Peer Review File
Article information: https://dx.doi.org/10.21037/jgo-24-42

Reviewer A

The paper titled “Neuroinflammation in the paraventricular nucleus of the hypothalamus precipitates visceral pain induced by pancreatic cancer in mice” is interesting. This study found that PVN microglia and astrocytes were involved in regulating PCVP. The results suggest that targeting glia may be a potential approach for alleviating visceral pain in patients. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) What are the mechanisms of microglia and astrocytes in chronic visceral pain concerning the release of cytokines, chemokines, and neuroactive substances and alterations in intracellular signaling pathways during the process? Suggest adding relevant content.

Reply: Thank you for your valuable advice. We have added relevant content as follows: The close proximity between glia (astrocytes and neurons) facilitates glial activation via neurotransmission, as glia express a diverse array of functional neurotransmitter receptors. These include ionotropic non-NMDA and NMDA receptors, as well as metabotropic glutamate (mGluR3 and mGluR5), purinergic, and substance P receptors. Upon activation of glia, signaling pathways such as mitogen-activated protein kinase 1 (MAPK1) and MAPK8 are initiated, leading to an upregulation in the synthesis of inflammatory factors including interleukin 1β (IL-1 β), IL-6, tumor necrosis factor-alpha (TNF α), prostaglandin E2 (PGE2), and nitric oxide (NO). Consequently, this cascade further contributes to the initiation and maintenance of chronic pain.

Changes in the terxt: Please see Page 3, Line 84-91.

2) What was the advantage of the evaluation of the animals from 12, 15, and 18 days? Suggest adding relevant content.

Reply: Thank you for your valuable advice. Our chosen time point corresponds to the findings reported in our previously published article (Ji et al. 2024), where mice exhibited visceral pain at 12, 15 and 18 days following pancreatic injection of tumor cell.

Reference: Ji NN, Cao S, Song XL, Pei B, Jin CY, Fan BF, Jiang H, Xia M. Glutamatergic neurons in the paraventricular nucleus of the hypothalamus participate in the regulation of visceral pain induced by pancreatic cancer in mice. Hepatobiliary Surg Nutr 2024. doi: 10.21037/hbsn-23-442

3) Activated astrocytes play important roles in visceral pain. Recent studies have shown reactive astrocytes are classified into A1 and A2 phenotypes. What are the precise roles in visceral pain? It is recommended to add relevant content.

Reply: Thank you for your advice. We have added relevant content as follows: Researchers have identified two distinct subtypes of reactive astrocytes, namely A1-reactive and A2-reactive astrocytes. Neuroinflammation induces the activation of A1 astrocytes, which can potentially secrete neurotoxins leading to rapid neuronal death. Reversely, ischemia triggers the activation of A2 astrocytes, contributing to more efficient neuronal preservation. The dominant role played by A1-reactive astrocytes in chronic pain development is widely acknowledged; hence extensive research efforts have been dedicated to unraveling their involvement in this specific field.
Changes in the terxt: Please see Page 8, Line 267-272.

4) This study lacks specific instructions on animal use, and it is recommended to supplement this.
Reply: Thank you for your advice. We supplemented the number of mice in each figure legend section.
Changes in the terxt: Please see figure legend section.

5) Why did this study not conduct research on target genes? If you increase the study of target genes, the results may be more convincing and meaningful.
Reply: Thank you for your invaluable guidance. Therapeutic gene transfer to the central nervous system (CNS) has been extensively studied for managing chronic pain in the past two decades, successfully alleviating neuropathic pain in rodent models. Additional experimental findings suggest that transferring anti-inflammatory cytokines through gene therapy may be a promising approach for treating refractory pain in humans. Therefore, in the future, we will look for effective genetic targets to alleviate chronic pain more accurately and efficiently.

6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Electroacupuncture attenuates spared nerve injury-induced neuropathic pain possibly by promoting the progression of AMPK/mTOR-mediated autophagy in spinal microglia, PMID: 36618785”. It is recommended to quote the article.
Reply: Thank you for your advice. We have quoted the article.
Changes in the terxt: Please see Page 11, Line 355-357.

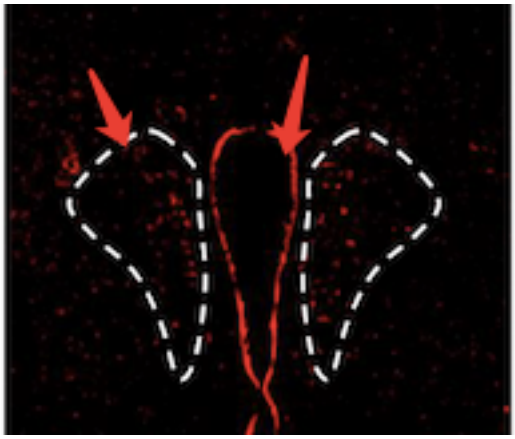
7) In this paper, it is best to supplement the research of signaling pathway. This is more conducive to support the conclusion of this paper.
Reply: Very insightful suggestion. However,The cellular signaling of glial cell activation has been extensively studied. Upon activation of glia, signaling pathways such as mitogen-activated protein kinase 1 (MAPK1) and MAPK8 are initiated, leading to an upregulation in the synthesis of inflammatory factors including interleukin 1β (IL-1 β), IL-6, tumor necrosis factor-alpha (TNF α), prostaglandin E2 (PGE2), and nitric oxide (NO). Consequently, this cascade further

contributes to the initiation and maintenance of chronic pain. Therefore, exploring new signaling pathways may be challenging but meaningful, and this will be our focus in the future.

Reviewer B

1. Figure 2

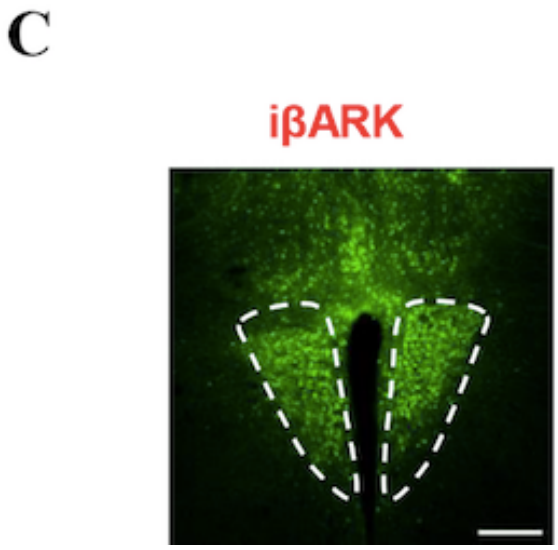
If applicable, please explain the white-dashed line and red line in the figure legend.



Reply: PVN is indicated by the white dashed line. The red line represents non-specific fluorescence staining. The content has been added in the figure legend. Please see Page 13, Line 437.

2. Figure 4

If applicable, please explain the white-dashed line in the figure legend.



Reply: PVN is indicated by the white dashed line. The content has been added in the figure legend. Please see Page 14, Line 454.

3. Citation

Please confirm if citations are needed, as you mentioned 'studies' in this sentence. Please revise. Please number references consecutively in the order in which they are first mentioned in the text.

Additionally, studies have also explored emerging chemogenetic methods to inhibit astrocytes and thus regulate central inflammation. However, it is not yet known whether targeting microglia and astrocytes effectively alleviates PCVP.

Reply: We have added the references. Please see Page 11, Line 370-375.