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Transcriptional Expression of Protein Arginine Methyltransferases in Dorsal Root Ganglion after Peripheral Nerve Injury in Mice (Postprint)

Authors: Xu Huali, Shiyuan Xu, Mo Kai

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Abstract

Objective To investigate the relationship between protein arginine methyltransferase (PRMT) transcriptional expression in dorsal root ganglion (DRG) and pain behavior following peripheral nerve injury. Methods A neuropathic pain model of peripheral nerve injury was established using bilateral L4 spinal nerve ligation (SNL) in C57BL/6 mice, with sham-operated group as control; DRG tissues were collected 7 days postoperatively from SNL or sham groups, and RNA-Seq sequencing was performed to comprehensively analyze the transcriptional expression patterns and tissue distribution of 9 PRMT family genes, screening for differentially expressed genes. A unilateral L4 SNL model was established (with sham group as control), paw withdrawal frequency (PWF) and paw withdrawal latency (PWL) were measured at preoperative (0 d) and 3, 7, and 14 days postoperatively, and ipsilateral and contralateral DRG from both groups at the aforementioned time points were collected for RT-qPCR validation of differential gene expression. A chronic constriction injury (CCI) model of sciatic nerve ligation was established, with sham-operated group as control; pain behavior detection methods and time points were the same as the SNL model, and RT-qPCR was used to further validate differential gene expression at the aforementioned time points post-CCI. Results (1) RNA-Seq sequencing results showed that all 9 PRMT genes were expressed in DRG, with Prmt2 and Prmt3 showing the highest basal expression levels and Prmt6 the lowest. Compared with the sham group, SNL nerve injury upregulated DRG Carm1 transcriptional expression (increased by 1.7-fold) and inhibited transcription of Prmt5, Prmt8, and Prmt9, with Prmt8 showing the most significant inhibition (decreased by 16.3-fold). (2) RT-qPCR validation showed that compared with the sham group, SNL peripheral nerve injury increased PWF and decreased PWL at 3, 7, and 14 days postoperatively, upregulated DRG Carm1 transcriptional expression, while only inhibiting Prmt8 transcriptional expression, with Prmt1, Prmt5, and Prmt9 showing no significant changes. (3) Similarly, CCI sciatic nerve injury

also increased PWF and decreased PWL at 3, 7, and 14 days postoperatively, upregulated DRG Carm1, and inhibited Prmt8 expression at the aforementioned time points. Conclusion Peripheral nerve injury induced mechanical hyperalgesia and thermal hyperalgesia while simultaneously upregulating DRG Carm1 transcriptional expression and inhibiting Prmt8 gene transcription.

Full Text

Preamble

XU Huali, XU Shiyuan, MO Kai
Department of Anesthesiology, Zhujiang Hospital, Southern Medical University, Guangzhou 510282, China

Abstract: Objective To investigate the changes in the transcription of protein arginine methylation enzyme family genes in the dorsal root ganglia (DRG) following peripheral nerve injury in mice. Methods C57BL6 mouse models of neuropathic pain induced by peripheral nerve injury were established by bilateral L4 spinal nerve ligation (SNL). At 7 days after SNL or sham operation, the DRG tissue was collected for transcriptional analysis of 9 protein arginine methylation enzyme genes (Prmt1-3, Carm1, and Prmt5-9) using RNA-Seq to identify the differentially expressed genes in the injured DRGs. We also established mouse models of lateral L4 SNL and models of chronic constriction injury (CCI) of the sciatic nerve and tested the paw withdrawal frequency (PWF) in response to mechanical stimulation and paw withdrawal latency (PWL) in response to thermal stimulation on 0, 3, 7 and 14 days after SNL or CCI; the expressions of the differentially expressed genes in the injured DRGs were verified in the two models using RT-qPCR. Results Among the 9 protein arginine methylation enzyme family genes that were tissue-specifically expressed in the DRG, Prmt2 and Prmt3 showed the highest and Prmt6 showed the lowest basal expression. Compared with the sham-operated mice group, the mice receiving SNL exhibited upregulated Carm1 gene transcription (by 1.7 folds) but downregulated Prmt5, Prmt8 and Prmt9 transcription in the injured DRG (Prmt8 gene showed the most significant down-regulation by 16.3 folds). In mouse models of SNL and CCI, Carm1 gene expression increased progressively with time while Prmt8 transcription was obviously lowered on days 3, 7 and 14 after the injury; the transcription levels of Prmt1, Prmt5 and Prmt9 presented with no significant changes following the injuries. Both SNL and CCI induced mechanical allodynia and thermal hypersensitivities in the mice shown by increased PWF and decreased PWL on days 3, 7 and 14 after the injuries. Conclusion Periphery nerve injury induces Carm1 upregulation and Prmt8 downregulation in the injured DRG in mice, which sheds light on new targets for treatment of neuropathic pain.

Keywords: protein arginine N-methyltransferase; periphery nerve injury; dorsal root ganglia; neuropathic pain

Neuropathic pain (NP) is caused by damage or disease of the somatosensory system, and peripheral nerve injury represents one of the most common etiologies in clinical practice. However, there remains no specific or curative treatment for NP, making the investigation of its pathological mechanisms crucial for discovering novel therapeutic approaches. The dorsal root ganglion (DRG) serves as the first-order neuron for pain signal transmission, and numerous studies have demonstrated that DRG-mediated peripheral mechanisms play a particularly important role in the pathogenesis of NP following peripheral nerve injury. These peripheral mechanisms primarily involve injury-induced alterations in the expression of pain-related genes in the DRG, including opioid, GABA, and purinergic receptor genes, as well as Na+ and K+ ion channel genes, leading to abnormal neuronal hyperexcitability and consequent cutaneous hyperalgesia and allodynia. Protein arginine methyltransferases (PRMTs) participate in critical cellular activities, including gene transcription regulation, RNA processing, signal transduction, and DNA damage repair, by specifically catalyzing the methylation of histone or non-histone arginine residues as a form of post-translational protein modification. These enzymes are widely distributed throughout the nervous system. However, whether PRMTs are involved in regulating the expression of pain-related genes in the DRG following peripheral nerve injury remains unclear. The PRMT family consists of 9 proteins (PRMT1-3, CARM1, and PRMT5-9) encoded by the Prmt1-3, Carm1, and Prmt5-9 genes. To determine which PRMTs exhibit altered transcriptional expression in the DRG following peripheral nerve injury, we employed RNA-Seq to comprehensively analyze the tissue distribution of these PRMTs and the impact of nerve injury on their transcriptional expression in the DRG of a spinal nerve ligation (SNL) pain model, followed by screening for differentially expressed genes. We further validated these findings using RT-qPCR in different nerve injury pain models to explore the effects of peripheral nerve injury on PRMT transcription, providing a foundation for subsequent in-depth investigation of the molecular mechanisms through which PRMTs mediate neuropathic pain.

Experimental Animals

Clean-grade, healthy adult male C57BL6 mice aged 8 weeks and weighing 25-30 g were used for RNA-Seq detection and RT-qPCR validation. All animals were obtained from the Laboratory Animal Center of Southern Medical University with approval from the Animal Ethics Committee, and all pain experiments complied with the guidelines of the International Association for the Study of Pain (IASP). The L4 spinal nerve ligation (SNL) model was established by making an incision along the mouse back, bluntly dissecting the unilateral erector spinae muscle, exposing and removing the L5 transverse process to reveal the L4 spinal nerve, ligating and transecting the L4 spinal nerve with 7-0 silk sutures, and then closing the wound in layers. The corresponding sham group underwent exposure of the L4 nerve without ligation. For the chronic constriction injury (CCI) model of the sciatic nerve, the left sciatic nerve trunk was exposed and four loose ligatures were placed around it using 4-0 chromic gut sutures spaced

1 mm apart; the ligation strength was sufficient to induce mild twitching of the lower leg muscles. The sham group for CCI underwent only separation of the left sciatic nerve without any further manipulation. Postoperative pain behaviors were assessed as required, and animals were monitored for autotomy of the injured limb.

Mechanical Allodynia Behavior Testing

Mechanical sensitivity was measured using the method described in our previous study. Briefly, calibrated 0.4 g von Frey filaments (Stoelting Co., Wood Dale, IL, USA) were applied perpendicularly to the middle of the plantar surface of the mouse hind paw. After the filament bent moderately, stimulation was maintained for 3 seconds, repeated 10 times with 5-second intervals between stimuli. A withdrawal response during stimulation was recorded as a positive response. The paw withdrawal frequency (PWF) was calculated as (number of positive responses/10) $\times$ 100% to reflect mechanical hypersensitivity.

Thermal Hyperalgesia Behavior Testing

Thermal sensitivity was assessed using the method described in our previous study. A thermal pain stimulator (Model 336, IITC Inc./Life Science Instruments, Woodland Hills, CA, USA) delivered radiant heat to the middle of the plantar surface of the mouse hind paw. Paw withdrawal latency (PWL) was used to determine thermal hypersensitivity. To avoid skin burns, the stimulation intensity was adjusted to yield a baseline PWL of approximately 10 seconds (50% light intensity) with a cutoff time of 20 seconds. Each mouse was tested 5 times with 5-minute intervals, and the average of the middle three values was taken as the PWL for that limb.

Total RNA Extraction

After pain behavior testing, mice were decapitated and L4 DRG tissues from the ipsilateral and contralateral sides were rapidly collected and immersed in RNAlater solution. To obtain sufficient L4 DRG tissue for total RNA extraction and RNA-Seq, eight L4 DRGs from the same group were pooled into one sample at 7 days post-SNL or sham surgery (when pain symptoms were most pronounced). For PCR validation, two DRGs from the same group were pooled per sample at different time points (preoperative, and 3, 7, and 14 days post-surgery). All experiments included three or more biological replicates. Total RNA was extracted using the miRNeasy kit (QIAGEN, Valencia, CA) following the manufacturer' s protocol. RNA concentration was measured using a NanoDrop 2000 spectrophotometer, with A260/A280 ratios between 1.97 and 2.08. RNA integrity was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA), yielding RNA integrity numbers between 7.5 and 8.4.

RNA-Seq Sequencing

Following total RNA extraction, ribosomal RNA (rRNA) was removed using the Ribo-Zero rRNA Removal Kit (Human/Mouse/Rat) (Illumina, San Diego, CA). RNA libraries were prepared using the TruSeq Stranded Total RNA Sample Preparation Kit without poly-A selection according to the manufacturer' s instructions. RNA-Seq was performed on the Illumina HiSeq 2500 platform using high-output mode with 2 × 100 bp paired-end sequencing, generating over 190 million total reads per channel (at least 60 million reads per sample). Gene expression levels were quantified as RPKM (Reads Per Kilobase per Million reads) using methods detailed in our previous publication.

RT-qPCR Validation of PRMT Gene Expression

Total RNA was reverse-transcribed into cDNA using the TaKaRa PrimeScript RT reagent kit with DNA eraser. The 10 µL PCR reaction contained 2 µL mRNA template, 2 µL Premix Taq Version 2.0, and 6 µL ddH2O. The PCR conditions were: initial denaturation at 95°C for 10 seconds, followed by 40 cycles of denaturation at 95°C for 5 seconds and extension at 72°C for 1 minute. Real-time quantitative PCR was performed using SYBR Green methodology. Primers were designed using Primer 3.0 online software and synthesized by Shanghai Sangon Biotech Co., Ltd. Primer sequences are listed in Table 1 . The 10 µL reaction mixture contained 1 µL cDNA template, 5 µL SYBR Green Master Mix, 3.6 µL ddH2O, and 0.2 µL each of forward and reverse primers. The reaction conditions were: initial denaturation at 95°C for 15 minutes and 85°C for 30 seconds; 40 cycles of 95°C for 10 seconds, 72°C for 3 minutes, and 95°C for 5 seconds; followed by a melting curve analysis from 65°C to 95°C. Fluorescence data were analyzed using Bio-Rad Prime PCR software to calculate Ct values. Relative gene expression was determined using the $2^{(-\Delta\Delta Ct)}$ method with Tubala (Tubulin alpha 1A gene) as the internal reference and compared to the contralateral DRG.

Statistical Analysis

Data were analyzed using SigmaPlot 12.0 software. Normally distributed data are presented as mean ± standard deviation. Inter-group comparisons were performed using independent t-tests, intra-group comparisons using one-way ANOVA, and multiple comparisons using two-way ANOVA. $P < 0.05$ was considered statistically significant.

Results

2.1 Transcriptional Expression Changes of PRMTs in DRG Following SNL Peripheral Nerve Injury

Current research has identified nine protein arginine methyltransferases encoded by the Prmt1-3, Carm1, and Prmt5-9 genes. Analysis of the sham group revealed

that all nine PRMT genes were expressed in the DRG, with Prmt2 and Prmt3 showing the highest basal expression levels and Prmt6 the lowest. Compared with the sham group, SNL nerve injury significantly upregulated Carm1 transcription (by 1.7-fold) while downregulating Prmt5, Prmt8, and Prmt9 expression in the injured DRG, with Prmt8 showing the most pronounced suppression (16.3-fold decrease) [Figure 1: see original paper].

2.2 RT-qPCR Validation of Differentially Expressed PRMT Genes After SNL Nerve Injury

Although RNA-Seq provides comprehensive transcriptional information, high coefficients of variation can lead to misinterpretation. Therefore, we established an SNL model and designed specific primers for Prmt1, Carm1, Prmt5, Prmt8, and Prmt9 (Table 1) to validate their expression at different postoperative time points. Behavioral measurements were performed preoperatively (0 d) and at 3, 7, and 14 days post-SNL. Compared with the sham group, SNL significantly increased PWF and decreased PWL in the ipsilateral hind paw at 3, 7, and 14 days post-surgery ($P < 0.01$), while no significant changes were observed in the sham group or contralateral paws ($P > 0.05$, $n = 6$) [FIGURE:2AB]. RT-qPCR analysis revealed that SNL upregulated Carm1 mRNA expression in the injured L4 DRG at 3, 7, and 14 days post-surgery ($P < 0.01$, $n = 4$) [Figure 2C: see original paper], while downregulating Prmt8 expression ($P < 0.01$, $n = 3$) [Figure 2D: see original paper]. In contrast, Prmt5, Prmt9, and Prmt1 showed no significant changes ($P > 0.05$, $n = 6$) [FIGURE:2EG].

2.3 CCI Nerve Injury Upregulates DRG Carm1 Expression and Inhibits Prmt8

To exclude model-specific effects, we used another common peripheral nerve injury model, CCI, for further validation. Behavioral testing methods and time points were identical to the SNL model. Compared with the sham group, CCI significantly increased PWF and decreased PWL in the ipsilateral hind paw at 3, 7, and 14 days post-surgery ($P < 0.05$), while no changes were observed in the sham group or contralateral paws ($P > 0.05$) [FIGURE:3AB]. RT-qPCR validation yielded consistent results with the SNL model: CCI time-dependently upregulated Carm1 expression [Figure 3C: see original paper] while downregulating Prmt8 expression [Figure 3D: see original paper] in the ipsilateral L4 DRG at the same time points.

Discussion

This study provides the first comprehensive analysis of PRMT transcriptional changes in the DRG following peripheral nerve injury, revealing significant upregulation of Carm1 and downregulation of Prmt5, Prmt8, and Prmt9, with Prmt8 showing the most pronounced suppression. Subsequent RT-qPCR validation in both SNL and CCI models confirmed that peripheral nerve injury

time-dependently upregulates Carm1 while specifically downregulating Prmt8 in the injured L4 DRG, with no significant changes in Prmt5 and Prmt9 expression. These expression changes correlate closely with neuropathic pain behaviors, providing a foundation for further investigation of the molecular mechanisms through which Carm1 or Prmt8 mediate peripheral nerve injury-induced NP.

DRG neurons contain not only neurotransmitters and modulators that transmit nociceptive signals, such as tachykinins and excitatory amino acids, but also presynaptic modulatory receptors including GABA, opioid, and purinergic receptors, as well as sodium, potassium, and calcium ion channels. Peripheral nerve injury alters the expression of these molecules in the DRG, leading to abnormal neuronal hyperexcitability that represents a crucial mechanism for NP development and maintenance. Therefore, elucidating the regulatory mechanisms governing these expression changes could provide potential therapeutic targets for pain management. RNA-Seq effectively reveals the quantity and activity of expressed genes, enabling multidimensional analysis of gene expression differences under various pathological conditions and identification of disease-associated genes. Using the HiSeq2500 platform with 2×100 bp paired-end sequencing, we performed deep transcriptome sequencing of DRG PRMTs in the mouse SNL model. Our analysis showed that basal PRMT expression was highest for Prmt2 and Prmt3 and lowest for Prmt6. Differential gene screening identified upregulated Carm1 expression and downregulated Prmt5, Prmt8, and Prmt9 transcription following SNL, with no significant changes in other genes. RT-qPCR validation in two different pain models (SNL and CCI) confirmed that peripheral nerve injury upregates Carm1 and specifically downregulates Prmt8 in the injured L4 DRG, while Prmt5 and Prmt9 remain unchanged—likely due to high coefficient of variation during RNA-Seq analysis.

Histone arginine methylation, catalyzed by the PRMT family, represents an important post-translational modification that participates in numerous cellular processes including transcriptional regulation, RNA splicing, signal transduction, DNA damage repair, and protein-protein interactions. The PRMT family comprises nine proteins (PRMT1-3, CARM1, and PRMT5-9) that catalyze arginine methylation of histone and non-histone proteins. Our comprehensive analysis of these nine PRMTs following nerve injury revealed consistent upregulation of DRG Carm1 and downregulation of Prmt8 in both SNL and CCI models. The functional implications of these transcriptional changes during nerve injury remain to be elucidated.

CARM1 plays important roles in DNA packaging, transcriptional regulation, pre-mRNA splicing, and RNA stability. Specifically, CARM1 catalyzes dimethylation of histone H3 at arginine residues 17 and 26 (H3R17me2 and H3R26me2), activating transcription of the associated DNA segment. Additionally, CARM1 participates in JAK/STAT signaling pathway regulation, which represents a key mechanism through which cytokines such as chemokines, growth factors, and interleukins mediate neuronal excitability and glial activa-

tion in the DRG and spinal dorsal horn, contributing to chronic pain including neuropathic and bone cancer pain. Therefore, peripheral nerve injury-induced upregulation of DRG Carm1 may contribute to neuropathic pain development and maintenance through histone H3R17me2 modification or JAK/STAT pathway activation—an hypothesis requiring further investigation.

Meanwhile, whether DRG Prmt8 downregulation contributes to pain generation or maintenance remains unreported. Membrane-associated PRMT8 can interact with the PI3K regulatory subunit p85, activating PI3K and increasing phosphatidylinositol (3,4,5)-trisphosphate (PIP3) synthesis to activate AKT, forming a PRMT8/PI3K/AKT axis that maintains pluripotency and regulates mesodermal differentiation in human pluripotent stem cells. The PI3K/AKT signaling pathway also plays a crucial role in chronic pain mediation in the DRG and central nervous system. Whether DRG Prmt8 influences neuropathic pain development through the PI3K/AKT pathway requires further investigation.

In summary, this study provides preliminary evidence for PRMT transcriptional alterations in the DRG following peripheral nerve injury, demonstrating upregulated Carm1 expression and downregulated Prmt8 expression. These findings establish a foundation for further investigation of the molecular mechanisms through which Carm1 or Prmt8 mediate peripheral nerve injury-induced neuropathic pain, potentially offering novel therapeutic targets for NP prevention and treatment.

References

[1] Campbell JN, Meyer RA. Mechanisms of neuropathic pain [J]. Neuron, 2006, 52(1): 77-92.
[2] Yan YY, Li CY, Zhou L, et al. Research progress of mechanisms and drug therapy for neuropathic pain [J]. Life Sci, 2017, 190: 68-77.
[3] Basbaum AI, Bautista DM, Scherrer GA. Cellular and molecular mechanisms of pain [J]. Cell, 2009, 139(2): 267-84.
[4] Krames ES. The role of the dorsal root ganglion in the development of neuropathic pain [J]. Pain Medicine, 2014, 15(10): 1669-85.
[5] Bedford MT, Richard S. Arginine methylation: An emerging regulator of protein function [J]. Mol Cell, 2005, 18(3): 263-72.
[6] Morales Y, Cáceres T, May K, et al. Biochemistry and regulation of the protein arginine methyltransferases (PRMTs) [J]. Arch Biochem Biophys, 2016, 590: 138-52.
[7] Zhao JY, Liang LL, Gu XY, et al. DNA methyltransferase DNMT3a contributes to neuropathic pain by repressing Kcna2 in primary afferent neurons [J]. Nat Commun, 2017, 8: 14712.
[8] Wu S, Marie Lutz B, Miao X, et al. Dorsal root ganglion transcriptome analysis following peripheral nerve injury in mice [J]. Mol Pain, 2016, 12: 1744806916629048.
[9] Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics [J]. Nat Rev Genet, 2009, 10(1): 57-63.

[10] Xian PF, Chen Y, Yang L, et al. Effects of bee venom on TrkA and TRPV1 expression in dorsal root ganglion of rats with collagen-induced arthritis inflammatory pain [J]. J South Med Univ, 2016, 36(6): 801-7.
[11] Jiang Q, Tu WF. Protective effect of sufentanil preconditioning on acute gastric mucosal lesion in rats and its relationship with acid-sensing ion channels [J]. J South Med Univ, 2010, 30(5): 1099-103.
[12] Liang LL, Gu XY, Zhao JY, et al. G9a participates in nerve injury-induced Kcna2 downregulation in primary sensory neurons [J]. Sci Rep, 2016, 6: 37704.
[13] Krames ES. The dorsal root ganglion in chronic pain and as a target for neuromodulation: a review [J]. Neuromodulation, 2015, 18(1): 24-32.
[14] Coutaux A, Adam F, Willer JC, et al. Hyperalgesia and allodynia: peripheral mechanisms [J]. Joint Bone Spine, 2005, 72(5): 359-71.
[15] Yi F, Yang F, Liu XQ, et al. RNA-seq identified a super-long intergenic transcript functioning in adipogenesis [J]. RNA Biol, 2013, 10(6): 991-1002.
[16] Wesche J, Kuehn S, Kessler BM, et al. Protein arginine methylation: a prominent modification and its demethylation [J]. Cell Mol Life Sci, 2017, 74(18): 3305-15.
[17] Simandi Z, Czipa E, Horvath A, et al. PRMT1 and PRMT8 regulate retinoic acid-dependent neuronal differentiation with implications to neuropathology [J]. Stem Cells, 2015, 33(3): 726-41.
[18] Shin HJ, Kim H, Oh S, et al. AMPK-SKP2-CARM1 signalling cascade in transcriptional regulation of autophagy [J]. Nature, 2016, 534(7608): 553-7.
[19] Cheng D, Côté J, Shaaban S, et al. The arginine methyltransferase CARM1 regulates the coupling of transcription and mRNA processing [J]. Mol Cell, 2007, 25(1): 71-83.
[20] Jacques SL, Aquino KP, Gureasko JA, et al. CARM1 preferentially methylates H3R17 over H3R26 through a random kinetic mechanism [J]. Cancer Res, 2015, 75(15): 1635-44.
[21] Selvi BR, Batta K, Kishore A, et al. Identification of a novel inhibitor of coactivator-associated arginine methyltransferase 1 (CARM1)-mediated methylation of histone H3 Arg-17 [J]. J Biol Chem, 2010, 285(10): 7143-52.
[22] Coughlan N, Thillainadesan G, Andrews J, et al. -Estradiol-dependent activation of the JAK/STAT pathway requires p/CIP and CARM1 [J]. Biochim Biophys Acta, 2013, 1833(6): 1463-75.
[23] Fang D, Kong LY, Cai J, et al. Interleukin-6-mediated functional upregulation of TRPV1 receptors in dorsal root ganglion neurons through the activation of JAK/PI3K signaling pathway: roles in the development of bone cancer pain in a rat model [J]. Pain, 2015, 156(6): 1124-44.
[24] Dominguez E, Rivat C, Pommier B, et al. JAK/STAT3 pathway is activated in spinal cord microglia after peripheral nerve injury and contributes to neuropathic pain development in rat [J]. J Neurochem, 2008, 107(1): 50-60.
[25] Jeong HC, Park SJ, Choi JJ, et al. PRMT8 controls the pluripotency and mesodermal fate of human embryonic stem cells by enhancing the PI3K/AKT/SOX2 axis [J]. Stem Cells, 2017, 35(9): 2037-49.
[26] Chen SP, Zhou YQ, Liu DQ, et al. PI3K/Akt pathway: a potential therapeutic target for chronic pain [J]. Curr Pharm Des, 2017, 23(12): 1860-8.

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