Proceedings of ICBioMed 2026 Symposium: Biomedical Engineering and Smart Medicine
DOI: 10.54254/2753-8818/2026.HZ38457

TGF-β2 Acts as a Context-Dependent Bidirectional Modulator at the Microglia-Neuron Crosstalk in Trigeminal Neuralgia

Yike Huang

Acabridge College, Shanghai, China
13564192558@163.com

Abstract. Trigeminal neuralgia (TN) is a severe chronic facial pain disorder with limited therapeutic options due to unclear underlying mechanisms. Neuroinflammation within the trigeminal ganglion (TG) microenvironment is increasingly recognized as a key driver of TN pathogenesis. Neuron-microglia crosstalk has been shown to be important for the neuroinflammation environment, yet the specific ligand–receptor interactions mediating this process remain poorly defined. This study aimed to identify critical neuroimmune signaling pathways in TN and to understand the functional role of the key ligand in microglia–neuron crosstalk. We performed single-cell RNA sequencing analysis followed by in vitro functional validation. In the human trigeminal microenvironment, microglia and neurons showed the strongest ligand–receptor coupling via TGFB2–TGFBR1/TGFBR2. Notably, transforming growth factor beta-2 (TGF-β2) tended to be downregulated in patients with TN. Exogenous TGF-β2 (10 ng/mL) protected both microglia and neurons from lipopolysaccharide (LPS)-induced cytotoxicity and promoted an M2-like phenotype in BV2 cells, upregulating arginase-1 (Arg1), interleukin-10 (IL-10) and mannose receptor (CD206) by over 2-fold. However, combined LPS and glutamate stimulation severely blunted endogenous TGF-β2 compensatory upregulation, mirroring the dual inflammatory and excitotoxic insults in TN. While TGF-β2 restored glutamate-suppressed Arg1, IL-10 and CD206 expression, the TβRI/II inhibitor LY2109761 failed to block this restorative effect in microglia, indicating that TGF-β2 exerts its effect on microglia through non canonical pathway. Yet, it completely reversed TGF-β2-mediated suppression of neuronal calcitonin gene-related peptide (CGRP). Our findings suggested that TGF-β2 acts as a context-dependent bidirectional modulator at the microglia–neuron interface. This discovery extended the conventional view of TGF-β2 as a purely anti-inflammatory factor and provided new therapeutic insights for TN by targeting neuroinflammation and cytotoxicity.

Keywords: Trigeminal neuralgia, Neuroinflammation, Excitotoxicity, TGF-β2

1. Introduction

Trigeminal neuralgia (TN) refers to a chronic facial pain condition that manifests as recurrent, severe paroxysmal pain affecting the ophthalmic, maxillary, and mandibular branches [1]. It is

© 2026 The Authors. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

DOI: 10.54254/2753-8818/2026.HZ38457

widely regarded as one of the most painful conditions experienced by humans [2] and leads to suicidal thoughts in over a third of patients interviewed [3]. The pathology and treatment of TN require substantial clinical attention, not only due to the intensity of pain, but also because of its tremendous damage to life quality, as pain attacks can be triggered by innocuous stimuli within widespread facial trigger zones during daily activities [1]. Current gold-standard therapeutic methods, including medications such as carbamazepine and oxcarbazepine and surgical interventions such as microvascular decompression, are often limited by significant side effects and high recurrence rates [4]. The severity, unpredictability and difficulty of treatment make TN a challenging clinical problem.

Apart from traditional therapies, recent studies have sought to address the problem at its root by investigating the role of neuroinflammation in TN pathogenesis. As a neuropathic pain disorder, TN is closely linked to immune dysregulation [5]. Evidence suggests that neuroimmune interaction within trigeminal microenvironment contribute to ion channel remodeling and demyelination, thereby promoting neuronal hypersensitivity and ectopic firing [5]. Elevated levels of classical inflammatory biomarkers—including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6)—have been detected in the serum, cerebrospinal fluid, and saliva of patients with TN [6]. These signaling molecules participate in bidirectional signaling among trigeminal ganglion neurons, microglia, astrocytes, oligodendrocytes, and infiltrating immune cells such as $CD8^+$ T cells. Recent advances have established using Mendelian Randomization causal relationships between presence of certain cytokines and TN risk [7, 8]. Furthermore, similar intercellular communication and increase in inflammation biomarkers have also been observed in TN animal models [9-12]. However, inflammation is mediated through intercellular communication rather than isolated cytokine elevation. Therefore, identifying critical ligand–receptor interactions and cell-type-specific signaling networks is essential for understanding how neuroinflammation is initiated and sustained in TN.

Publicly available human trigeminal tissue datasets from the GEO database [13-15] were utilized to perform scRNA-seq analysis, with the aim of better characterizing the local neuroinflammatory microenvironment in TN. By using cell-type annotation with intercellular communication analysis, we aimed to systematically identify key ligand–receptor interactions associated with TN pathogenesis. Candidate signaling pathways identified through CellChat analysis were further evaluated though in-vitro cell experiments to explore their potential roles between cellular crosstalk in neuroimmune regulation. Through focusing on local neuroinflammatory communication within human trigeminal tissues, this study may provide new insights into the cellular mechanisms underlying TN and reveal potential therapeutic targets for intervention.

2. Methods

2.1. Data acquisition

The data used for scRNA-seq was obtained from GSE197289 [13, 15] on gene expression omnibus. The file used for Seurat processing is snRNA-seq_human_raw_counts.RDS.

2.2. Seurat processing

We used R version 4.3.3 for analyzation. After quality control, cells with nFeature_RNA between 200 and 6,000 and percent.mt < 20% were retained. Normalization of the data was subsequently carried out using the LogNormalize approach with a scaling factor of 10,000. Genes with high

DOI: 10.54254/2753-8818/2026.HZ38457

variability were extracted via the variance-stabilizing transformation (VST), and the 2,000 most variable were retained for downstream procedures. Following PCA, the first 30 principal components (PCs) were utilized for graph-based clustering via the FindClusters algorithm at a resolution of 0.5. Uniform manifold approximation and projection (UMAP) was then adopted for data visualization.

2.3. Cell annotation

After clustering, marker genes for each cluster were identified using the FindAllMarkers function. Cell types were assigned to individual clusters based on the expression of well-established canonical marker genes reported in the literature.

2.4. CellChat analysis

Intercellular communication was inferred using CellChat with the CellChatDB.human ligand–receptor database. Interactions with fewer than 10 cells (min.cells = 10) were filtered out to ensure robustness. Global communication analysis revealed prominent crosstalk between microglia and neurons. We subsequently focused on these two cell types. All communication probabilities were exported for both directions (microglia-to-neuron and neuron-to-microglia), as well as for autocrine signaling (microglia-to-microglia and neuron-to-neuron).

2.5. Gene expression analysis

Following CellChat, we obtained RNA sequencing data of whole blood cells in patients with trigeminal neuralgia and pain-free healthy comparisons from GSE186505 [16] on gene expression omnibus. We conducted Limma analysis on genes of interest and compared expression level of TGF-β2 between control group and TN group for the purpose of deducing the role of TGF-β2 in inflammation. The analyzation and visualization were made using https://www.bioinformatics.com.cn.

2.6. Cell culture and reagents

BV2 murine microglial and HT22 murine hippocampal neuronal cell lines were obtained from Procell Life Science Technology Co., Ltd. (Wuhan, China). These cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 mg/mL streptomycin, and 100 mg/mL amphotericin, at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. Culture medium was refreshed every two days.

For experiments requiring microglia–neuron crosstalk, a non-contact co-culture system was established using 6-well Transwell plates. BV2 cells were seeded in the lower chamber at 2×10^5 cells/well, while HT22 cells were seeded in the upper insert at 1×10^5 cells/insert. After overnight attachment, the co-cultures were subjected to the indicated treatments. This system allows free exchange of soluble factors while keeping the two cell populations physically separate, enabling subsequent independent analysis of BV2 and HT22 cells.

Key reagents included: L-glutamate, recombinant human Transforming Growth Factor Beta-2 (TGF-β2), lipopolysaccharide (LPS), and the Cell Counting Kit-8 (Sangon, Cat#ZEF1DX8). ELISA kit used was Mouse calcitonin gene-related peptide (CGRP) Elisa kit (Jonlnbio, Cat#JL11651).

DOI: 10.54254/2753-8818/2026.HZ38457

2.7. Cell viability test

BV2 and HT22 cells were individually seeded in 96-well plates at 5×10^3 cells per well and left to adhere overnight. Following the experimental protocols, the cells received corresponding treatments and were then incubated. After 48 h, 10 μL of CCK-8 solution was introduced into each well, and the plates were further incubated for 2 h at 37 °C. Optical density was subsequently read at 450 nm on a BioTek Synergy H1 microplate reader.

2.8. Phalloidin staining

Sterile glass coverslips positioned in 24-well plates were seeded with cells at a density of 2×10^4 cells per well. After overnight attachment, cells were treated according to experimental settings for 48 h. After treatment, the cells underwent two washes with preheated PBS and were then fixed in 4% PFA for 15 minutes at ambient temperature. After permeabilization with 0.1% Triton X-100 in PBS for 10 min, cells were stained with Phalloidin for 30 min in the dark to label F-actin. Coverslips were mounted and imaged using fluorescence microscope. For each experimental group, at least 6 random fields were captured. A BV2 cell was classified as "activated" if it displayed processes and intense phalloidin staining, in contrast to the typical round resting shape. We calculated the percentage of activated cells by dividing the number of activated cells by the total cell count and then multiplying the quotient by 100%.

2.9. Quantitative real-time PCR analysis of inflammatory and anti-inflammatory markers

We isolated cellular total RNA with the RNA extraction kit (Accurate Biotechnology, Hunan) following the manufacturer's protocol. A NanoDrop One spectrophotometer (Thermo Scientific) was used to measure RNA concentration and purity. From each sample, 1 μg of total RNA was reverse-transcribed into cDNA using the Hifair III 1st Strand cDNA Synthesis SuperMix (Yeasen, Cat# HB221101). Real-time qPCR was then run on a BIO-RAD CFX96 system with Hieff qPCR SYBR Green Master Mix under the following thermal profile: 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s, 60 °C for 20 s, and 72 °C for 20 s. All primer sequences are given in Table 1.

Table 1. The primer sequences used are as followed

Gene	Forward Primer	Reverse Primer
GAPDH	CGACTTCAACAGCAACTCCCACTCTTCC	TGGGTGGTCCAGGGTTTCTTACTCCTT
IL-1β	GTGGCTGTGGAGAAGCTGTG	GAAGGTCCACGGGAAAGACAC
TNF-α	TCTTCTCATTCCTGCTTGTGG	ATGAGAGGGAGGCCATTTG
IL-6	CAAAGCCAGAGTCCTTCAGAG	AGCATTGGAAATTGGGGTAG
IL-10	TCACTCTTCACCTGCTCCAC	CTATGCTGCCTGCTCTTACTC
CD206	CCCTCAGCAAGCGATGTGC	GGATACTTGCCAGGTCCCCA
TGF-β2	CCGCATCTCCTGCTAATGTTG	AATAGGCGGCATCCAAAGC

We determined relative mRNA expression by the 2^−ΔΔCt method, using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the endogenous reference.

DOI: 10.54254/2753-8818/2026.HZ38457

2.10. ELISA

The concentration of CGRP was measured using Mouse CGRP Elisa kit (Jonlnbio, Cat#JL11651) strictly following the manufacturer's protocol. We recorded the absorbance at 450 nm with a BioTek Synergy H1 plate reader.

2.11. Statistical analysis

All data were analyzed using GraphPad Prism 11. Results are expressed as mean ± SEM from at least three independent experiments. We performed one-way ANOVA with Tukey's post hoc test for multiple-group comparisons. A P-value < 0.05 was set as the significance threshold. Significance levels are indicated as *P < 0.05, **P < 0.01, ***P < 0.001, and **P < 0.0001.

3. Results

3.1. Identification of major cell populations including neurons and microglia

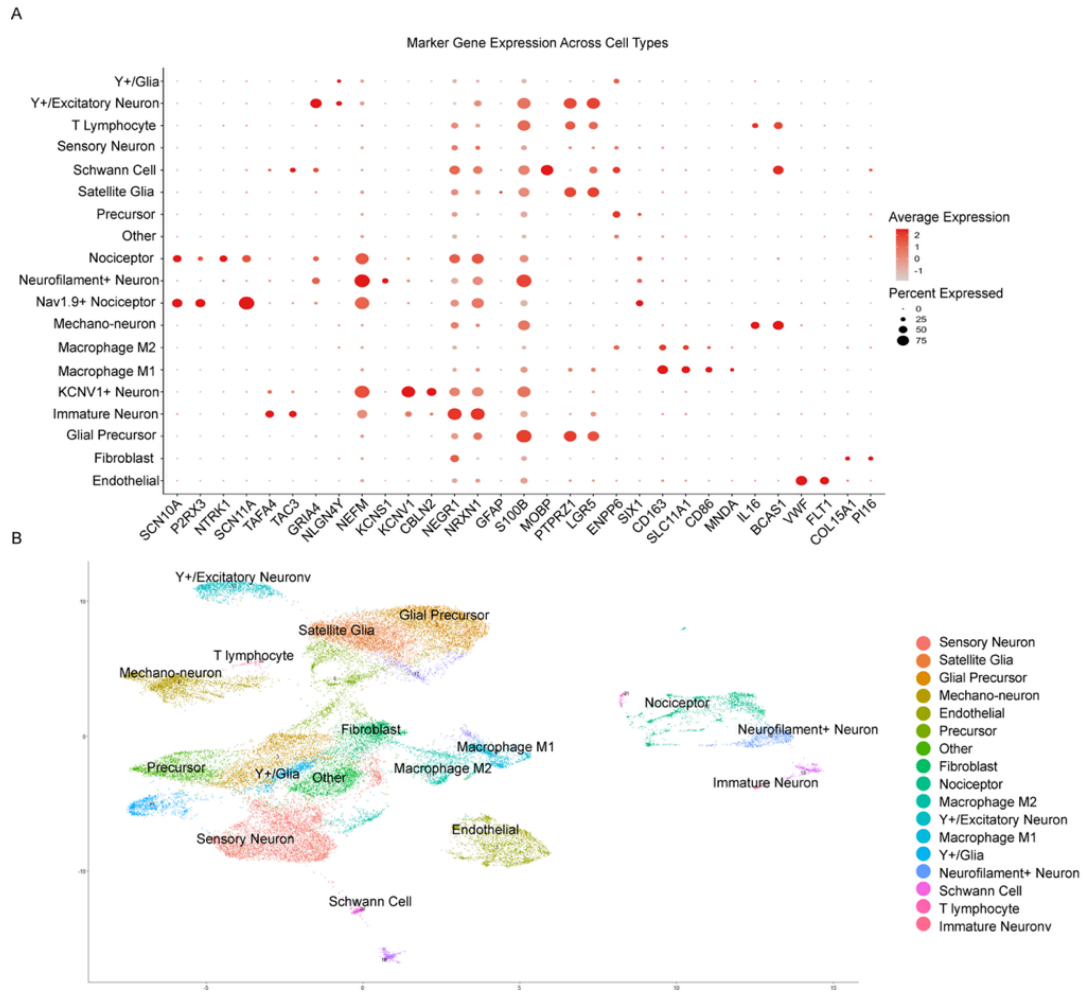
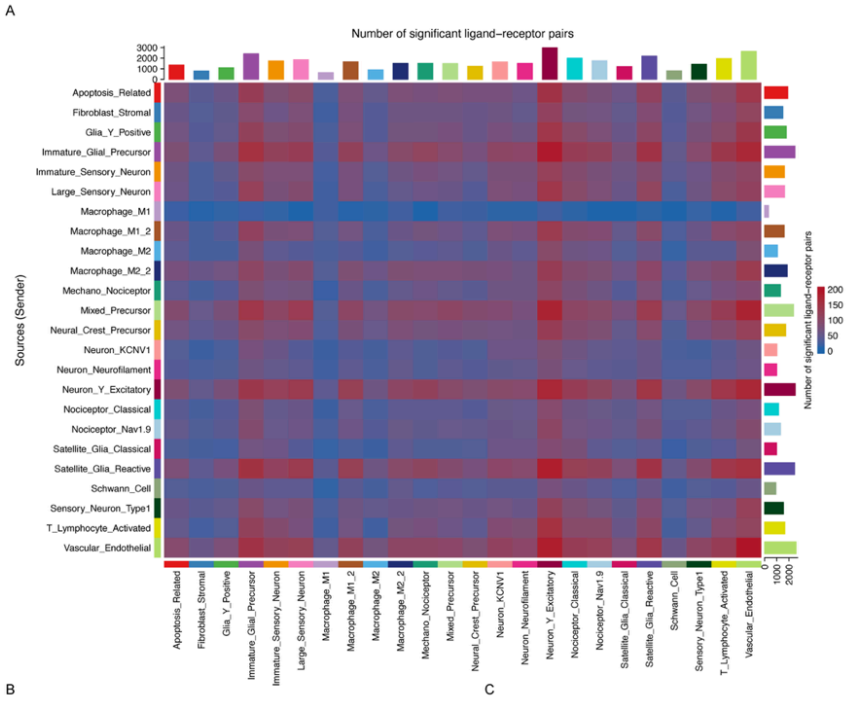


Figure 1. Characterization of major cell clusters and marker gene signatures.(A). The marker gene expression across cell types. Deeper color indicates higher average expression level. Larger dot indicates expression by higher percentage of cells. (B). The UMAP visualization of cell clustering result and annotations

DOI: 10.54254/2753-8818/2026.HZ38457

By using data from GSE197289, we were able to investigate the major cell types in trigeminal region. 31672 genes were included in the processing. A total of 36575 cells out of 38028 were analyzed following the quality control. Based on their different marker genes (Figure 1A), we were able to conduct clustering and cell-type annotation. UMAP visualized the result, as shown in Figure 1B. 23 cell types are identified, including endothelial, fibroblast, glial precursor, immature neuron, KCNV1+ neuron, macrophage M1, macrophage M2, mechano-neuron, Nav1.9+ Nociceptor, neurofilament+ neuron, nociceptor, precursor, satellite glia, Schwann cell, sensory neuron, T lymphocyte, Y+/excitatory neuron, Y+/Glia.

3.2. Identification of microglia–neuron crosstalk via cell-cell communication analysis



B

Table: major inflammation pathways in order of communication probabilaty

source	target	ligand	receptor	prob
Sensory_Neuron_Type1	Sensory_Neuron_Type1	FGF7	FGFR1	2.3E-08
Sensory_Neuron_Type1	Large_Sensory_Neuron	FGF7	FGFR1	9.3E-09
Sensory_Neuron_Type1	Macrophage_M2_2	FGF7	FGFR1	8.2E-09
Sensory_Neuron_Type1	Macrophage_M2	FGF7	FGFR1	6.2E-09
Sensory_Neuron_Type1	Neuron_Neurofilament	FGF7	FGFR1	4.9E-09
Sensory_Neuron_Type1	Sensory_Neuron_Type1	IGF1	IGF1R	3.7E-09
Neuron_Neurofilament	Sensory_Neuron_Type1	FGF1	FGFR1	3.6E-09
Neuron_KCNV1	Sensory_Neuron_Type1	FGF1	FGFR1	3.6E-09
Macrophage_M2_2	Sensory_Neuron_Type1	FGF7	FGFR1	3E-09
Sensory_Neuron_Type1	Sensory_Neuron_Type1	TGFB2	TGFbR1_R2	3E-09
Sensory_Neuron_Type1	Macrophage_M2_2	FGF7	FGFR2	2.9E-09
Large_Sensory_Neuron	Sensory_Neuron_Type1	FGF2	FGFR1	2.8E-09
Sensory_Neuron_Type1	Sensory_Neuron_Type1	TGFB2	ACVR1B_TGFbR2	2.3E-09
Macrophage_M2_2	Sensory_Neuron_Type1	TGFB2	TGFbR1_R2	1.6E-09
Sensory_Neuron_Type1	Large_Sensory_Neuron	IGF1	IGF1R	1.6E-09
Neuron_Neurofilament	Large_Sensory_Neuron	FGF1	FGFR1	1.5E-09
Sensory_Neuron_Type1	Macrophage_M1_2	FGF7	FGFR1	1.5E-09
Neuron_Neurofilament	Sensory_Neuron_Type1	FGF9	FGFR1	1.5E-09

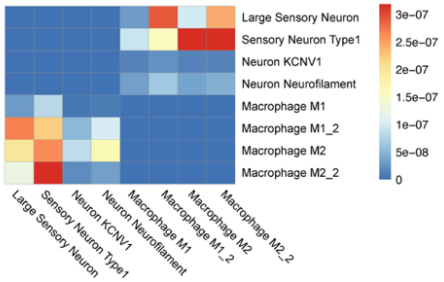


Figure 2. Global landscape of cell-cell communication networks and inflammatory signaling. (A). Number of significant ligand-receptor pairs. Deep red color indicates more significant ligand-receptor pairs between corresponding source cell types. Summary of major inflammation pathways in order of communication probability.(C). Bidirectional communication weight between neuron and macrophage. Deep red color indicates higher communication weight

DOI: 10.54254/2753-8818/2026.HZ38457

To systematically characterize intercellular communication in the human trigeminal microenvironment, we applied CellChat to infer ligand–receptor interactions among the annotated cell types. Quantitative analysis of the significant ligand-receptor pairs and the distribution of cell category in intercellular communication network revealed that microglia and neurons formed prominent signaling axis (Figure 2A). This observation aligns with the established role of microglia–neuron crosstalk in neuroinflammation and neuropathic pain, and it prompted us to focus subsequent analyses on this cell pair.

To pinpoint the specific molecular mediators, we next examined individual ligand–receptor pairs. A total of 201 communicating pairs were identified. Among the top-ranked pairs, there were ligands of fibroblast growth factor (FGF), insulin-like growth factor (IGF) and transforming growth factor beta (TGF-β) families (Figure 2B). Since IGF and FGF exhibit complex, context-dependent, and often opposing effects on pain modulation, TGF-β is a more direct and mechanistically tractable target for studying neuron–microglia crosstalk. So we targeted primarily on TGF-β family. TGFB2–TGFBR1 and TGFB2–TGFBR2 emerged as the most prominent interactions within the TGF-β pathway, ranking top in communication probability apart from growth factors.

This result is consistent with the visualization of bidirectional communication weight by specific cell type between macrophage and neuron. The source and target cell of TGF-β pathway are the most frequently communicating cell types in the trigeminal ganglion region (Figure 2C).

Given the known immunomodulatory and neuroprotective functions of TGF-β signaling, these findings raise the possibility that microglia-derived TGF-β2 may play a homeostatic role in the trigeminal microenvironment, a hypothesis that warrants further experimental investigation.

3.3. Potential importance of TGF-β2 as a target in the TN pathway

According to current literature, TGF-β2 mainly plays anti-inflammatory and protective roles in the nervous system and also participates in maintaining immune homeostasis [17]. Researches support that it acts on microglia to inhibit their excessive activation, thereby mitigating neuroinflammation [18]. The analyzation of gene expression data from GSE186505 showed a trend of decrease in TGFB2 expression in TN patients (Figure 3), which provided insight for the hypothesis that the protective function of TGF-β2 might be exhausted under inflammation.

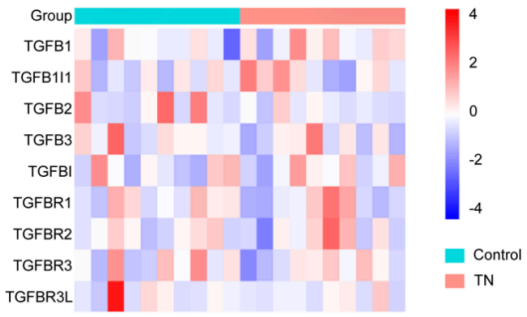


Figure 3. A heat map visualizing the relative expression of TGFB related genes

3.4. Protective effect of TGF-β2 on microglia and neuron against inflammatory injury

We first examined the dose-dependent effect of TGF-β2 on the viability of BV2 microglia and HT22 neurons at 0, 1, 5, 10, 20 ng/mL. In HT22 cells, TGF-β2 at concentrations ranging from 1 to 20 ng/mL did not produce any significant change in CCK-8 absorbance compared with the untreated

DOI: 10.54254/2753-8818/2026.HZ38457

control (0 ng/mL) (Figure 4A, $P > 0.05$ for all concentrations). In contrast, treatment of BV2 cells with 5 and 10 ng/mL TGF-β2 resulted in a significant increase in cell viability relative to the control group (Figure 4B, $P < 0.01$). Lower concentrations (1 ng/mL) and the higher concentration (20 ng/mL) did not significantly alter BV2 viability (Figure 4B). These results indicate that 10 ng/mL TGF-β2 may play a protective role to BV2 microglial under normal culturing conditions, but its function under inflammation conditions as in the case of TN is still unknown.

We next determined whether TGF-β2 could counteract inflammation-associated cytotoxicity. Exposure to 1 ng/mL LPS for 48 h led to a marked reduction in cell viability in both BV2 and HT22 cells when compared with the respective untreated controls (Figure 4C, Figure 4D). Co-treatment with 10 ng/mL TGF-β2 significantly attenuated the LPS-induced viability decline in both cell lines. In BV2 cells, viability in the LPS+TGF-β2 group was significantly higher than that in the LPS-alone group (Figure 4C). Similarly, in HT22 cells, the presence of TGF-β2 restored viability to a level significantly above the LPS group (Figure 4D). These findings demonstrated that TGF-β2 protects both microglia and neurons from LPS-triggered viability loss, consistent with a homeostatic, anti-inflammatory role for TGF-β2 in the trigeminal microenvironment.

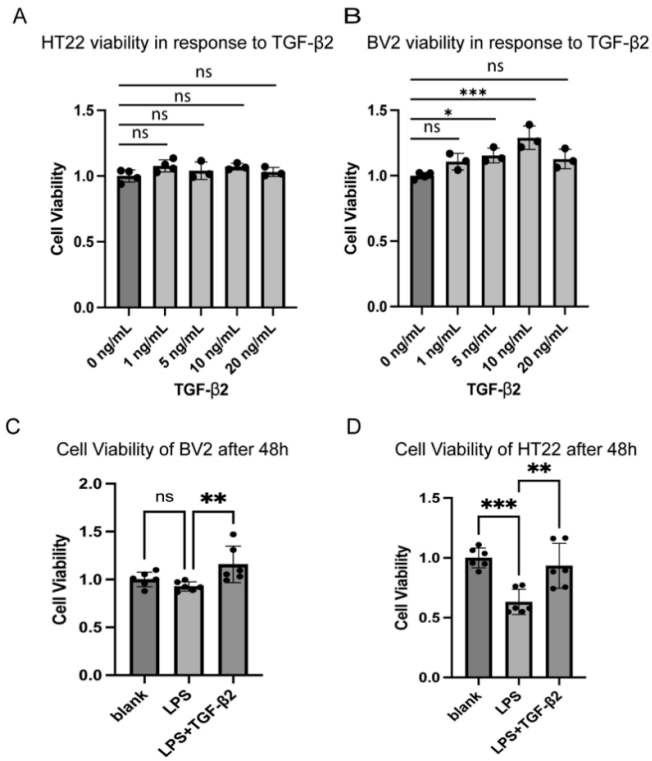


Figure 4. Concentration-response of TGF-β2 in BV2 and HT22 cells. (A-B). Dose-dependent effect of TGF-β2 on BV2 and HT22 cell viability. BV2 and HT22 cells were exposed to different concentrations of TGF-β2 for 48 h. Cell viability was assessed by CCK-8 assay. (C-D). TGF-β2 protects against LPS-induced cytotoxicity. BV2 and HT22 cells were exposed to 1 ng/mL LPS in the absence or presence of 10 ng/mL TGF-β2 for 48 h. Cell viability was assessed by CCK-8 assay. All data are mean ± SEM (n = 6). One-way ANOVA with Tukey's post hoc test

DOI: 10.54254/2753-8818/2026.HZ38457

3.5. Counteraction of LPS-induced BV2 activation by TGF-β2 in a co-culture environment

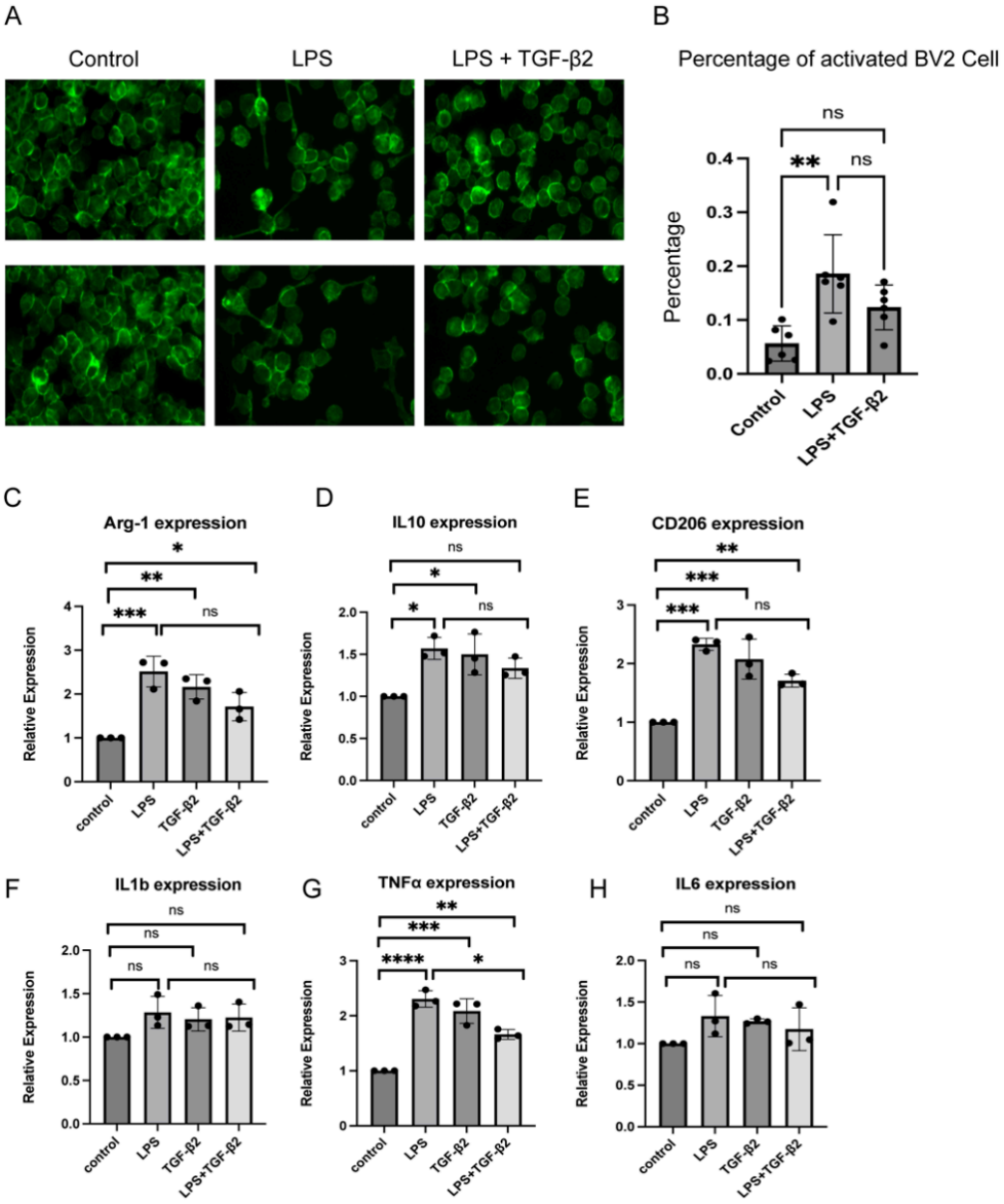


Figure 5. TGF-β2 suppresses microglial activation and modulates Inflammatory gene expression. (A-B). TGF-β2 attenuates LPS-induced BV2 morphological activation in co-culture. A. Representative images of F-actin (phalloidin, green) in BV2 cells after 48 h of co-culture with HT22 cells under the indicated treatments. B. Quantification of the percentage of activated BV2 cells. (C-H). TGF-β2 modulates inflammatory gene expression in BV2 cells. BV2 cells were treated with control, LPS (1 ng/mL), TGF-β2 (10 ng/mL), or LPS+TGF-β2 for 48 h. mRNA levels of (C) Arg-1, (D) IL-10, (E) CD206 (F) IL-1β, (G) TNF-α, (H) IL-6, were measured by qPCR and normalized to GAPDH. Data are mean ± SEM (n = 3). One-way ANOVA with Tukey's post hoc test.

To directly visualize the effect of TGF-β2 on microglial activation in the presence of neurons, BV2 and HT22 cells were co-cultured for 48 h with or without LPS and TGF-β2, and BV2 morphology was assessed by phalloidin staining. The control co-culture group showed a low

DOI: 10.54254/2753-8818/2026.HZ38457

proportion of activated BV2 cells (Figure 5A, Figure 5B). LPS stimulation (1 ng/mL) significantly increased the percentage of activated cells compared with control ($P < 0.01$). Co-treatment group with TGF-β2 (10 ng/mL) showed decreasing trend in ratio of activated cell compared to the LPS alone group, and the level in the LPS+TGF-β2 group was not statistically different from the control condition ($P > 0.05$). These morphological data demonstrate that TGF-β2 effectively attenuates LPS-induced microglial activation within a neuron–microglia interactive milieu.

To further dissect the polarization state of BV2 cells, we performed qPCR for a panel of pro-inflammatory (IL-1β, TNF-α, IL-6) and anti-inflammatory (IL-10, Arg1, CD206) markers after 48 h of treatment with LPS and/or TGF-β2 in BV2 monoculture. LPS induced a broad upregulation of all markers, consistent with a mixed inflammatory response (Figure 5C–H). TGF-β2 alone affected both kinds of inflammatory cytokines and markedly elevated the anti-inflammatory markers Arg1 and CD206 to more than 2-fold of control levels ($P < 0.01$ for Arg1, $P < 0.001$ for CD206), indicating a shift toward an anti-inflammatory M2-like phenotype. When co-administered, TGF-β2 showed slight attenuation to the LPS-driven surge of cytokines, although these cytokines remained modestly above control. These results suggest that TGF-β2, even in the presence of a strong M1-polarizing stimulus, promotes an anti-inflammatory, M2-biased microglial phenotype.

3.6. Blunted compensatory upregulation of microglial TGF-β2 upon co-exposure to LPS and glutamate

To model the combined inflammatory and excitotoxic stress that occurs in trigeminal neuralgia, BV2-HT22 co-cultures were exposed to LPS (1 ng/mL), glutamate (100 μM), or their combination for 48 h. TGFB2 mRNA levels in BV2 cells were then measured by qPCR. LPS alone increased TGFB2 expression to approximately 4-fold of the control level, and glutamate alone raised it to about 3-fold (Figure 6). Strikingly, the combined LPS + glutamate treatment resulted in only a modest, less than 1.5-fold increase. This blunted response suggests that when multiple pathogenic stimuli converge, microglia fail to mount a sufficient compensatory upregulation of protective TGF-β2, which may contribute to the progression of neuroinflammation.

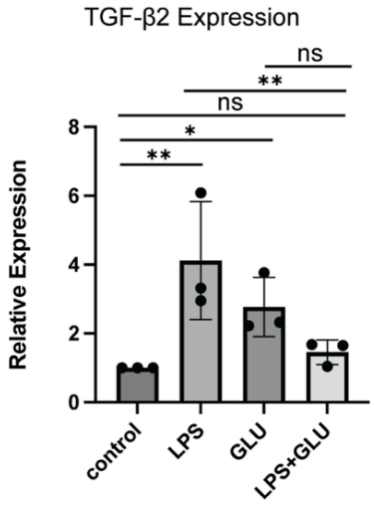


Figure 6. Combined LPS and glutamate stress blunts microglial TGF-β2 upregulation. BV2-HT22 co-cultures were treated with LPS (1 ng/mL), glutamate (100 μM), or LPS + glutamate for 48 h. TGFB2 mRNA in BV2 cells was quantified by qPCR. Values are expressed as fold change relative to control. Mean ± SEM, n = 3. One-way ANOVA with Tukey's post hoc test

DOI: 10.54254/2753-8818/2026.HZ38457

3.7. TGF-β2 restores anti-inflammatory gene expression despite TGF-β receptor inhibition

We next examined whether supplying exogenous TGF-β2 could rescue the anti-inflammatory capacity of BV2 cells under excitotoxic conditions, and whether this effect was receptor-dependent. BV2-HT22 co-cultures were treated with glutamate (100 μM) alone or in combination with TGF-β2 (10 ng/mL) and the TβRI/II inhibitor LY2109761 (20 nM) for 24 h. Glutamate significantly suppressed the expression of Arg1, CD206, and IL-10 in BV2 cells compared with untreated co-cultures (Figure 7). The addition of TGF-β2 upregulated all three markers relative to the glutamate group. Interestingly, co-treatment with LY2109761 didn't abrogate the TGF-β2-mediated increase in Arg1, CD206, and IL-10 back to levels comparable to the glutamate-alone group. These results demonstrated that TGF-β2 might not only act through canonical TGF-β receptor signaling to reinstate microglial anti-inflammatory gene expression in an excitotoxic microenvironment.

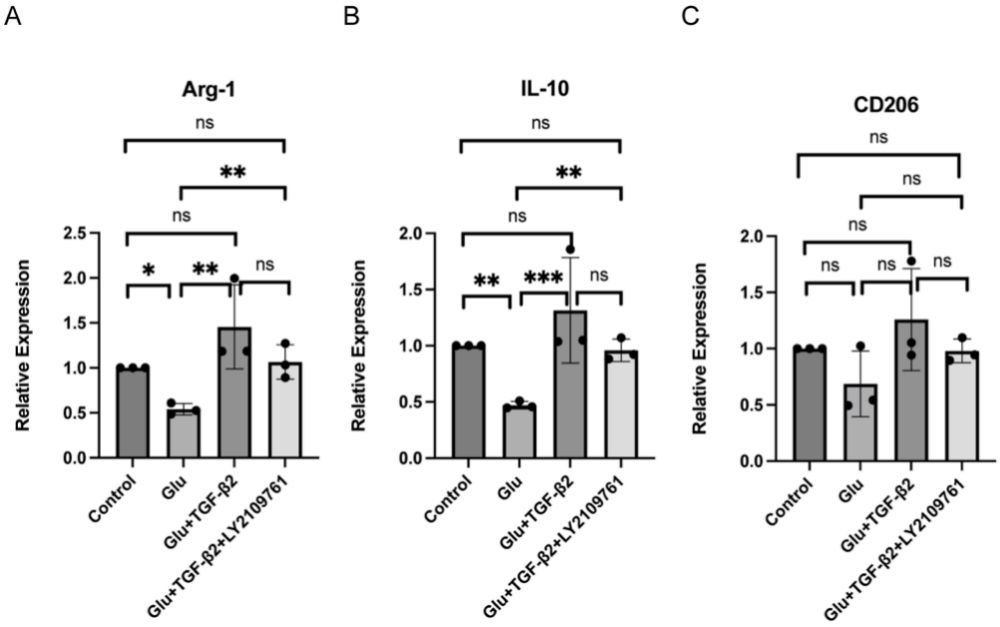


Figure 7. TGF-β2 rescues anti-inflammatory gene expression in glutamate-exposed BV2. BV2-HT22 co-cultures were treated with control, glutamate (100 μM), Glu+TGF-β2 (10 ng/mL), or Glu+TGF-β2+LY2109761 (20 nM) for 24 h. qPCR analysis of (A) Arg1, (B) IL-10, and (C) CD206 in BV2 cells. Data are mean ± SEM, n = 3. One-way ANOVA with Tukey's post hoc test

3.8. Reduction of neuronal CGRP and reversal of hyperexcitability by TGF-β2 via receptor-dependent signaling

To directly assess the functional consequence on neurons, we measured CGRP protein levels—a marker of neuronal hyperexcitability—in HT22 cell lysates from the same co-culture experiments. Glutamate stimulation caused a robust increase in CGRP expression (Figure 8). Co-treatment with TGF-β2 (10 ng/mL) completely normalized CGRP levels, which became statistically indistinguishable from the untreated control ($P > 0.05$). Crucially, when LY2109761 was added together with TGF-β2, the CGRP level rebounded to a level not different from the glutamate alone group ($P > 0.05$ vs. Glutamate). This indicates that TGF-β2, through its cognate receptor, effectively suppresses the glutamate-induced hyperexcitability of trigeminal neurons, providing a mechanistic basis for its analgesic potential.

DOI: 10.54254/2753-8818/2026.HZ38457

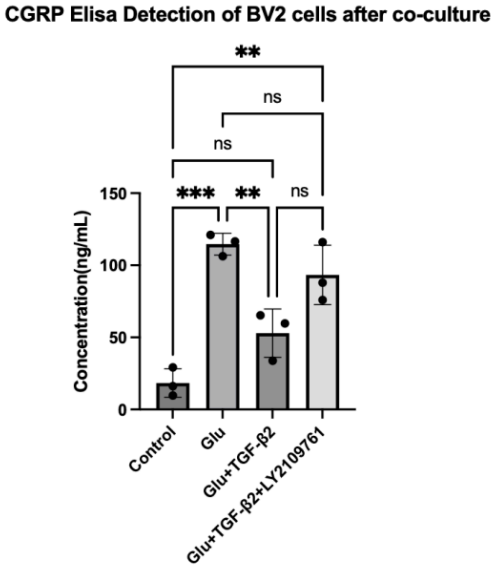


Figure 8. TGF-β2 suppresses glutamate-induced CGRP expression in HT22 neurons. BV2-HT22 co-cultures were treated as described in Figure. CGRP protein levels in HT22 lysates were measured by ELISA and normalized to total protein. Mean ± SEM, n = 3. One-way ANOVA with Tukey's post hoc test

4. Discussion

In the present study, we revealed that TGF-β2 acts as a context-dependent bidirectional modulator at the microglia–neuron interface within the trigeminal microenvironment, with potential implications for the pathophysiology of trigeminal neuralgia. Through single-cell RNA sequencing of human trigeminal ganglia, we identified TGFB2–TGFBR1/TGFBR2 as one of the most prominent ligand–receptor pairs between microglia and neurons, and analysis of whole-blood transcriptomic data from TN patients showed a trend toward reduced TGFB2 expression. Functionally, exogenous TGF-β2 protected both microglia and neurons from LPS-induced cytotoxicity, promoted an M2-like anti-inflammatory phenotype in microglia, and suppressed glutamate-induced CGRP expression in neurons. However, we also found that TGF-β2 was not merely a protective signal: it exerted cell-type-specific and context-dependent effects, acting through canonical receptor signaling on neurons while appearing to engage non-canonical pathways on microglia under excitotoxic conditions. These findings extend the conventional view of TGF-β2 as a purely anti-inflammatory factor and suggest that its role in TN is shaped by the local inflammatory and excitotoxic milieu.

A key observation was that the endogenous compensatory upregulation of microglial TGF-β2, which occurred robustly in response to LPS alone (~4-fold) or glutamate alone (~3-fold), was severely blunted when both stimuli were combined, resulting in only a modest increase (<1.5-fold). This failure of endogenous TGF-β2 feedback under dual inflammatory and excitotoxic stress, a condition that mirrors the pathological microenvironment of TN, suggests that when microglia face concurrent TLR4 activation and neuronal damage-associated signals, their homeostatic transcriptional program may be overwhelmed, preventing sufficient TGF-β2 production. This phenomenon is consistent with previous reports that inflammatory activation can disrupt TGF-β signaling [19, 20], and may reflect a state of "microglial paralysis" or transcriptional tolerance. Importantly, we also observed that glutamate alone suppressed anti-inflammatory markers (Arg1, IL-10, CD206) in microglia, whereas LPS alone increased them, highlighting the fundamentally

DOI: 10.54254/2753-8818/2026.HZ38457

different nature of these two stimuli. LPS triggers a classic inflammatory response with intrinsic negative-feedback mechanisms, while glutamate acts indirectly through neuronal injury and damage-associated molecular patterns, which may drive microglia toward a dysfunctional, metabolically stressed state rather than a classical inflammatory program [21, 22]. These findings indicate that in TN, where both inflammation and excitotoxicity coexist, the loss of endogenous TGF-β2 compensation may contribute to the persistence and aggravation of neuroinflammation.

When we examined the effects of exogenous TGF-β2 on microglia, we found a more complex picture. In BV2 monoculture, TGF-β2 upregulated not only anti-inflammatory markers (Arg1, IL-10, CD206) but also pro-inflammatory cytokines (IL-1β, TNF-α, IL-6), suggesting that TGF-β2 may induce a broader activated state rather than a simple M2 polarization. This seemingly contradictory effect may reflect the inherent heterogeneity of microglial activation, where both inflammatory and repair-related genes are transcriptionally initiated, or may arise from branched TGF-β signaling that engages not only canonical Smad2/3 but also MAPK cascades (ERK, JNK, p38) and NF-κB, whose net outcome depends on the balance between these pathways and the cellular context [23]. The most striking mechanistic finding, however, was that in glutamate-injured co-cultures, exogenous TGF-β2 restored Arg1, IL-10 and CD206 expression, yet the TβRI/II dual inhibitor LY2109761, which effectively blocks canonical Smad2/3 phosphorylation, failed to abolish this restorative effect. This indicates that TGF-β2 may act through non-canonical (Smad-independent) pathways, or even via alternative receptors, to rescue anti-inflammatory gene expression in microglia under excitotoxic conditions. This observation contrasts with studies where LY2109761 successfully inhibited TGF-β1-mediated microglial/macrophage polarization in traumatic brain injury [24], raising the possibility of isoform-specific differences between TGF-β1 and TGF-β2 or context-dependent variations in signaling pathway utilization.

In marked contrast to its effects on microglia, TGF-β2's action on neurons was entirely receptor-dependent. Glutamate stimulation caused a robust increase in CGRP protein levels in HT22 neurons, a well-established marker of neuronal hyperexcitability and nociceptive activation [25], and co-treatment with TGF-β2 completely normalized CGRP levels. This suppression was fully reversed by LY2109761, demonstrating that TGF-β2 acts through its canonical receptors to directly dampen neuronal hyperexcitability. This cell-type-specific signaling divergence—non-canonical on microglia versus canonical on neurons—has important therapeutic implications. In TN, neuronal hyperexcitability within the trigeminal ganglion is a core pathological feature [1], and the ability of TGF-β2 to suppress CGRP through receptor-dependent signaling suggests direct analgesic potential. At the same time, the preservation of beneficial microglial functions through non-canonical pathways raises the possibility of designing interventions that bypass full receptor activation, potentially avoiding unwanted neuronal side effects. Several limitations should be acknowledged: our use of immortalized BV2 microglia and HT22 hippocampal neurons may not fully recapitulate primary or human cell behavior; the scRNA-seq analysis relied on publicly available datasets with limited TN-specific samples; and in vivo validation in TN animal models is needed to assess pain behavior and inflammatory markers following TGF-β2 administration. Despite these, our findings demonstrate that TGF-β2 is not a uniform protective signal but a critical, context-dependent regulator at the microglia–neuron interface and suggest that future therapeutic strategies should be tailored according to the severity of inflammation and cell-type-specific signaling rather than simply enhancing or blocking TGF-β signaling as a whole.

DOI: 10.54254/2753-8818/2026.HZ38457

5. Conclusion

In summary, this study demonstrated that TGF-β2 is not merely a protective signal but a critical bidirectional modulator at the microglia–neuron interface. Exogenous TGF-β2 exhibits context-dependent effects. It can promote both inflammatory and anti-inflammatory programme in microglia, yet under pathological conditions it selectively restores repair-associated genes through non-canonical pathways while simultaneously suppressing neuronal hyperexcitability through canonical receptor signaling. These findings deepen our understanding of the neuroimmune mechanisms underlying TN and suggest that future therapeutic strategies should be tailored according to the severity of inflammation and cell-type-specific signaling, rather than simply enhancing or blocking TGF-β signaling as a whole. The recognition of non-canonical TGF-β2 actions opens new avenues for developing targeted interventions that preserve beneficial microglial functions while avoiding unwanted neuronal side effects.

References

[1] Cruccu, G., Stefano, G. D., & Truini, A. (2020). Trigeminal neuralgia. *N. Engl. J. Med., 383*(8), 754–762. doi: 10.1056/NEJMra1914484.
[2] Granzer-Corno, L., Rana, R., Dick, B. D., & Sankar, T. (2025). Different scale, different pain? Discordant pain measurements after surgery for trigeminal neuralgia. *World Neurosurg., 194*, 123481. doi: 10.1016/j.wneu.2024.11.064.
[3] Fishbein, N. S., Bakhshaie, J., & Greenberg, J. (2025). Suicidal ideation and self-injury in trigeminal neuralgia. *J. Pain Res., 18*, 2003–2010. doi: 10.2147/JPR.S493649.
[4] Worm, J., et al. (2025). Five-year prospective outcomes of medical management and microvascular decompression in trigeminal neuralgia. *J. Neurol., 272*(10), 701. doi: 10.1007/s00415-025-13447-9.
[5] Li, Z., et al. (2025). Molecular mechanisms of chronic pain and therapeutic interventions. *MedComm, 6*(8), e70325. doi: 10.1002/mco2.70325.
[6] Dong, B., Xu, R., & Lim, M. (2023). The pathophysiology of trigeminal neuralgia: A molecular review. *Journal of Neurosurgery, 139*(5), 1471–1479. https://doi.org/10.3171/2023.2.JNS23274
[7] Lai, S., Li, H., Xing, Y., Wu, D., Wang, L., & Liang, Q. (2024). Exploring the role of inflammatory biomarkers in trigeminal neuralgia. *Brain, Behavior, & Immunity - Health, 43*, 100930. https://doi.org/10.1016/j.bbih.2024.100930
[8] Shang, H., et al. (2025). Causal relationship between circulating inflammatory cytokines and the risk of trigeminal neuralgia: A Mendelian randomization study. *Brain and Behavior, 15*(4), e70463. https://doi.org/10.1002/brb3.70463
[9] Demartini, C., et al. (2025). Insights into the involvement of TRPA1 channels in the neuro-inflammatory machinery of trigeminal neuralgia. *Molecules, 30*(9), 1884. https://doi.org/10.3390/molecules30091884
[10] Koizumi, M., et al. (2021). P2X3 receptor upregulation in trigeminal ganglion neurons through TNFα production in macrophages contributes to trigeminal neuropathic pain in rats. *Journal of Headache and Pain, 22*(1), 31. https://doi.org/10.1186/s10194-021-01244-4
[11] Liu, M., Li, Y., Zhong, J., Xia, L., & Dou, N. (2021). The effect of IL-6/Piezo2 on the trigeminal neuropathic pain. *Aging, 13*(10), 13615–13625. https://doi.org/10.18632/aging.202887
[12] Shi, S., et al. (2020). PD-L1 and PD-1 expressed in trigeminal ganglia may inhibit pain in an acute migraine model. *Cephalalgia International Journal of Headache, 40*(3), 288–298. https://doi.org/10.1177/0333102419883374
[13] Barrett, T., et al. (2013). NCBI GEO: Archive for functional genomics data sets--update. *Nucleic Acids Research, 41*(Database issue), D991–995. https://doi.org/10.1093/nar/gks1193
[14] Edgar, R., Domrachev, M., & Lash, A. E. (2002). Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Research, 30*(1), 207–210. https://doi.org/10.1093/nar/30.1.207
[15] Yang, L., et al. (2022). Human and mouse trigeminal ganglia cell atlas implicates multiple cell types in migraine. *Neuron, 110*(11), 1806–1821.e8. https://doi.org/10.1016/j.neuron.2022.03.003
[16] Clough, E., et al. (2024). NCBI GEO: Archive for gene expression and epigenomics data sets: 23-year update. *Nucleic Acids Research, 52*(D1), D138–D144. https://doi.org/10.1093/nar/gkad965

DOI: 10.54254/2753-8818/2026.HZ38457

[17] Wang, M.-Y., Liu, W.-J., Wu, L.-Y., Wang, G., Zhang, C.-L., & Liu, J. (2023). The research progress in transforming growth factor-β2. *Cells, 12*(23), 2739. https://doi.org/10.3390/cells12232739
[18] Zhang, S.-Z., et al. (2019). NG2 glia regulate brain innate immunity via TGF-β2/TGFBR2 axis. *BMC Medicine, 17*(1), 204. https://doi.org/10.1186/s12916-019-1439-x
[19] Affram, K. O., Mitchell, K., & Symes, A. J. (2017). Microglial activation results in inhibition of TGF-β-regulated gene expression. *Journal of Molecular Neuroscience, 63*(3–4), 308–319. https://doi.org/10.1007/s12031-017-0980-3
[20] Freudlsperger, C., et al. (2013). TGF-β and NF-κB signal pathway cross-talk is mediated through TAK1 and SMAD7 in a subset of head and neck cancers. *Oncogene, 32*(12), 1549–1559. https://doi.org/10.1038/onc.2012.171
[21] Butovsky, O., & Weiner, H. L. (2018). Microglial signatures and their role in health and disease. *Nature Reviews Neuroscience, 19*(10), 622–635. https://doi.org/10.1038/s41583-018-0057-5
[22] Noda, M. (2016). Dysfunction of glutamate receptors in microglia may cause neurodegeneration. *Current Alzheimer Research, 13*(4), 381–386. https://doi.org/10.2174/1567205013666151116125810
[23] Zhang, Y. E. (2017). Non-Smad signaling pathways of the TGF-β family. *Cold Spring Harbor Perspectives in Biology, 9*(2), a022129. https://doi.org/10.1101/cshperspect.a022129
[24] Zhao, J., et al. (2020). TGFβ1 alleviates axonal injury by regulating microglia/macrophages alternative activation in traumatic brain injury. *Brain Research Bulletin, 161*, 21–32. https://doi.org/10.1016/j.brainresbull.2020.04.011
[25] Iyengar, S., Johnson, K. W., Ossipov, M. H., & Aurora, S. K. (2019). CGRP and the trigeminal system in migraine. *Headache, 59*(5), 659–681. https://doi.org/10.1111/head.13529