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Original Article

Serotonin promotes lip sensory recovery after inferior alveolar nerve transection via histone serotonylation

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KEYWORDS
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Peripheral nerve injury

Abstract *Background/purpose:* Sensory impairment due to Inferior alveolar nerve injury in mandibular tumor ablation severely impairs the survivors' quality of life. This study explored the role of serotonin in lip sensory recovery after inferior alveolar nerve transection.
Materials and methods: We established an *in vivo* inferior alveolar nerve transection model using C57BL/6J mice and an *in vitro* model of primary neurons in trigeminal ganglia. Serotonin or transglutaminase 2 inhibitor (GK921) were administrated to mice or neurons. The degree of lip sensory recovery was confirmed by quantitative sensory testing with Von Frey filaments. The axonal length was directly measured under optical microscopes. The changes of histone serotonylation and axon regeneration-related molecules were validated by quantitative real-time polymerase chain reaction, Western blot, immunofluorescence, and enzyme-linked immunosorbent assay.
Results: Lip sensory functions could recover without additional interventions within 4 weeks. Serotonin production, histone serotonylation, and transglutaminase 2 were upregulated in the ipsilateral trigeminal ganglia. Exogenous serotonin could improve axon growth with histone serotonylation. Additional serotonin could slightly compress the lip sensory recovery time, and an increase in histone serotonylation and axon growth-related molecules were observed

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in the ipsilateral trigeminal ganglia. Oppositely, GK921 significantly reduced the axon length and delayed the lip sensory recovery after inferior alveolar nerve transection.
Conclusion: Serotonin could accelerate the lip sensory recovery after inferior alveolar nerve transection via increasing histone serotonylation. These results may offer a potential therapeutic approach for peripheral nerve injury and studies may be needed to validate the efficacy in other peripheral nerve injury models.

Introduction

The inferior alveolar nerve (IAN, a branch of the mandibular division of the trigeminal nerve) innervate the skin and mucosa of the lower lip.[1] As the IAN travels through the mandibular canal within the mandible, it is frequently at risk of transection during mandibular tumor ablation, making lower lip numbness a common complication after ablation of head and neck tumors, severely impacting patients' quality of life.[2,3] The current gold standard for reconstructing segmental peripheral nerve defects is autologous nerve grafting.[4] However, its limitations, including donor site morbidity, restricted nerve availability, and suboptimal recovery outcomes, have hindered its widespread clinical application.[5] Therefore, it is crucial to explore novel methods of recovering the sensory function of the lower lip. Notably, our previous study found that most patients who underwent IAN sacrifice exhibited varying degrees of sensory recovery in the lower lip during clinical follow-ups, suggesting an alternative mechanism of sensory recovery after IAN injury.[6]

Serotonin, also known as 5-hydroxytryptamine (5-HT), is a crucial neurotransmitter involved in both central and peripheral nervous system functions. Recent studies have highlighted its role in metabolic regulation and tissue regeneration, with serotonergic axons demonstrating regenerative capacity following brain and spinal cord injuries.[7,8] Specifically, serotonin has been shown to promote axonal regeneration and partially restore neural function.[9–11] Despite its well-established role in sensory and pain transmission within the trigeminal nerve, the involvement of serotonin in lip sensory recovery has not yet been explored.

In injured neurons, histone methylation plays a key role in regulating the expression of genes associated with nerve regeneration.[12] Histone serotonylation at glutamine 5 of histone H3 (H3Q5ser) is widely observed in mammalian cells and tissues and plays a crucial role in modulating histone methylation.[13] Trimethylation of lysine 4 on histone H3 (H3K4me3) is one of the earliest discovered epigenetic modifications on histones.[14] H3Q5ser often co-localizes with H3K4me3 at promoters of actively transcribed genes. While H3Q5ser does not affect H3K4 methylation or the binding of H3K4me3 to reader proteins, it prevents the demethylation of H3K4me3. By stabilizing the active H3K4me3 mark, H3Q5ser facilitates the expression of key genes involved in neuronal differentiation.[15–17] Although histone serotonylation has been shown to influence the epigenetic regulation of gene expression, its potential role in sensory recovery following nerve injury, particularly in the context of lip sensory function, remains unexplored.

In this study, we investigated the role of serotonin and histone serotonylation in sensory recovery following inferior alveolar nerve transection. We sought to uncover the underlying mechanisms and identify novel therapeutic targets for enhancing sensory recovery after peripheral nerve injury.

Materials and methods

Animal models

This study was approved by the Institutional Review Board of Sichuan University West China Hospital of Stomatology (WCHSIRB-D-2024-158). Thirty 8-week-old male C57BL/6J mice, weighing approximately 20 g, were purchased from Chengdu Dossy Experimental Animals Co., Ltd. (Chengdu, China). Mice were anesthetized with isoflurane. The inferior alveolar nerve transection model was established by making a small incision over the mental foramen and transecting the nerve as we previously described (Fig. 1A).[6] In the control group, the nerve was exposed but left intact. When establishing inferior alveolar nerve transection model, drugs were administered through the infraorbital foramen if needed. The drug injection was performed with a 29 G syringe. The tip of the needle was then inserted through the infraorbital foramen, infraorbital canal, and foramen rotundum, and finally was positioned in the trigeminal ganglia. 100 μM Serotonin hydrochloride (HY—B1473, MedChemExpress, Middlesex, NJ, USA) or 50 μM transglutaminase 2 inhibitor GK921 (HY-12337, MedChemExpress).

Cell culture

The trigeminal ganglion tissues of postnatal mice were separated from the cranial base under a microscope. The tissues were placed in PBS containing 0.125% trypsinase (Gibco, Waltham, MA, USA) for digestion (20 min, 37C). The cells were then mechanically dissociated with a Pasteur pipette in DMEM/F12 medium (Gibco) containing 10% fetal bovine serum (Gibco) and 50 μg/ml ampicillin (Sigma—Aldrich, St. Louis, MO, USA). Once the trigeminal ganglion neurons adhered to the plastic or glass surfaces (about 3 h), the culture medium was changed to another one without

fetal bovine serum. The TGNs were cultured in DMEM/F12 medium (Gibco) supplemented with N2 (1:100 dilution, Invitrogen, USA), B27 (1:50 dilution, Invitrogen, USA), and 50 μg/ml ampicillin (Sigma–Aldrich) at 37 °C, 5% CO2 for 2 days. For cells in the group of drug administration, 100 μM Serotonin hydrochloride or 50 μM transglutaminase 2 inhibitor GK921 were added into the cell culture system. An inverted microscope was used to observe and take images of growth edges. NeuronJ was used to measure and record the longest axons in 50 cells in each image for analysis.

Sensory function assessment

The mice were placed in steel wire cages, with their head out of the cage. The measuring tip of the electronic Von Frey meter was used to vertically compress the same site of the mice' left and right lower lips, and the force was gradually applied. The behaviors of the mice, such as foot retraction, vocalization, head retraction, and avoidance, were regarded as signs, and the values of the five signs were recorded continuously for statistical analysis. Each mouse was measured several times before recording to make the mouse adapt to the contact of the measuring tip. We limited the maximum force to 100 gr to avoid the risk of puncturing the lower lip skin during the measurement (1 gr = 0.064 8 g). Five mice in each group were measured before and every four days after the operation.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from mouse trigeminal ganglia samples or primary neurons using TRIzol reagent (15596026; Thermo Fisher Scientific, Waltham, MA, USA). Total RNA (1 μg) was used to synthesize the first strand of cDNA with the TaqMan MicroRNA Reverse Transcription Kit (RR047A, Takara, Shiga, Japan). Real-time PCR was conducted using SYBR Green (RR820A, Takara) on a 7900HT Fast Real-Time PCR system (4329001, Applied Biosystems, Waltham, MA, USA). The relative expression levels of target genes were normalized to GAPDH and calculated using the 2−ΔΔCt method. The sequences of primers are listed in Table 1.

Western blot

Tissues and cells were lysed in cold RIPA buffer (BioSharp, Hefei, China), supplemented with 1 mM phenylmethylsulfonyl fluoride (Biosharp) and a protease inhibitor cocktail (MedChemExpress). Protein samples (30 μg per well) were loaded onto gels prepared using the Epizyme Gel Kit (Epizyme, Shanghai, China). Electrophoresis was performed at 80 V for 30 min, followed by 120 V for approximately 1.5 h. Proteins were transferred onto a 6 × 8 cm polyvinylidene fluoride membrane (Sigma–Aldrich) preactivated with methanol. The membranes were blocked in 5% BSA in Tris-buffered saline with Tween 20 (TBST), and then incubated overnight with the primary antibody. The used primary antibodies included H3Q5ser (PTM-1420, PTM Bio, Hangzhou, China), H3K4me3 (PTM-5019, PTM Bio), H3 (PTM-6613, PTM Bio), TGM2 (3557, Cell Signaling Technology, Boston, MA, USA), ATF3 (18665, Cell Signaling Technology), GAPDH (GB15002, Servicebio, Wuhan, China) After washing in TBST, membranes were incubated with the secondary antibody (ZSGB-BIO, Beijing, China), washed again in TBST, and visualized. The grayscale values were quantified using ImageJ software.

Enzyme-linked immunosorbent assay (ELISA)

Levels of serotonin or nerve growth factor (NGF) in tissue homogenates or cell supernants were measured using ELISA kits (Serotonin: D751013, Sangon Biotech, Shanghai, China; NGF: M0815, Elabscience, Wuhan, China). For animal samples, trigeminal ganglia were homogenized in cold PBS, centrifuged at 12,000×*g* for 10 min at 4 °C, and the supernatants were collected; For cell samples, the culture medium was collected, centrifuged at 12,000×*g* for 10 min at 4 °C, and the supernatants were collected. Samples and standards were added to a 96-well plate, incubated, and washed before adding a detection antibody. After substrate reaction and stop solution, absorbance was measured at 450 nm using a microplate reader.

Immunofluorescence

Samples were fixed in 10% formalin for 1 day, followed by gradient dehydration, paraffin embedding, sectioning, dewaxing, and rehydration. Antigen retrieval was performed using citric acid buffer (Beyotime, Shanghai, China) in an autoclave. SGs were treated with 3% H_2O_2 to block endogenous peroxidase activity, washed with TBS 2–3 times, and blocked in 10% goat serum (BioSharp). Primary antibodies were diluted 1:100 and incubated overnight at approximately 50 μL per section. The used primary antibodies included H3Q5ser (PTM-1420, PTM Bio), H3K4me3 (PTM-5019, PTM Bio), and Tuj1 (5568, Cell Signaling Technology). After washing with TBS, sections were incubated with secondary antibodies (1:200, ZSGB-BIO) and then washed in TBS. Slides were mounted with Antifade Mounting Medium for Fluorescence (with DAPI) (Biosharp). Analysis was performed using ImageJ software.

Statistical analysis

Statistical analyses were performed to evaluate the differences between experimental groups. ANOVA or t-tests were used to compare the outcomes of sensory recovery, gene expression, and molecular markers across groups. Data were reported as mean ± standard deviation (SD), and a *P*-value of less than 0.05 was considered statistically significant.

Results

Levels of serotonin production and histone serotonylation were improved in trigeminal ganglia after inferior alveolar nerve transection

We assessed the impact of mental nerve transection on serotonin levels and histone modifications to better understand the mechanisms behind sensory recovery. As depicted in Fig. 1A, the mental nerve transection was successfully performed in mice, as evidenced by visible

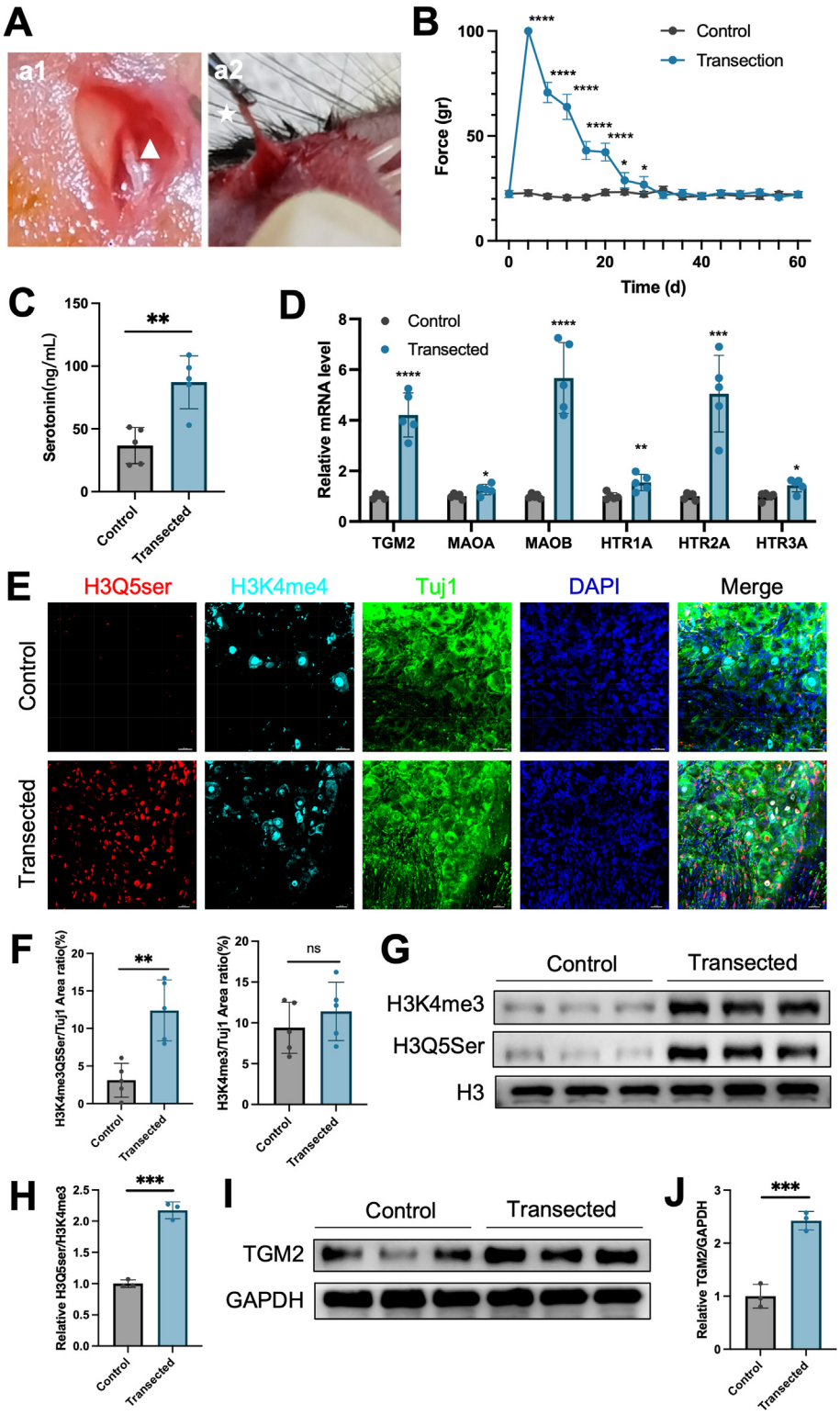

Figure 1 Levels of serotonin production and histone serotonylation were improved in trigeminal ganglia after inferior alveolar nerve transection. (A) Photos of inferior alveolar nerve transection mice model. The triangle in (a1 shows the exposure of the inferior alveolar nerve. The asterisk in (a2) shows the transection of the inferior alveolar nerve. (B) Quantitative sensory testing with Von Frey filaments showed the lower lip sensation after sham surgery (Control group) versus inferior alveolar nerve transection (Transected group) in mice, measured every four days for 8 weeks (n = 5). (C) ELISA showed the serotonin level in trigeminal ganglia (n = 5). (D) Quantitative real-time polymerase chain reaction showed the genes related to serotonin production (including TGM2, MAOA, MAOB, HTR1A, HTR2A, and HTR3A) in trigeminal ganglia (n = 5). (E) Immunofluorescence showed the level of H3Q5Ser, H3K4me3, Tuj in the trigeminal ganglia. Bar indicated 20 μm. (F) Quantitative analysis of immunofluorescence strength of

surgical alterations in the lower lip (Fig. 1A(a2)). Quantitative sensory testing using Von Frey filaments (Fig. 1B) showed that the transection group displayed significantly poorer sensory recovery compared to the control group, with a delayed and reduced response to mechanical stimuli ($P < 0.0001$). Following the transection, serotonin levels in the trigeminal ganglia were markedly elevated in the transection group compared to controls (Fig. 1C, $P < 0.01$).

To examine the molecular changes induced by nerve injury, we analyzed the expression of serotonin synthesis-related genes using quantitative PCR (Fig. 1D). The results indicated significant upregulation of genes such as TGM2, MAOA, and MAOB, suggesting that serotonin production is enhanced following the nerve transection. Immunofluorescence analysis (Fig. 1E) revealed a marked increase in histone serotonylation (H3Q5ser) in the transection group, along with enhanced axonal growth as indicated by Tuj1 staining. Quantitative analysis of the immunofluorescence (Fig. 1F) confirmed that the H3Q5ser-positive area was 45% greater in the transection group compared to the control ($P < 0.01$). Western blot analysis (Fig. 1G) further supported these findings, showing elevated levels of H3Q5ser in the transection group. Quantitative analysis of the blots (Fig. 1H) revealed a significant increase in the H3Q5Ser/H3K4me3 ratio ($P < 0.01$), suggesting that while both H3Q5Ser and H3K4me3 levels increased, the increase in H3Q5Ser was more pronounced. Additionally, TGM2, a key gene involved in serotonin signaling, was also upregulated at the protein level (Fig. 1I and J), further confirming the role of serotonin in modulating the transcriptional activity associated with nerve repair. These results suggest that serotonin, via histone serotonylation, plays a significant role in sensory recovery following nerve injury.

Exogenous serotonin improved the axonal length of primary neurons of trigeminal ganglia via histone serotonylation *in vitro*

To investigate the impact of serotonin on axonal growth and its potential mechanism, we cultured trigeminal neurons under control and serotonin-treated conditions. The results show that serotonin significantly enhances axonal elongation and regulates key neurotrophic factors involved in nerve regeneration.

In vitro, serotonin treatment significantly promoted axonal growth in trigeminal neurons. As shown in Fig. 2A, phase-contrast microscopy revealed that serotonin-treated neurons exhibited longer axons compared to the control group. Quantitative analysis (Fig. 2B) confirmed that serotonin significantly increased axonal length ($P < 0.01$). Western blot analysis (Fig. 2C) demonstrated that serotonin treatment increased the expression of H3Q5ser, a key histone modification, while H3K4me3 levels also showed some change, though the increase was less pronounced. The relative ratio of H3Q5ser/H3K4me3 was significantly elevated in serotonin-treated neurons (Fig. 2D, $P < 0.01$), indicating that serotonin promotes histone serotonylation, which may play a more prominent role in axonal elongation.

Furthermore, qPCR results (Fig. 2E) showed that serotonin treatment significantly upregulated the expression of several neurotrophic factors, including NGF, ATF3, NTF3, NTF5, GDNF, as well as lipid metabolism-related molecules such as FASN, SREBF1, and APOD, all of which are involved in neuronal growth and regeneration.[18–20] Western blot

Table 1 Sequences of primers used for qRT-PCR.

Gene	Primer sequences (5′-3′)
TGM2	Forward: GCTGGACCAACAGGACAATGT
	Reverse: CTCTAGGCTGAGACGGTACAG
MAOA	Forward: AACAAAAGCGATGTGATCGTGG
	Reverse: GTCCCACATAAGCTCCACCA
MAOB	Forward: AACAAAAGCGATGTGATCGTGG
	Reverse: GCCCAACATAAGATCCTCCAAGG
HTR1A	Forward: ACAGGCGGCAACGATACTG
	Reverse: AGCACCGCGCAGAAAATGA
HTR2A	Forward: TAATGCAATTAGGTGACGACTCG
	Reverse: GAGGCTTCGGAAGTGTTAGCA
HTR3A	Forward: TCCTGAGGACTTCGACAATGT
	Reverse: CCCCACGTCCACAAACTCAT
NGF	Forward: TGATCGGCGTACAGGCAGA
	Reverse: GCTGAAGTTTAGTCCAGTGGG
ATF3	Forward: TTTGCTAACCTGACACCCTTTG
	Reverse: AGAGGACATCCGATGGCAGA
NTF3	Forward: AGTTTGCCGGAAGACTCTCTC
	Reverse: GGGTGCTCTGGTAATTTTCCTTA
NTF5	Forward: TGAGCTGGCAGTATGCGAC
	Reverse: CAGCGCGTCTCGAAGAAGT
GDNF	Forward: GCCGGACGGGACTCTAAGAT
	Reverse: CGTCATCAAACTGGTCAGGATAA
FASN	Forward: GGAGGTGGTGATAGCCGGTAT
	Reverse: TGGGTAATCCATAGAGCCCAG
SREBF1	Forward: TGACCCGGCTATTCCGTGA
	Reverse: CTGGGCTGAGCAATACAGTTC
APOD	Forward: TCACCACAGCCAAAGGACAAA
	Reverse: CGTTCTCCATCAGCGAGTAGT

TGM2, transglutaminase 2; MAOA, monoamine oxidase A; MAOB, monoamine oxidase B; HTR1A, 5- hydroxytryptamine (serotonin) receptor 1A; HTR2A, 5-hydroxytryptamine (serotonin) receptor 2A; HTR3A, 5-hydroxytryptamine (serotonin) receptor 3A; NGF, nerve growth factor; ATF3, activating transcription factor 3; NTF3, neurotrophin-3; NTF5, neurotrophin-5; GDNF, glial cell line-derived neurotrophic factor; FASN, fatty acid synthase; SREBF1, sterol regulatory element-binding transcription factor 1; APOD, apolipoprotein D.

H3Q5Ser and H3K4me3 in Tuj-positive area (n = 5). (G) Western blot showed the level of H3Q5Ser and H3K4me3 in the trigeminal ganglia. (H) Quantitative analysis of the relative ratio of H3Q5Ser per H3K4me3 in the blots (n = 3). (I) Western blot showed the protein expression level of TGM2 in the trigeminal ganglia. (J) Quantitative analysis of the relative ratio of TGM2 per GAPDH in the blots (n = 3). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. TGM2, transglutaminase 2; MAOA, monoamine oxidase A; MAOB, monoamine oxidase B; HTR1A, 5-hydroxytryptamine receptor 1A; HTR2A, 5-hydroxytryptamine receptor 2A; HTR3A, 5-hydroxytryptamine receptor 3A; H3Q5Ser, histone H3 glutamine 5 serotonylation; H3K4me3, histone H3 lysine 4 trimethylation; Tuj1, neuron-specific class III beta-tubulin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

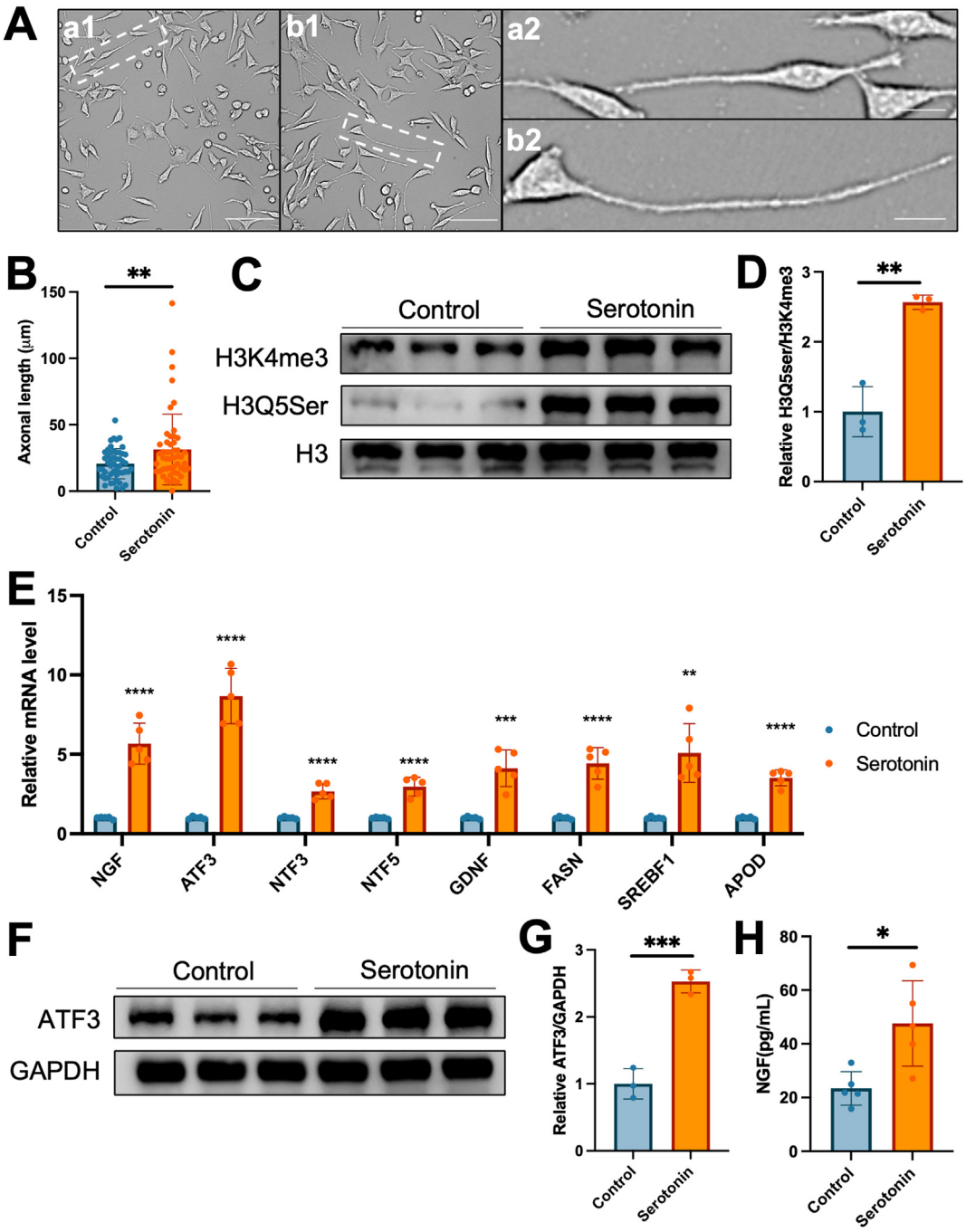

Figure 2 Exogenuous serotonin improved the axonal length of primary neurons of trigeminal ganglia via histone serotonylation *in vitro*. (A) Images of Cultured primary neurons. (a1) and (a2) were cells in the control group. (b1) and (b2) were cells in the serotonin group (addition of 100 μM Serotonin hydrochloride). Bar in (a1) and (b1) indicated 100 μm. Bar in (a2) and (b2) indicated 20 μm. (B) Quantitative analysis of axon length of top 50 neurons in the images. (C) Western blot showed the level of H3Q5Ser and H3K4me3 in the neurons. (D) Quantitative analysis of the relative ratio of H3Q5Ser per H3K4me3 in the blots (n = 3). (E) Quantitative real-time polymerase chain reaction showed the genes related to axon regeneration (including NGF, ATF3, NTF3, NTF5, and GDNF) and lipid metabolism (FASN, SREBF1, and APOD) in the neurons (n = 5). (F) Western blot showed the protein expression level of ATF3 in the trigeminal ganglia. (G) Quantitative analysis of the relative ratio of ATF3 per GAPDH in the blots (n = 3). (H) ELISA showed the NGF level in the nerons (n = 5). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. NGF, nerve growth factor; ATF3, activating transcription factor 3; NTF3, neurotrophin-3; NTF5, neurotrophin-5; GDNF, glial cell line-derived neurotrophic factor; FASN, fatty acid synthase; SREBF1, sterol regulatory element-binding transcription factor 1; APOD, apolipoprotein D; H3Q5Ser, histone H3 glutamine 5 serotonylation; H3K4me3, histone H3 lysine 4 trimethylation; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

analysis (Fig. 2F) also revealed that ATF3, a key gene involved in neurogenesis, was upregulated in the serotonin-treated group ($P < 0.001$). Moreover, the NGF protein levels were significantly higher in the serotonin-treated group compared to the control group (Fig. 2H, $P < 0.05$), further supporting the role of serotonin in promoting sensory nerve regeneration. These results suggest that serotonin, through histone serotonylation and the upregulation of neurotrophic factors, enhances axonal regeneration and supports the repair of sensory neurons following injury.

Serotonin promotes sensory recovery after inferior alveolar nerve transection via histone serotonylation *in vivo*

To explore the effects of serotonin on sensory recovery following inferior alveolar nerve transection, we examined sensory function and histone modifications in the transection model. As shown in Fig. 3A, serotonin treatment significantly improved sensory recovery, as demonstrated by a marked increase in the force exerted by the transected group compared to controls ($P < 0.01$). Immunofluorescence analysis (Fig. 3B) showed a significant increase in histone serotonylation (H3Q5ser) in serotonin-treated neurons, with a higher ratio of H3Q5ser-positive areas (Fig. 3C, $P < 0.01$), whereas no significant changes were observed in H3K4me3 (Fig. 3C). Western blot analysis (Fig. 3D) confirmed these results, with serotonin treatment leading to a significant increase in the H3Q5ser/H3K4me3 ratio ($P < 0.01$, Fig. 3E), suggesting that serotonin promotes histone serotonylation in sensory neurons.

Further, to assess the impact of serotonin on gene expression related to axon regeneration, we measured the mRNA levels of several key neurotrophic factors: NGF, ATF3, NTF3, NTF5, and GDNF. As shown in Fig. 3F, serotonin significantly upregulated the expression of these neurotrophic factors, which are associated with nerve regeneration ($P < 0.05$). Western blot analysis (Fig. 3G) further revealed an increase in ATF3 protein expression ($P < 0.01$, Fig. 3H). Additionally, the NGF protein level in the culture medium was significantly higher in serotonin-treated samples compared to controls ($P < 0.05$, Fig. 3I). These findings suggest that serotonin promotes sensory recovery by enhancing the production of neurotrophic factors through the mechanism of histone serotonylation.

GK921 inhibited the axonal length of primary neurons of trigeminal ganglia by reducing histone serotonylation *in vitro*

To further confirm the effects of serotonin on axonal growth and neurotrophic factor regulation, we treated trigeminal neurons with serotonin and GK921 (Histone Deacetylase Inhibitor) compared with the serotonin group to reversely verify the effect of serotonin on neural growth. As shown in Fig. 4A and B, while serotonin significantly promoted axonal growth, neurons treated with both serotonin and GK921 exhibited shorter axons, suggesting that GK921 hindered axonal growth. ($P < 0.001$). West blot analysis (Fig. 4C) demonstrated decreased histone serotonylation (H3Q5Ser) and H3K4me4 in serotonin and GK921-treated neurons compared with the serotonin-treated group. Notably, the decrease in H3Q5Ser expression was more pronounced (Fig. 4D, $P < 0.001$).

We also evaluated the expression of key neurotrophic factors related to nerve regeneration. As shown in Fig. 4E, GK921 significantly downregulated the mRNA expression of NGF, ATF3, NTF3, NTF5, GDNF, FASN, SREBF1, and APOD ($P < 0.05$). Western blot analysis further confirmed that GK921 treatment reduced the protein expression of ATF3, a key molecule involved in nerve repair (Fig. 4F and G, $P < 0.001$). Additionally, the level of NGF in the culture medium was significantly decreased following GK921 treatment (Fig. 4H, $P < 0.05$). These results suggest that blocking serotonin reuptake disrupts the beneficial effects of serotonin on sensory recovery, supporting the hypothesis that serotonin signaling plays a key role in nerve repair after injury.

GK921 delayed the lip sensory recovery after inferior alveolar nerve transection by reducing histone serotonylation *in vivo*

To further confirm the effects of serotonin on sensory recovery after nerve injury, we examined the GK921's impact in the mental nerve transection model, specifically analyzing its impact on histone serotonylation. As shown in Fig. 5A, GK921 treatment significantly inhibited sensory recovery, evidenced by the increased force measured in the transected group treated with GK921 (Trans + GK921) compared to the transected-only group ($P < 0.01$). Immunofluorescence analysis (Fig. 5B) revealed that GK921 treatment led to a significant decrease in histone serotonylation (H3Q5Ser) in neurons, as shown by the reduced H3Q5Ser-positive area ratio (Fig. 5C, $P < 0.01$). However, no significant change was observed in H3K4me3 levels (Fig. 5C). Western blot analysis (Fig. 5D and E) confirmed these results, with GK921 treatment leading to a significant decrease in the ratio of H3Q5Ser to H3K4me3 ($P < 0.05$).

We also examined the expression of neurotrophic factors involved in axon regeneration. As shown in Fig. 5F, GK921 significantly downregulated the expression of key neurotrophic factors, including NGF, ATF3, NTF3, NTF5, GDNF, as well as lipid metabolism-related molecules such as FASN, SREBF1, and APOD ($P < 0.05$). Western blot analysis (Fig. 5G and H) further confirmed these findings, with GK921 treatment significantly decreasing ATF3 protein levels ($P < 0.001$), a crucial factor for neurogenesis and neuronal repair. Additionally, NGF levels in the culture medium were significantly higher in transection-only samples compared to GK921 treatment groups (Fig. 5I, $P < 0.05$). These results suggest that GK921 inhibits sensory recovery by decreasing the level of histone serotonylation and by diminishing the production of neurotrophic factors, thereby hindering nerve regeneration following injury. This inversely confirms the significant role of serotonin and histone serotonylation in the repair of the mental nerve.

Discussion

Previous studies have demonstrated that peripheral nerve injury can trigger strong regeneration, leading to the

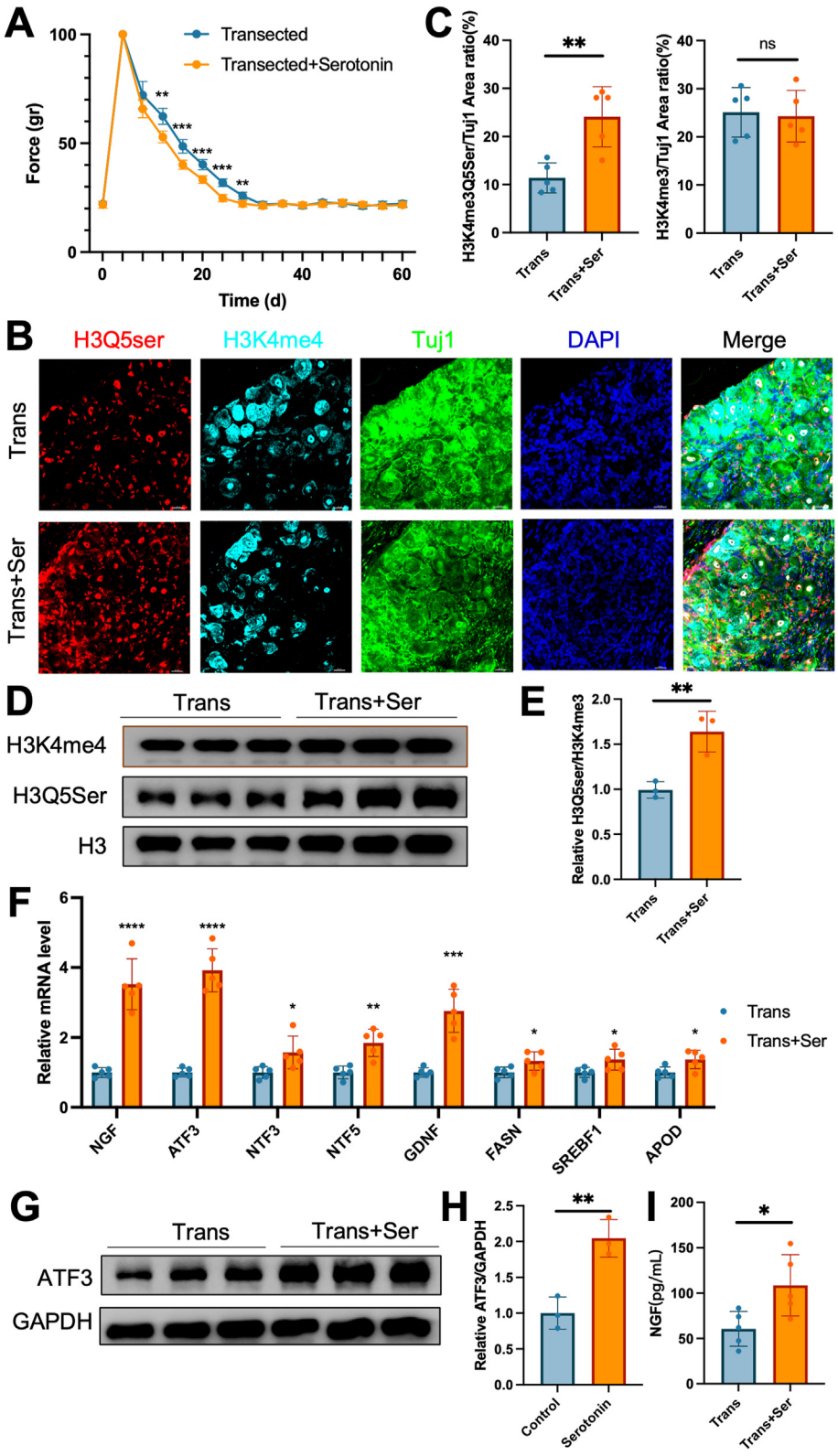

Figure 3 Exogenous serotonin improved the lip sensory recovery after inferior alveolar nerve transection via histone serotonylation *in vivo*. (A) Quantitative sensory testing with Von Frey filaments showed the lower lip sensation after transection alone (Trans group) versus transection plus exogenous serotonin group (administration of 100 μM Serotonin hydrochloride) (Trans + Ser group) in mice, measured every four days for 8 weeks (n = 5). (B) Immunofluorescence showed the level of H3Q5Ser, H3K4me3, Tuj in the trigeminal ganglia. Bar indicated 20 μm. (C) Quantitative analysis of immunofluorescence strength of H3Q5Ser and H3K4me3 in Tuj-positive area (n = 5). (D) Western blot showed the level of H3Q5Ser and H3K4me3 in the trigeminal ganglia. (E) Quantitative analysis of the relative ratio of H3Q5Ser per H3K4me3 in the blots (n = 3). (F) Quantitative real-time polymerase chain reaction showed the genes related to

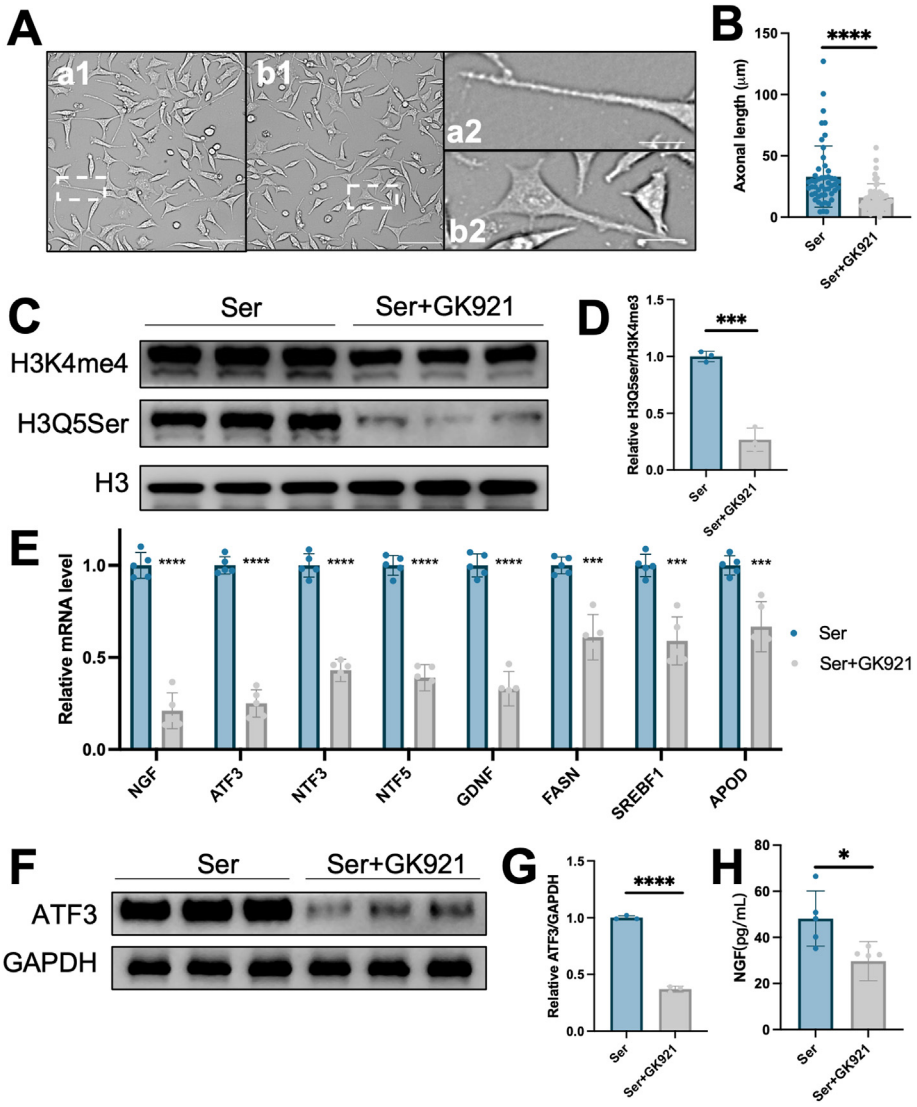

Figure 4 GK921 inhibited the serotonin-induced axonal growth of primary neurons of trigeminal ganglia by reducing histone serotonylation *in vitro*. (A) Images of Cultured primary neurons. (a1) and (a2) were cells in the serotonin (Ser) group (addition of 100 μM Serotonin hydrochloride). (b1) and (b2) were cells in the serotonin + GK921 (Ser + GK921) group (addition of 100 μM Serotonin hydrochloride plus 50 μM GK921). Bar in (a1) and (b1) indicated 100 μm. Bar in (a2) and (b2) indicated 20 μm. (B) Quantitative analysis of axon length of top 50 neurons in the images. (C) Western blot showed the level of H3Q5Ser and H3K4me3 in the neurons. (D) Quantitative analysis of the relative ratio of H3Q5Ser per H3K4me3 in the blots (n = 3). (E) Quantitative real-time polymerase chain reaction showed the genes related to axon regeneration (including NGF, ATF3, NTF3, NTF5, and GDNF) and lipid metabolism (FASN, SREBF1, and APOD) in the neurons (n = 5). (F) Western blot showed the protein expression level of ATF3 in the trigeminal ganglia. (G) Quantitative analysis of the relative ratio of ATF3 per GAPDH in the blots (n = 3). (H) ELISA showed the NGF level in the nerons (n = 5). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. NGF, nerve growth factor; ATF3, activating transcription factor 3; NTF3, neurotrophin-3; NTF5, neurotrophin-5; GDNF, glial cell line-derived neurotrophic factor; FASN, fatty acid synthase; SREBF1, sterol regulatory element-binding transcription factor 1; APOD, apolipoprotein D; H3Q5Ser, histone H3 glutamine 5 serotonylation; H3K4me3, histone H3 lysine 4 trimethylation; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Tuj1, neuron-specific class III β-tubulin.

axon regeneration (including NGF, ATF3, NTF3, NTF5, and GDNF) and lipid metabolism (FASN, SREBF1, and APOD) in the trigeminal ganglia (n = 5). (G) Western blot showed the protein expression level of ATF3 in the trigeminal ganglia. (H) Quantitative analysis of the relative ratio of ATF3 per GAPDH in the blots (n = 3). (I) ELISA showed the NGF level in the trigeminal ganglia (n = 5). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. NGF, nerve growth factor; ATF3, activating transcription factor 3; NTF3, neurotrophin-3; NTF5, neurotrophin-5; GDNF, glial cell line-derived neurotrophic factor; FASN, fatty acid synthase; SREBF1, sterol regulatory element-binding transcription factor 1; APOD, apolipoprotein D; H3Q5Ser, histone H3 glutamine 5 serotonylation; H3K4me3, histone H3 lysine 4 trimethylation; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Tuj1, neuron-specific class III β-tubulin.

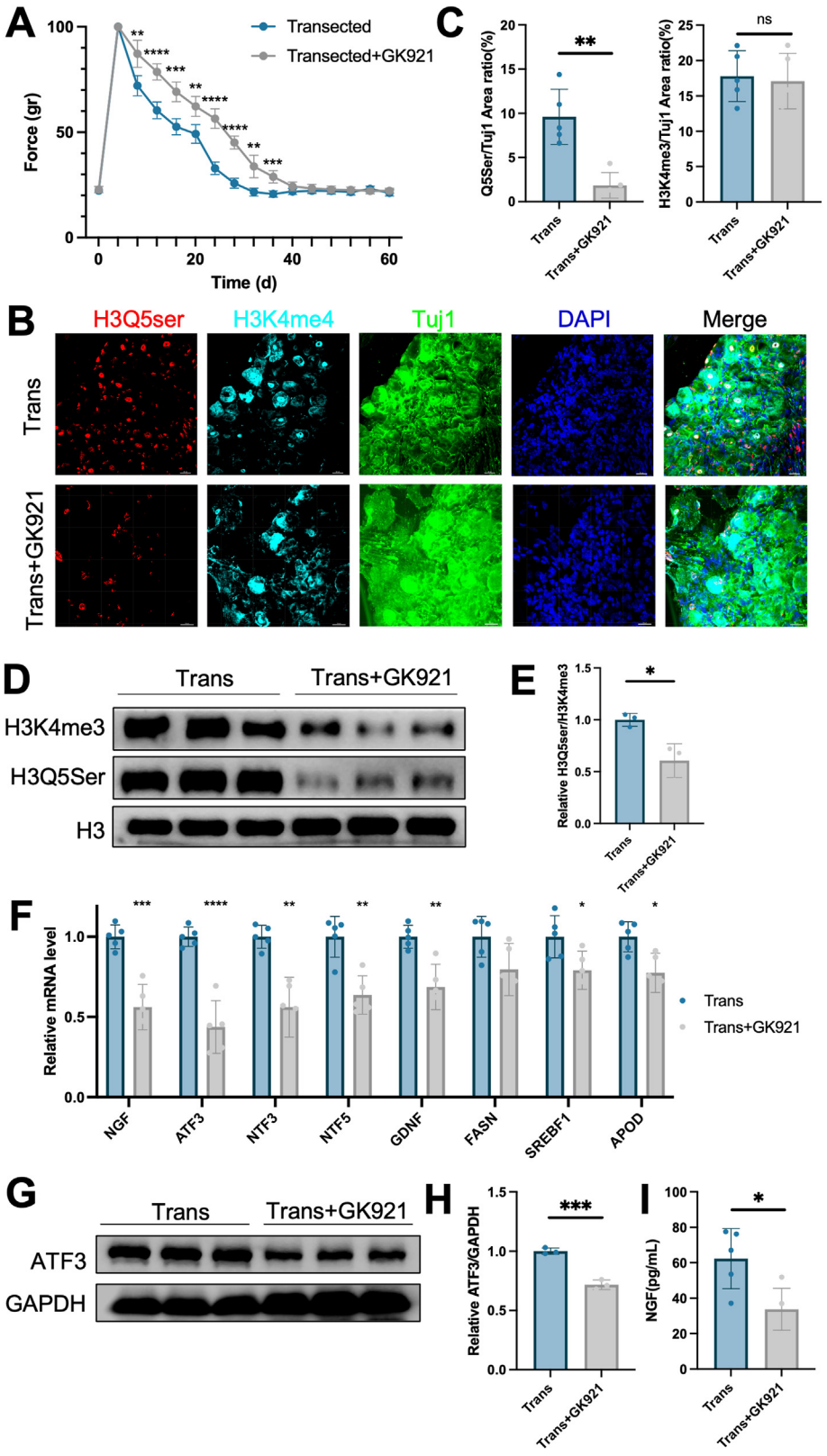

Figure 5 GK921 delayed the lip sensory recovery after inferior alveolar nerve transection by reducing histone serotonylation *in vivo*. (A) Quantitative sensory testing with Von Frey filaments showed the lower lip sensation after transection alone (Trans group) versus transection plus exogenous serotonin group (administration of 50 μM GK921) (Trans + GK921 group) in mice, measured every four days for 8 weeks (n = 5). (B) Immunofluorescence showed the level of H3Q5Ser, H3K4me3, Tuj in the trigeminal ganglia. Bar indicated 20 μm. (C) Quantitative analysis of immunofluorescence strength of H3Q5Ser and H3K4me3 in Tuj-positive area (n = 5). (D) Western blot showed the level of H3Q5Ser and H3K4me3 in the trigeminal ganglia. (E) Quantitative analysis of the

restoration of sensory and motor functions.[21–23] This regeneration requires extensive transcriptional and epigenetic changes in the injured neurons,[24] with histone modifications playing a key role in regulating gene expression necessary for nerve repair. While histone methylation has been well-documented in promoting nerve regeneration, the role of histone serotonylation in this process remains underexplored. In this study, we demonstrate that serotonin, through its interaction with histones—particularly histone H3 at glutamine 5 (H3Q5ser), a relatively novel mechanism discovered to regulate gene expression[16,25]—promotes sensory neuron regeneration after inferior alveolar nerve transection. Our results show that serotonin significantly enhances sensory recovery *in vivo*, as evidenced by improved mechanical sensitivity in the transected group treated with exogenous serotonin. Notably, this effect was not associated with changes in H3K4me3, indicating a specific role of serotonylation. Additionally, in the serotonin treatment group, we found upregulated neurotrophic factors like NGF, ATF3, and GDNF, which are crucial for axonal growth and neuronal survival. These findings hold potential value for future research into the specific mechanisms by which serotonin promotes peripheral nerve recovery. Our results highlight serotonin-based therapies as a promising, regulated approach for peripheral nerve repair.

Additionally, during the experiment, we observed an increase in molecules involved in lipid metabolism, such as FASN, SREBF1, and APOD, in the serotonin-treated group. This highlights the interaction between serotonin and lipid metabolism in promoting nerve recovery. Previous studies, including those from our lab, have shown that lipid metabolism plays a pivotal role in peripheral nerve regeneration.[6,18] Specifically, we identified APOD as a key player in lipid metabolism during nerve repair, which acts to regulate the TGF-β pathway involved in axonal regrowth. The upregulation of APOD in serotonin-treated neurons suggests that serotonin influences lipid metabolism to promote neuroregeneration, providing a direction for future research into the underlying mechanisms.

Furthermore, effect of serotonin extends beyond neuronal cells. Non-neuronal cells, including Schwann cells and fibroblasts, may also respond to serotonin through similar histone modifications. However, the exact role of these modifications in non-neuronal cells and their impact on nerve repair is still under investigation. Schwann cells, which are crucial for myelin formation and nerve regeneration,[18] may also benefit from serotonin-mediated epigenetic changes, though the specific mechanisms remain unclear. The role of serotonin in promoting peripheral nerve regeneration has been well documented, but its interaction with epigenetic mechanisms, particularly histone modifications, has not been extensively studied. Our findings suggest that serotonin may work through a complex network of signaling pathways to regulate the expression of genes involved in axon growth, nerve survival, and differentiation. By stabilizing H3K4me3 through serotonin-induced H3Q5ser, serotonin might contribute to the long-term maintenance of neurogenic signaling, ensuring efficient nerve regeneration.

However, there is something more that remains unrevealed. The precise molecular pathways through which serotonin induces histone serotonylation remain unclear. Further research is needed to elucidate the downstream signaling pathways involved in serotonin's action on histone modifications. Also, we did not examine the effects of serotonin in other models of peripheral nerve injury, such as sciatic nerve injury or spinal cord injury. It is important to determine whether the observed effects of serotonin-mediated histone serotonylation are consistent across different types of nerve injury. Additionally, research should be expanded to include different types of nerves, rather than being limited to the inferior alveolar nerve. It will also be important to assess the involvement of serotonin receptors in nerve repair and to explore potential interactions between serotonin and other epigenetic modifications, such as DNA methylation or histone acetylation, which may work together to modulate gene expression.

Furthermore, although our results demonstrate the beneficial effects of serotonin on sensory recovery and axonal regeneration, we did not explore whether this effect is dose-dependent. Future studies should examine the dose–response relationship to optimize therapeutic efficacy and determine appropriate clinical dosing strategies. In addition, this study administered serotonin systemically. For clinical translation, it would be critical to develop targeted delivery approaches, such as local injection or nanoparticle-based carriers, which may enhance therapeutic precision while minimizing systemic exposure and off-target effects. Finally, potential side effects associated with elevated serotonin levels should not be overlooked. Excessive systemic serotonin may lead to serotonin toxicity (also known as serotonin syndrome), a rare but potentially serious condition characterized by neuromuscular, autonomic, and cognitive symptoms.[26] Although no adverse effects were observed in our study, this highlights the need for careful dose selection and monitoring in future preclinical and clinical trials.

In conclusion, serotonin could accelerate the lip sensory recovery after inferior alveolar nerve transection via

relative ratio of H3Q5Ser per H3K4me3 in the blots (n = 3). (F) Quantitative real-time polymerase chain reaction showed the genes related to axon regeneration (including NGF, ATF3, NTF3, NTF5, and GDNF) and lipid metabolism (FASN, SREBF1, and APOD) in the trigeminal ganglia (n = 5). (G) Western blot showed the protein expression level of ATF3 in the trigeminal ganglia. (H) Quantitative analysis of the relative ratio of ATF3 per GAPDH in the blots (n = 3). (I) ELISA showed the NGF level in the trigeminal ganglia (n = 5). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. NGF, nerve growth factor; ATF3, activating transcription factor 3; NTF3, neurotrophin-3; NTF5, neurotrophin-5; GDNF, glial cell line-derived neurotrophic factor; FASN, fatty acid synthase; SREBF1, sterol regulatory element-binding transcription factor 1; APOD, apolipoprotein D; H3Q5Ser, histone H3 glutamine 5 serotonylation; H3K4me3, histone H3 lysine 4 trimethylation; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Tuj1, neuron-specific class III β-tubulin.

increasing histone serotonylation. These results may offer a potential therapeutic approach for peripheral nerve injury and studies may be needed to validate the efficacy in other peripheral nerve injury models.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

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