Identification of Molecular Mechanisms in Primary Sensory Neurons and Glial Cells in a Mouse Neuropathic Pain Model

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INTRODUCTION:
Chronic neural compression due to spinal degeneration can cause neuropathic pain, which significantly reduces quality of daily life. Therefore, in-depth investigation into primary sensory neurons and glial cells in the dorsal root ganglion (DRG) is essential for identifying therapeutic targets that can counteract persistent pain. The aim of this study was to investigate the roles of primary sensory neurons and glial cells in neuropathic pain, as well as the molecular mechanisms involved.

METHODS:
We used the spared nerve injury (SNI) mouse model of neuropathic pain, with specific focus on post-SNI timepoints (1 and 3 weeks) and corresponding control groups in order to investigate the mechanisms of neuropathic pain. (Approved by the Animal Experiment Review Committee of Tokyo Medical and Dental University)

We first prepared DRG sections and performed immunofluorescence staining to quantitatively analyze primary sensory neurons (using anti-NeuN antibody), satellite glial cells (using anti-glutamine synthetase antibody), macrophages (using anti-IBA1 antibody), and P2X7 receptors (using anti-P2X7 receptor antibody). P2X7 is a protein involved in various cellular functions, including inflammation and pain signaling.

To evaluate differentially expressed genes (DEGs), RNA-seq technology was used for comprehensive transcriptomic analyses of DRG glial cells on both the ipsilateral and contralateral sides at 1 week and 3 weeks post-SNI surgery. Subsequently, these genes were further explored through a bioinformatics approach using gene set enrichment analysis (GSEA).

RESULTS SECTION:
Through detailed quantitative analyses in the SNI model, immunohistochemistry (IHC) revealed that the reduction in the number of primary sensory neurons was less pronounced on the affected side of the L3 DRG (1 week: 85%; 3 weeks: 82%) compared with the L4 DRG (1 week: 86%; 3 weeks: 71%). Moreover, changes in the proportion of satellite glial cells were more prominent on the affected side of the L4 DRG (1 week: 190%; 3 weeks: 134%) compared with the L3 DRG (1 week: 167%; 3 weeks: 137%). Immunofluorescence staining of frozen sections of mouse DRG tissue was performed. The results showed a notable reduction in the numbers of primary sensory neurons, about 15% and 18% in the L3 DRG at 1 week and 3 weeks post-SNI (p < 0.01), respectively, as well as a decrease of about 29% in the L4 DRG compared with the control group at 3 weeks post-SNI (p < 0.005). In contrast, satellite glial cells exhibited a distinct increasing trend, particularly at 1 week post-SNI (p < 0.001), with an approximately 90% surge observed in the L4 DRG. These observations strongly underscore the pivotal role of satellite glial cells in neuropathic pain.

Next, the Sox10 Venus-YFP mouse model was used to validate the increase in satellite glial cells. Fluorescent area analysis demonstrated a significant increase in the Venus-positive fluorescent area of satellite glial cells in the DRG on the affected side at 1 week and 3 weeks post-SNI (p < 0.01), thereby supporting the observed increase.

In addition, IHC results from the SNI model showed that the overlap of immunofluorescence regions significantly increased between macrophages and glial cells, and between macrophages and P2X7 receptor (p < 0.01).

Further utilization of RNA-seq analysis revealed potential molecular mechanisms. Differential gene expression analysis showed significant upregulation of several kinds of DEGs post-SNI, which play pivotal roles in immune regulation, neural transmission, and inflammatory responses. Moreover, certain differentially expressed genes were enriched in signal pathways highlighted through GSEA.

DISCUSSION:
In the SNI mouse model of neuropathic pain, the activation of P2X7 receptors associated with neural inflammation and pain signaling provided evidence for the potential significance of glial cells in the development of neuropathic pain. These DEGs and their regulated signal pathways likely play vital roles in pain signal transmission, inflammatory responses, and neural regulation. Our research will further focus on in-depth exploration of their functions and mechanisms in satellite glia and their surrounding cells.

SIGNIFICANCE/CLINICAL RELEVANCE:
The findings of this study hold significant implications for our understanding of neuropathic pain and its potential molecular mechanisms. Neuropathic pain often arises from conditions such as spinal degeneration and nerve injuries, posing considerable challenges in clinical practice due to its intricate and often refractory nature. The significance of this research lies in providing insights that could offer leads towards potential therapeutic avenues for addressing this debilitating condition.

IMAGES AND TABLES: