pain- relieving opioid effects in neuronal cells [124, 125].

Glia, once thought to be merely supporting cells of the CNS, are now recognized to play a central role in the formation and maintenance of morphine tolerance[126]. It is suggested that morphine-induced migration of reactive microglia produce locally elevated concentrations of proinflammatory cytokines and chemokines[127]. Morphine-induced microglial migration is an μ-opioid receptor and PI3K dependent [128]. It was reported that inhibiting PI3K reduces migration and ATP-induced Akt phosphorylation, implicating that the PI3K/Akt pathway in purinergic receptor mediated migration [129, 130]. The PI3K/Akt pathway have been demonstrated to be involved in ADP-induced microglial migration and chemo taxis by the P2Y12 receptor, which contribute to the development of pain [130].

The contribution of opioid-induced neuroinflammation is well documented [131] among the extensive studies regarding the mechanism underlying morphine tolerance. It is considered that morphine tolerance and neuropathic pain share common cellular mechanisms [132]. In a chronic morphine tolerance rat model, it was observed that Akt phosphorylation, cleaved Caspase-1-dependent NALP1 inflammasome activation and IL-1β maturation in spinal cord neurons were significantly enhanced by morphine. This revealed the role of μ-opioid/PI3K-Akt signaling/NALP1 inflammasome cascade in the development of morphine tolerance, and how treatment with LY294002 significantly reduced Caspase-1 cleavage, NALP1 inflammasome activation and

attenuated morphine tolerance[133]. It is well known that repeated and long-term exposure to opioids causes opioid receptor-mediated adaptive changes within the nervous system, including desensitization, internalization, downregulation, and phosphorylation of opioid receptors [134] or heterodimerization with other receptors[135]. The findings demonstrated that the μ opioid receptor-triggered PI3K/Akt/mTOR pathway in promoting morphine-induced spinal protein translation changes and is associated with morphine tolerance and hyperalgesia [136]. Besides, inhibition of the spinal PI3K/Akt not only reduces morphine-induced increase in p-mTOR, but also attenuates the development of morphine tolerance [136]. Thus the PI3K/Akt pathway is likely a novel target for preventing and/or treating chronic morphine tolerance and morphine-induced hyperalgesia.

Conclusion

By reviewing the current evidence, we discussed the role of PI3K/Akt pathway in chronic pain (Figure 1, 2, 3 and 4). These studies provided solid evidence that the PI3K/Akt pathway plays a pivotal role in the pathogenesis of bone cancer pain, neuropathic pain and inflammatory pain. Treatment with PI3K or the Akt inhibitor could attenuate mechanical allodynia and thermal hyperalgesia caused by pathological pain, implying that they may be beneficial and more effective therapeutic tools for chronic pain management. However, future extensive exploration should be performed with more selective and clinically relevant drugs targeting PI3K/Akt pathway.

Abbreviations

BCP: bone cancer pain; PSL: partial sciatic nerve ligation; MCP-1: monocyte chemoattractant protein-1; CCR2: CC chemokine receptor-2; MAPK: mitogen-activated protein kinases; ERK: extracellular signal-related kinase; PI3K: phosphatidylinositol 3-kinase; TRPV1: transient receptor potential vanilloid subfamily member 1; TNF: tumor necrosis factor; NMDA receptor: N-methyl-D-aspartic acid receptor; PAR-2: proteinase-activated receptor-2; SP: substance P; NKIR: neurokinin-1 receptor; NGF: nerve growth factor; TrkA receptor: tropomyosin receptor kinase A receptor; PDK1: pyruvate dehydrogenase kinase 1; mTOR: mammalian target of rapamycin; PKC: protein kinase C; 4EBP: 4E-binding protein; ET-1: endothelin-1; ETA-R: endothelin type A receptor; MEK: methyl ethyl ketone; GSK3: glycogen synthase kinase 3; Smad1: drosophila mothers against decapentaplegic 1; NALP1: neutrophilic alkaline phosphatase 1

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Conflicts of interest

All authors have no conflicts of interest to disclose.

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Figure legends

Figure 1. Schematic illustration of potential mechanisms of PI3K/Akt pathway in the processing of bone cancer pain. MCP-1: monocyte chemoattractant protein-1; CCR2: CC chemokine receptor-2; MAPK: mitogen-activated protein kinases; ERK: extracellular signal-related kinase; PI3K: phosphatidylinositol 3-kinase; PKB/Akt: protein kinase B; TRPV1: transient receptor potential vanilloid subfamily member 1;

Figure 2. Schematic illustration of potential mechanisms of PI3K/Akt pathway in the processing of neuropathic pain. ET-1: endothelin-1; ETA-R: endothelin type A receptor; MAPK: mitogen-activated protein kinases; ERK: extracellular signal-related kinase; PI3K: phosphatidylinositol 3-kinase; PKB/Akt: protein kinase B; MEK: mitogen-activated ERK-regulating kinase; GSK3: glycogen synthase kinase 3; Smad1: drosophila mothers against decapentaplegic 1.

Figure 3. Schematic illustration of potential mechanisms of PI3K/Akt pathway in the processing of inflammatory pain. TNF: tumor necrosis factor; NMDA receptor: N-methyl-D-aspartic acid receptor; PAR-2: proteinase-activated receptor-2; SP: substance P; NKIR: neurokinin-1 receptor; NGF: nerve growth factor; TrkA receptor: tropomyosin receptor kinase A receptor; PI3K: phosphatidylinositol 3-kinase; ERK: extracellular signal-related kinase; PKB/Akt: protein kinase B; PDK1: pyruvate dehydrogenase kinase 1; mTOR: mammalian target of rapamycin; PKC: protein kinase C; 4EBP: 4E-binding protein.

Figure 4. Schematic illustration of potential mechanisms of PI3K/Akt pathway in the processing of morphine tolerance. PI3K: phosphatidylinositol 3-kinase; PKB/Akt: protein kinase B; mTOR: mammalian target of rapamycin; NALP1: neutrophilic alkaline phosphatase 1.
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