Research Article

Application of Bioinformatics Methods to Identify Key Genes and Functions in Chronic Pelvic Pain

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Neuropathologic pain (NPP) occurs in most patients with chronic pelvic pain (CPP), and the unique physiological characteristics of visceral sensory neurons make the current analgesic effect of CPP patients not optimistic. Therefore, this study explored the possible biological characteristics of key genes in CPP through the bioinformatics method. CPP-related dataset GSE131619 was downloaded from Gene Expression Omnibus to investigate the differentially expressed genes (DEGs) between lumbar dorsal root ganglia (DRG) and sacral DRG, and the functional enrichment analysis was performed. A protein-protein interaction (PPI) network was constructed to search subnet modules of specific biological processes, and then, the genes in the subnet were enriched by single gene set analysis. A CPP mouse model was established, and the expression of key genes were identified by qPCR. The results showed that 127 upregulated DEGs and 103 downregulated DEGs are identified. Functional enrichment analysis showed that most of the genes involved in signal transduction were involved in the pathway of receptor interaction. A subnet module related to neural signal regulation was identified in PPI, including CHRNB4, CHRNA3, and CHRNB2. All three genes were associated with neurological or inflammatory activity and are downregulated in the sacral spinal cord of CPP mice. This study provided three key candidate genes for CPP: CHRNB4, CHRNA3, and CHRNB2, which may be involved in the occurrence and development of CPP, and provided a powerful molecular target for the clinical diagnosis and treatment of CPP.

1. Introduction

Chronic pelvic pain (CPP) refers to acyclic pain in the pelvic area lasting more than 3 months, including interstitial cystitis/bladder pain syndrome (IC/BPS) common in women and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) specific to men [1, 2]. CPP is commonly seen in endometriosis, chronic prostatitis, irritable bowel syndrome, and many serious psychiatric disorders [2–4]. Unfortunately, pain and injury persist even after additional surgery and/or prolonged medication [4]. This undoubtedly has a disastrous impact on the quality of life of patients, resulting in a serious decrease in productivity, impaired sexual function, and serious psychological and life disorders [4–6]. Unfortunately, CPP has not received due attention like other chronic pain [7], and it is urgent to find out the pain mechanism of CPP to reduce the functional impairment of patients.

Studies have found that when CPP lasts much longer than the expected healing time, it often causes damage to specific organs and develops into the neuropathic pain (NPP) state over time, causing visceral allodynic pain and hyperalgesia of the bladder and adjacent pelvic organs [8, 9]. In addition, some CPP patients are highly similar to some known neurotic syndromes in their pain state [9]. On the other hand, more than half of CPP patients will develop NPP even if no obvious pathological changes were observed during laparoscopy [10], which leads us to speculate that finding the unique expression pattern of neurons in CPP may reduce unnecessary surgery for patients and facilitate drug selection in clinical practice.

While visceral pain is the source of CPP, visceral sensory neurons are often parallel with somatosensory neurons, and patients’ pain cannot be significantly improved if visceral pain fibers are ignored in CPP [9, 11]. Due to its unique physiological and pathological characteristics, visceral pain...
fibers often have a complex path, leading to poor perception of traditional analgesic drugs and traditional analgesic therapy in CPP patients [9, 11, 12]. The present study analyzed the datasets of the dorsal root ganglion (DRG) of the lumbar segment, which contains only somatosensory neurons, and the DRG of the sacral segment, which contains both somatosensory and visceral sensory neurons, by bioinformatics methods [13], to find the key to the differentially expressed genes for help to improve the understanding of the pathogenesis of the CPP, which provided new molecular targets for the treatment.

2. Materials and Methods

2.1. Data Acquisition. Lumbosacral dorsal root ganglion of the GSE131619 dataset included L4-5 and L6-S1 DRG of 5 adult male and 7 adult male C57BL/6 mice, as well as 12 cases of lumbar DRG sample, and 12 cases of sacral segment DRG sample were downloaded from Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi).

2.2. Screening of Differentially Expressed Genes (DEGs). R package limma analysis was conducted to screen DGR between samples, filter condition was set as $|\log_2(FC)| > 0.5$, $P < 0.05$, and R package ggrepplot2 was used to map the gene expression.

2.3. Function and Pathway Enrichment Analysis of DEGs. Online tool Metascape (https://metascape.org/gp/index.html#/main/step1) [14] of DEGs based on gene ontology (GO) was used to describe the biological process (BP), molecular function (MF), and cellular components (CCs) of DEGs. The pathway enrichment analysis is also conducted according to the Kyoto Encyclopedia of Genes and Genomes (KEGG). Threshold is $P < 0.01$, minimum count is 3, and enrichment factor $\geq 1.5$.

2.4. Protein-Protein Interaction (PPI) Network Analysis. The PPI network was constructed based on DEGs using String (https://string-db.org/), Visualization with Cytoscape, and the Molecular Complex (MCODE) plug-in in Cytoscape was used to identify network modules with similar functions in PPI.

2.5. Single Gene Set Enrichment Analysis (GSEA). According to the median expression of key genes as the partition condition, the R package msigdbr was used to match the corresponding species, and the differential enrichment of pathways was performed by using the function gseKEGG in the R package clusterProfiler. The screening conditions were $P < 0.05$ and FDR $< 25\%$. Finally, the visualization was performed by using the function gseaplot2 in the R package clusterProfiler.

2.6. Experimental Animals and Model Construction. Sixteen C57BL/6 mice (6–8 weeks) were purchased from Beijing Vitong Lihua Experimental Animal Technology Co., Ltd., with half male and half female. A CPP mouse model was built according to the previous study [15], and mice were divided into a CPP group and control group (CON), with 8 mice in each group. Mice in the CPP group were intraperitoneally injected with 0.2 mL of a mixture containing complete Freund's fluid (Beyotime, P2036) and normal saline, while mice in the CON group were injected with the same amount of normal saline. Seven days after the operation, the mice were anesthetized with pentobarbital sodium, and the mice were killed by neck breaking. Spinal cord tissue of the L6-S1 segment was collected and fixed with 4% paraformaldehyde, and frozen sections (7 mm) were made for subsequent experiments. All the procedures in this experiment complied with the National Institutes of Health guidelines for the use and care of laboratory animals [16] and were approved by the Medical Ethics Committee.

2.7. RT-qPCR. Total RNA was extracted from tissues using Trizol (Sigma-Aldrich, T9424). The cDNA library was synthesized using the PrimeScript™ One Step RT-PCR Kit RR055A). PCR amplification was performed on Takara PCR Thermal Cycler Dice™ Touch (TP350). The primer sequence is shown in Table 1. The experimental procedures were strictly used in accordance with the manufacturer’s protocol, with GAPDH as the reference gene, and $2^{-\Delta\Delta CT}$ was used for calculation, $\Delta\Delta CT = \Delta CT$ experimental group $- \Delta CT$ control group; $\Delta CT$ experimental group $= \Delta CT$ target gene, experimental group $- \Delta CT$ internal reference gene, experimental group $- \Delta CT$ control group $= \Delta CT$ target gene, control group $- \Delta CT$ internal reference gene, control group.

2.8. Statistical Methods. Experimental data were expressed as mean $\pm$ standard deviation. The $T$ test and One-way ANOVA were performed by GraphPad Prism 9.0. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Recognition of DEGs. The expression levels of 12,286 genes in lumbar DRG and sacral DRG were analyzed. The median expression values of all the genes in the samples were in a straight line, and the quality control results were good (Figure 1(a)). The expression pattern cluster analysis of the samples was conducted (Figure 1(b)), of which 127 were upregulated and 103 were downregulated (Figure 1(c)).

3.2. GO and KEGG Analysis of DEGs. We conducted GO functional enrichment analysis on 230 DEGs and found 337 BP-, 57 CC-, and 58 MF-related GO terms (Figures 2(a)–2(c)). Most GO terms were related to BP, in which most of the genes were closely related to the regulation of membrane potential, behavior, sensory perception of pain, signal release, etc. KEGG analysis results showed that the most significant enrichment pathways were involved in the conduction of nerve signals, such as ECM-receptor interaction, neuroactive ligand-receptor interaction, and axon guidance (Figure 2(d)).
Table 1: Primer sequences.

|         | Sequence (5’-3’) | Length | Tm   | Location   |
|---------|------------------|--------|------|------------|
| CHRNA3  | Forward          | TCCAGTTTGAGGTGTCTATGTCT | 23    | 60.4       | 182–204    |
|         | Reverse          | TGGTAGTCAGAGGGTTCCATTT  | 23    | 60.9       | 308–286    |
| CHRN2   | Forward          | ATTCGATGGATCCCGAAGACT   | 23    | 60.9       | 240–262    |
|         | Reverse          | TCAAAACGTCCGTCAGCATT    | 21    | 60.8       | 350–330    |
| CHRN4   | Forward          | TGGATGATCTCCTGAACAAAACC | 23    | 60.2       | 89–111     |
|         | Reverse          | GTTCTCGGCATTCACACTGAT   | 22    | 60.4       | 214–193    |
| GAPDH   | Forward          | AGCTTCGGCACATTTCTCAGC   | 23    | 61.0       | 624–646    |
|         | Reverse          | CGTTCACTCCCATGACAAACA   | 21    | 60.5       | 712–692    |

Figure 1: Continued.
3.3. PPI. According to the String database, the PPI network was constructed (Figure 3), and 41 nodes and 282 edges were identified by consensus. MCODE algorithm was used to identify a protein cluster containing three genes of CHRNB4, CHRNA3, and CHRNB2. The enrichment analysis results showed that this subnet module was related to biological processes such as regulation of membrane potential, behavior, signal release, and regulation of neurotransmitter. The expression of CHRNB4, CHRNA3, and CHRNB2 in normal lumbar DRG was lower than that in sacral DRG (Table 2).

3.4. GSEA of Key Genes. In order to analyze the potential functions of CHRNA3, CHRNB2, and CHRNB4, GSEA was performed on KEGG in the highly expressed samples, and the results showed that these three genes were all related to nerve or inflammatory activity (Figure 4).

3.5. Identification of Key Genes. RT-qPCR was used to identify the expression of key genes in the spinal cord tissue of mice at the L6-S1 segment. The results showed that the expression levels of CHRNA3, CHRNB2, and CHRNB4 in the spinal cord tissue of mice in the CPP group were significantly downregulated compared with those in the CON group ($P < 0.05$, Figure 5).

4. Discussion

Most of the time, only when pain occurred can we become aware of the presence of viscera and the potential or substantial tissue damage [12]. NPP is often associated with the activation of glial cells and changes in immune signals [17]. Injuries such as inflammation and tissue damage are transmitted to the dorsal horn of the spinal cord, and unlike the harmful signals of somatic structure in a specific spinal cord segment, visceral sensation is often transmitted to synapses at multiple spinal cord segment levels, so visceral pain tended to be diffuse and poorly localized [12, 18]. The viscera can project sensory signals to the dorsal horn of the spinal cord, thereby activating the neural reflex pathway, while peripheral or central sensitization can promote the induction and persistence of chronic pain signals [18, 19].

On the other hand, due to the overlap of the regions regulating visceral pain and psychological stress, when the nerve signal was strong enough, the brain stem or cortical region will provide emotional or conscious regulation, resulting in increased pain perception, hyperalgesia, and an adverse cycle between mental stress and pain state [18, 20–22]. Therefore, patients with visceral pain are vulnerable in psychological and physiological aspects and are easily interfered by the environment, inflammation, nervous system, and other factors [22]. Therefore, it is urgent to explore the molecular mechanism associated with visceral pain, in order to interrupt the neural cascade, relieve pain, and improve the quality of life of patients.

The visceral sensory neurons had unique functional characteristics that are different from those of somatosensory neurons [23, 24], which results in that the efficacy of traditional somatosensory analgesic drugs for CPP patients is not up to expectations [11, 13]. Studies had pointed out that nerve injury will lead to adaptive changes of sensory neurons and corresponding pathways in the central nervous system, resulting in abnormal occurrence of neuron-induced signals and changes in epigenetic regulation of gene expression, leading to increased visceral sensitivity [18, 25]. In this study, we used the dataset created by Smith et al. [13], which suggested DRGs in lumbar-sacral DRG were caused by differences in visceral sensory neurons in the sacral segment. By reidentifying the unique genes uniquely expressed in
Figure 2: Enrichment analysis results of DEGs. (a) BP, (b) CC, (c) MF, and (d) KEGG enrichment analysis are performed for 230 DEGs. BP: biological process; CC: cell component; MF: molecular function; and KEGG: Kyoto Encyclopedia of Genes and Genomes.
Figure 3: Protein-protein interaction network of DEGs. Note. The dots in the figure represent the protein members in PPI, and the dots in the upper left corner represent the subnet module with CHRNB4 as the core.

Table 2: Difference of expression of key genes in normal lumbar DRG and sacral DRG.

|          | Log FC | Ave. expression | t      | P value       | Adj. P value | B        | Expression |
|----------|--------|-----------------|--------|---------------|--------------|----------|------------|
| Chrna3   | 2.804  | 3.919           | 11.758 | 1.53 × 10⁻¹¹  | 8.59 × 10⁻⁸  | 15.754   | Down       |
| Chrnb2   | 1.498  | 2.621227581     | 6.545  | 8.36 × 10⁻⁷   | 0.000311285  | 5.873    | Down       |
| Chrnb4   | 2.570  | 4.011           | 11.918 | 1.16 × 10⁻¹¹  | 8.59 × 10⁻⁸  | 15.996   | Down       |

Note. Lumbar DRG samples are used as the comparison matrix for analysis.
visceral sensory neurons, it was helpful to select candidate analgesic targets for patients with CPP [23].

The consensus of this study was that 230 DEGs in lumbar DRG and sacral DRG were identified. GO and KEGG analysis showed that these DRGs were closely related to multiple processes of nerve signaling (regulation of membrane potential, sensory perception of pain, and signal release). It might mediate the synaptic connection between splanchnic sensory neurons by participating in multiple receptor interactions (ECM-receptor interaction, neuroactive ligand-receptor interaction, and atron guidance). MCODE was a unique algorithm used to detect tightly connected regions in the PPI network [26], which was often used to explore subnet modules with specific biological functions, and the genes in the subnet were often considered as key genes [27, 28]. Enrichment analysis showed that the subclusters composed of three genes, CHRNB4, CHRNA3, and CHRNB2, were related to the BP associated with the regulation of membrane potential, signal release, and regulation of neurotransmitter, and these genes were highly expressed in normal sacral segment DRG.

Figure 4: Single gene set enrichment analysis of key genes (GSEA). Note. (a) GSEA highly expressed by CHRNA3; (b) GSEA highly expressed by CHRNB2; and (c) GSEA highly expressed by CHRNB4.
GSEA had now become an indispensable part of high-throughput gene expression data analysis, focusing on gene sets and analyzing their common biological pathways or potential functions [29, 30]. In this study, GSEA results showed that CHRNA3 was enriched in neuroactiveligand-receptor interaction, ECM-receptor interaction, and IL-17 signaling pathway-related pathways, while CHRNB2 and CHRNB4 were related to ferroptosis, the toll-like receptor signaling pathway, the hedgehog signaling pathway, cell cycle, the mTOR signaling pathway, and inflammatory mediator regulation of TRP channels, respectively. Most of these signaling pathways mediate nerve injury and the continuation of NPP by participating in neurotoxicity [31], neural activity [32], inflammatory response [33, 34], hyperalgesia [35], nerve healing [36, 37], microglia polarization [38], and oxidative stress [39].

The nicotine and nicotinic acetylcholine receptors (NACHRs) subtype, by a variety of subunits of combinatorial synthesis channel receptor complexes, its structure, and function in the nervous system of diversity, had been found to enhance the presynaptic neurotransmitter and release and affect the excitability of neurons, and when its function declines, it would cause the nervous system dysfunction [40, 41]. A growing number of studies had found that NACHRs had great potential in the treatment of chronic pain as targets for NPP and inflammation control [42, 43]. In this study, we found that the expression of the three NACHR gene clusters consisting of CHRNA4, CHRNA3, and CHRNA2 decreased in the sacral DRG of the CPP model, which was consistent with the results of previous reports related to nerve injury [44, 45].

5. Conclusions

In conclusion, this study found the gene differentially expressed in the visceral sensory neurons of the CPP model, which might be the key to the occurrence of CPP, and provided a new analytical target for the drug therapy of CPP. However, few studies have revealed the contribution of these genes in nerve pain, and further studies are needed to verify this.

Data Availability

The data used and/or analyzed during the current study are available from all the authors.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] J. Q. Clemens, C. Mullins, C. Mullins et al., "Urologic chronic pelvic pain syndrome: insights from the MAPP Research Network," Nature Reviews Urology, vol. 16, no. 3, pp. 187–200, 2019.
[2] S. Kothari, "Neuromodulatory approaches to chronic pelvic pain and coccygodynia," Operative Neuromodulation, pp. 365–371, Springer, Vienna, Austria, 2007.
[3] L. M. Speer, S. Mushkbar, and T. Erbele, "Chronic pelvic pain in women," American Family Physician, vol. 93, no. 5, pp. 380–387, 2016.
[4] C. Smith, "Male chronic pelvic pain: an update," Indian Journal of Urology, vol. 32, no. 1, pp. 34–39, 2016.
[5] S. R. Till, S. As-Sanie, and A. Schrepf, "Psychology of chronic pelvic pain: prevalence, neurobiological vulnerabilities, and treatment," Clinical Obstetrics & Gynecology, vol. 62, no. 1, pp. 22–36, 2019.
[6] C. Allaire, C. Williams, S. Bodmer-Roy et al., "Chronic pelvic pain in an interdisciplinary setting: 1-year prospective cohort," American Journal of Obstetrics and Gynecology, vol. 218, no. 1, pp. 114.e1–114.e12, 2018.
[7] L. R. Miller-Matero, C. Saulino, S. Clark, M. Bugenski, A. Eshelman, and D. Eisenstein, "When treating the pain is not enough: a multidisciplinary approach for chronic pelvic pain," Archives of Women’s Mental Health, vol. 19, no. 2, pp. 349–354, 2016.
[8] F. M. Howard, "Endometriosis and mechanisms of pelvic pain," Journal of Minimally Invasive Gynecology, vol. 16, no. 5, pp. 540–550, 2009.
[9] C. Hunter, N. Davé, S. Diwan, and T. Deer, "Neuro-modulation of pelvic visceral pain: review of the literature and case series of potential novel targets for treatment," Pain Practice, vol. 13, no. 1, pp. 3–17, 2013.
[10] L. H. Whitaker, J. Reid, A. Choa et al., "An exploratory study into objective and reported characteristics of neuropathic pain
in women with chronic pelvic pain,” PLoS One, vol. 11, no. 4, Article ID e0151950, 2016.

[11] A. Kansal and J. Hughes, “Visceral pain,” Anesthesia & Intensive Care Medicine, vol. 17, no. 11, pp. 543–547, 2016.

[12] L. Grundy, A. Erickson, and S. M. Brierley, “Visceral pain,” Annual Review of Physiology, vol. 81, pp. 261–284, 2019.

[13] C. J. A. Smith-Alltala, E. A. Mason, C. A. Wells, B. J. Aronow, P. B. Osborne, and J. R. Keast, “Identification of a sacral, visceral sensory transcriptome in embryonic and adult mice,” eNeuro, vol. 7, no. 1, pp. 397–419, 2020.

[14] Y. Zhou, B. Zhou, L. Pache et al., “Metascape provides a biologist-oriented resource for the analysis of systems-level datasets,” Nature Communications, vol. 10, no. 1, pp. 1523–1610, 2019.

[15] M. Tang, R. Ullah, J. Wazir et al., “Effect of oral T2 antigen on chronic prostatitis/chronic pelvic pain syndrome in mice model,” Inflammation, vol. 42, no. 6, pp. 2086–2094, 2019.

[16] National Research Council, Guide for the Care and Use of Laboratory Animals, National Academies Press, Washington, DC, USA, 2010.

[17] P. M. Grace, M. R. Hutchinson, S. F. Maier, and L. R. Watkins, “Pathological pain and the neuroimmune interface,” Nature Reviews Immunology, vol. 14, no. 4, pp. 217–231, 2014.

[18] B. Greenwood-Van Meerveld and A. C. Johnson, “Stress-induced chronic visceral pain of gastrointestinal origin,” Frontiers in Systems Neuroscience, vol. 11, p. 86, 2017.

[19] D. Fornasari, “Pain mechanisms in patients with chronic pain,” Clinical Drug Investigation, vol. 32, no. 1, pp. 45–52, 2012.

[20] L. Grundy and S. M. Brierley, “Visceral pain,” Journal of the Association of Urologists of Canada, vol. 233, no. 1, pp. 49–67, 2012.

[21] M. Larauche, A. Mulak, and Y. Taché, “Stress and visceral pain: from animal models to clinical therapies,” Experimental Neurology, vol. 233, no. 1, pp. 49–67, 2012.

[22] D. A. Tripp, “Managing psychosocial correlates of uroligic chronic pelvic pain syndromes: advice from a uroligic pain psychologist,” Canadian Urological Association Journal = Journal de l’Association des urologues du Canada, vol. 12, no. 6, pp. S175–S157, 2018.

[23] J. R. F. Hockley, R. Gonzalez-Can, S. McMurray et al., “Visceral and somatic pain modalities reveal NaV1.7-independent visceral nociceptive pathways,” The Journal of Physiology, vol. 595, no. 8, pp. 2661–2679, 2017.

[24] L. Rinaman, “Visceral sensory inputs to the endocrine hypothalamus,” Frontiers in Neuroendocrinology, vol. 28, no. 1, pp. 50–60, 2007.

[25] C. A. von Hehn, R. Baron, and C. J. Woolf, “Deconstructing the neuropathic pain phenotype to reveal neural mechanisms,” Neuron, vol. 73, no. 4, pp. 638–652, 2012.

[26] G. D. Bader and C. W. Hogue, “An automated method for finding molecular complexes in large protein interaction networks,” BMC Bioinformatics, vol. 4, no. 1, p. 2, 2003.

[27] F. Liu, Y. Wu, Y. Mi, L. Gu, M. Sang, and C. Geng, “Identification of core genes and potential molecular mechanisms in breast cancer using bioinformatics analysis,” Pathology Research and Practice, vol. 215, no. 7, Article ID 152436, 2019.

[28] B.-F. Xu, R. Liu, C.-X. Huang et al., “Identification of key genes in ruptured atherosclerotic plaques by weighted gene correlation network analysis,” Scientific Reports, vol. 10, no. 1, Article ID 10847, 2020.

[29] A. Subramanian, P. Tamayo, V. K. Mootha et al., “Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles,” Proceedings of the National Academy of Sciences, vol. 102, no. 43, pp. 15545–15550, 2005.

[30] L. Geislinger, G. Csaba, M. Santarelli, M. Ramos, L. Schiffer, and N. Turaga, “Toward a gold standard for benchmarking gene set enrichment analysis,” Bioinformatics, vol. 10, no. 1, pp. 1–10, 2020.

[31] J. Wei, J. Liu, S. Liang, M. Sun, and J. Duan, “Low-dose exposure of silica nanoparticles induces neurotoxicity via neuroactive ligand-receptor interaction signaling pathway in zebrafish embryos,” International Journal of Nanomedicine, vol. 15, pp. 4407–4415, 2020.

[32] M. Zille, A. Kumar, N. Kundu et al., “Ferropotaxis in neurons and cancer cells is similar but differentially regulated by histone deacetylase inhibitors,” Eurevo, vol. 6, no. 1, pp. 263–318, 2019.

[33] J. Zhou, Y. Jin, R. Ma et al., “Electroacupuncture alleviates experimental chronic inflammatory pain by inhibiting calcium voltage-gated channel-mediated inflammation,” Evidence-based Complementary and Alternative Medicine, vol. 2020, Article ID 7061972, 10 pages, 2020.

[34] F. Richter, G. Natura, M. Ebbinghaus et al., “Interleukin-17 sensitizes joint nociceptors to mechanical stimuli and contributes to arthritic pain through neuronal interleukin-17 receptors in rodents,” Arthritis & Rheumatism, vol. 64, no. 12, pp. 4125–4134, 2012.

[35] J. A. Stokes, J. Cheung, K. Eddinger, M. Corr, and T. L. Yaksh, “Toll-like receptor signaling adapter proteins govern spread of neuropathic pain and recovery following nerve injury in male mice,” Journal of Neuroinflammation, vol. 10, no. 1, p. 925, 2013.

[36] N. Moreau and Y. Boucher, “Hedging against neuropathic pain: role of Hedgehog signaling in pathological nerve healing,” International Journal of Molecular Sciences, vol. 21, no. 23, p. 9115, 2020.

[37] J. Wu, Z. Zhao, X. Zhu, C. L. Renn, S. G. Dorsey, and A. I. Faden, “Cell cycle inhibition limits development and maintenance of neuropathic pain following spinal cord injury,” Pain, vol. 157, no. 2, pp. 488–503, 2016.

[38] J.-R. Guo, H. Wang, X.-J. Jin, D.-L. Jia, X. Zhou, and Q. Tao, “Effect and mechanism of inhibition of PI3K/Akt/mTOR signal pathway on chronic neuropathic pain and spinal microglia in a rat model of chronic constriction injury,” Oncotarget, vol. 8, no. 32, pp. 52923–52934, 2017.

[39] C. Carrasco, M. Nazíróglu, A. B. Rodríguez, and J. A. Pariente, “Neuropathic pain: delving into the oxidative origin and the possible implication of transient receptor potential channels,” Frontiers in Physiology, vol. 9, p. 95, 2018.

[40] J. A. Dani and D. Bertrand, “Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system,” Annual Review of Pharmacology and Toxicology, vol. 47, no. 1, pp. 699–729, 2007.

[41] Y. Yan, C. Peng, M. C. Arvin et al., “Nicotinic cholinergic receptors in VTA glutamate neurons modulate excitatory transmission,” Cell Reports, vol. 23, no. 8, pp. 2236–2244, 2018.

[42] D. Bagdas, M. S. Gurun, P. Flood, R. L. Papke, and M. I. Damaj, “New insights on neuronal nicotinic acetylcholine receptors as targets for pain and inflammation: a focus on α7 nAChRs,” Current Neuropharmacology, vol. 16, no. 4, pp. 415–425, 2018.

[43] A. J. Hone and J. M. McIntosh, “Nicotinic acetylcholine receptors in neuropathic and inflammatory pain,” FEBS Letters, vol. 592, no. 7, pp. 1045–1062, 2018.
[44] J. R. Perkins, A. Antunes-Martins, M. Calvo et al., "A comparison of RNA-seq and exon arrays for whole genome transcription profiling of the L5 spinal nerve transection model of neuropathic pain in the rat," *Molecular Pain*, vol. 10, no. 1, p. 7, 2014.

[45] L. Topham, S. Gregoire, H. Kang et al., "The transition from acute to chronic pain: dynamic epigenetic reprogramming of the mouse prefrontal cortex up to 1 year after nerve injury," *Pain*, vol. 161, no. 10, pp. 2394–2409, 2020.