Immunohistochemical analysis of histone H3 acetylation in the trigeminal root entry zone in an animal model of trigeminal neuralgia

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OBJECTIVE The trigeminal root entry zone (TREZ) is a transitional zone between the central nervous system (CNS) and peripheral nervous system (PNS), adjacent to the brainstem. Microvascular compression of the TREZ has been considered to be the primary etiology in most cases of trigeminal neuralgia (TN), but whether epigenetic regulation is involved in the pathogenesis of TN is still unclear. Therefore, this study was designed to investigate the epigenetic regulation of histone H3 acetylation in the TREZ in an animal model of TN.

METHODS An animal model of TN was established, and adult male Sprague-Dawley rats were randomly assigned to a TN group with trigeminal nerve root compression, sham operation group, TN+HDACi group (TN plus selective histone deacetylase inhibitor injection into the TREZ), or TN+Veh group (TN plus vehicle injection into the TREZ). To measure the length of the central portion of the TREZ from the junction of the trigeminal nerve root entering the pons to the interface of the dome-shaped CNS-PNS transitional zone, immunofluorescent staining of glia and glial nuclei was performed using glial fibrillary acidic protein (GFAP) antibody and DAPI, respectively. To investigate the acetylation of histone H3 within the TREZ in a TN animal model group and a sham operation group, localization of histone H3K9, H3K18, and H3K27 acetylation was examined via immunohistochemical staining methods.

RESULTS Measurements of the CNS-PNS transitional zone in the TREZ revealed that the average length from the junction of the trigeminal nerve root connecting the pons to the glial fringe of the TREZ in the TN group was longer than that in the sham operation group ($p < 0.05$) and that the interface gradually migrated distally. Cells that stained positive for acetylated histone H3K9, H3K18, and H3K27 were distributed around both sides of the border of the CNS-PNS junction in the TREZ. The ratio of immunoreactive H3K9-, H3K18- and H3K27-positive cells in the TN group was obviously higher than that in the sham operation group on postoperative days 7, 14, 21, and 28 ($p < 0.05$).

CONCLUSIONS These results suggested that chronic compression of the trigeminal nerve root may be involved in the pathogenesis of TN in an animal model by influencing the plasticity of the CNS-PNS transitional zone and the level of histone acetylation in the TREZ.

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KEYWORDS histone H3; acetylation; trigeminal root entry zone; chronic compression; trigeminal neuralgia; animal model; functional neurosurgery; pain

An epigenetic trait is a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence.³ Histone acetylation is one of the best-characterized epigenetic modifications, which results from acetylation of the lysine residues

of the histone core of the nucleosome by a family of enzymes known as histone acetyltransferases (HATs). Acetylation of histones is known to increase the expression of genes through transcriptional activation.³⁰

Histone H3 is one of the most extensively modified of

ABBREVIATIONS CNS = central nervous system; DMSO = dimethyl sulfoxide; GFAP = glial fibrillary acidic protein; HAT = histone acetyltransferase; HDACi = histone deacetylase inhibitor; mAb = monoclonal antibody; PBS = phosphate-buffered saline; PNS = peripheral nervous system; SAHA = suberoylanilide hydroxamic acid; TN = trigeminal neuralgia; TREZ = trigeminal root entry zone.

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the five primary histone proteins, and acetylation at different lysine residues of histone H3 may play key roles in gene regulation. Studies have shown that acetylated H3K9, H3K18, and H3K27 are enriched near transcription start sites (TSSs),³¹ and each of these histone acetylation sites has activation functions.¹⁸ Acetylated H3K9 was observed in actively transcribed promoters, and acetylated H3K9 mediated a switch from transcription initiation to elongation.¹⁴ Acetylated H3K27 was defined as an active enhancer marker for its higher activation of transcription.⁵ Studies have shown that epigenetic regulation may interfere with the process of histone acetylation, influence expression of nociceptive genes, and affect pain behavior in chronic pain states,^{2,7} which implies the potential involvement of an epigenetic process in chronic neuropathic pain.

Trigeminal neuralgia (TN) is one of the most severe types of neuropathic pain, and its pathogenesis is still unknown.²¹ Accumulating evidence suggests that aberrant microvascular compression of the trigeminal root entry zone (TREZ) may be the main etiology of TN. Because the anatomical structure and physiological functions of the TREZ, a transitional zone between the central nervous system (CNS) and the peripheral nervous system (PNS), are highly complex, we suspect that this zone may be particularly vulnerable to noxious stimulation from various external environments, which may cause an imbalance in homeostasis and even affect the epigenetic regulation of gene expression.

We hypothesize that chronic mechanical compression stimulation of the TREZ induces epigenetic changes, which may subsequently affect the pathogenesis of TN. However, little is known regarding epigenetic regulation in the TREZ, especially under conditions of TN. Therefore, we designed the present study to examine the acetylation of histone H3K9, H3K18, and H3K27 in the TREZ in an animal model of TN that was induced by chronic compression of the trigeminal nerve root.

Methods

Antibodies

Acetyl histone H3K9 (C5B11) rabbit monoclonal antibody (mAb; 1:50 dilution), acetyl histone H3K18 (D8Z5H) rabbit mAb (1:50 dilution), and acetyl histone H3K27 (D5E4) rabbit mAb (1:50 dilution) primary antibodies were purchased from Cell Signaling Technology. Rabbit anti–glial fibrillary acidic protein (GFAP) antibody (1:2000 dilution) was obtained from Abcam. Mouse anti-GFAP antibody (1:1500, Proteintech), mouse anti-P75 nerve growth factor receptor (NGFR; 1:200, Abcam), and rabbit anti-IBA1 (1:200, WAKO) were also used as glial markers. Biotinylated anti–rabbit IgG (H+L) (1:200 dilution) and biotinylated anti–mouse IgG (H+L) (1:200 dilution) secondary antibodies were purchased from Vector Laboratories. Alexa Fluor 488 and Cy3 antibodies (1:1000 dilution) were purchased from Jackson ImmunoResearch, and DAPI (1:1000 dilution) was obtained from Invitrogen.

Animals

Adult male Sprague-Dawley rats weighing 150 ± 20 g were obtained from Fujian Medical University Labora-

tory Animal Center. Rats were placed in plastic cages and housed in a temperature- and humidity-controlled room under a 12-hour light/dark cycle. Water and food were available ad libitum. Rats were randomly assigned to the TN group with trigeminal nerve root compression ($n = 24$), sham operation group ($n = 24$), TN+HDACi group (TN plus selective histone deacetylase inhibitor injection into the TREZ; $n = 12$), or TN+Veh group (TN plus vehicle injection into the TREZ; $n = 12$). All animal experimental procedures were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council Institute for Laboratory Animal Research, Washington, DC: National Academies Press, 1996) and were approved by the Fujian Medical University Institutional Animal Care and Use Committee. The number of animals used and their suffering were minimized in our study.

TN Animal Model of Trigeminal Nerve Root Compression

An animal model of TN was established by retrograde insertion of a plastic filament from the right inferior orbital fissure to the TREZ in rats, as described elsewhere.¹⁹ Briefly, rats were anesthetized with pentobarbital (40 mg/kg, intraperitoneally), and an anterior-posterior curve skin incision was made above the right eye by sterile technique. The fascia and muscle near the medial wall of the orbit were gently moved aside to expose the right infraorbital nerve traveling through the infraorbital groove. A small, round plastic filament with a diameter of 0.1 cm was slowly inserted into the intracalvarium from the inferior orbital fissure to reach and compress the trigeminal nerve root. For the sham operation group, the right infraorbital nerve was exposed and left intact without filament compression of the trigeminal nerve. The incision was closed using silk sutures (5-0).

Behavioral Testing on Orofacial Mechanical Allodynia

The behavioral testing for orofacial mechanical allodynia was performed as previously described.¹⁹ Briefly, all 72 rats were habituated to behavioral testing for 3 days as baseline testing. von Frey hairs were applied to the vibrissal pad of the rats to determine the orofacial mechanical allodynia threshold. Each von Frey filament was applied five times. Stimulation always began with the filament that produced the lowest force and stopped when the threshold was found within the vibrissal pad of the rats.

Histone Deacetylase Inhibitor Administration

To better understand the role of H3 histone acetylation and illuminate the relationship between histone acetylation and TN, the selective histone deacetylase inhibitor (HDACi) suberoylanilide hydroxamic acid (SAHA; Selleck Chemicals, USA) was used in the TN animal model experiments. The SAHA was dissolved in dimethyl sulfoxide (DMSO) and diluted with 0.01 M phosphate-buffered saline (PBS). Animals in the TN+HDACi group or TN+Veh group received 50 mg/kg of SAHA or equivalent vehicle (equivalent administration dosage of DMSO diluted with 0.01 M PBS without SAHA) via microinjection into the TREZ during the TN operation, respectively.

Because of the difficulty in successive daily local administrations of SAHA or vehicle in the TN animal model, drug administration was performed only once during the animal operation. Given that the medication's effect may not last very long in vivo, we performed the behavioral testing for orofacial mechanical allodynia and immunohistochemical analysis of H3 histone acetylation in the TN+HDACi or TN+Veh group within postoperative day 14.

Tissue Preparation

Animals in the TN and sham operation groups were euthanized at days 7, 14, 21, and 28 after operation (6 rats from each group were euthanized each day). Briefly, animals were deeply anesthetized with sodium pentobarbital (200 mg/kg) and were transcardially perfused through the left ventricle with a solution containing 30% saturation picric acid and 4% paraformaldehyde phosphate buffer (pH 7.4). The segment of the trigeminal nerve root from the trigeminal ganglia to the junction of the trigeminal nerve root in the pons was dissected and then cryoprotected with a 30% (weight/volume) sucrose solution in 0.1 M phosphate buffer for 24 hours at 4°C.

Trigeminal nerve roots from the TN groups and sham operation group were embedded in mounting medium, and serial longitudinal sections with a 10- μ m thickness were cut using the Cryostat Microtome (Leica CM1950) at -20°C. The tissue sections were collected for immunohistochemical staining of GFAP, H3K9, H3K18, and H3K27.

Immunohistochemical Analysis

Immunohistochemical analysis was performed according to standard protocols. Cryosections were washed three times in 0.01 M PBS for 10 minutes each time and preincubated with 5% normal goat IgG for 1 hour. The blocking solution was later removed, and the tissue sections were incubated with GFAP, H3K9, H3K18, or H3K27 primary antibodies at 4°C overnight.

To study the types of acetylated cells in the TREZ, one of the glial markers GFAP, P75 NGFR, or IBA1 was incubated with H3K9, H3K18, or H3K27 separately. It was known that most of the cell types in the TREZ were different glia, including oligodendrocytes and astrocytes in the central part of the TREZ and Schwann cells in the peripheral part of the TREZ. The acetylated glial cells in the TREZ were distinguished not only by specific glial markers, but also by the nuclear morphological characteristics and arrangement of the nuclei. We selected specimens from the same TN group on postoperative day 21 as representative to distinguish acetylated cell types in the TREZ.

After incubation, sections were washed three times with 0.01 M PBS for 10 minutes each time. Subsequently, the sections were incubated with biotinylated anti-rabbit IgG secondary antibodies for 4 hours at room temperature. Fluorescence immunohistochemical staining was performed after washing the sections three times with 0.01 M PBS, and then the sections were incubated with Alexa Fluor 488 or Cy3 in 1% bovine serum albumin (BSA) in PBS for 1 hour. After washing three times with 0.01 M PBS, the sections were counterstained with DAPI.

The sections were observed under a confocal laser scanning microscope (Leica TCS SP8). Histone H3 acetylation levels were calculated as the ratio of the total number of immunoreactive cells to the number of DAPI-stained nuclei in each group by using Image-Pro Plus 6.0 software (Media Cybernetics Corp., USA).

Statistical Analysis

All data were expressed as the means $\pm$ standard deviation. Two-way ANOVA with post hoc Tukey tests was used for statistical analyses. A $p < 0.05$ was considered to be statistically significant. All statistical analyses were performed with the IBM SPSS 19.0 statistical software (IBM Corp.).

Results

Behavioral Results

The behavioral results of orofacial mechanical stimulation were similar to what we have previously seen in establishing a TN animal model (data not shown); that is, rats in the TN group showed significant mechanical hypersensitivity compared to that in the sham operation group. The threshold of orofacial mechanical allodynia in the TN+HDACi group with SAHA administration increased on postoperative days 7 and 14, compared with that in the TN+Veh group (with equivalent vehicle DMSO administration; $p < 0.05$; Fig. 1).

Measurement of the Interface of the CNS-PNS Transitional Zone in the TREZ

As the glial fringe of the CNS-PNS transitional zone in the TREZ was fornical and irregular, we measured the length of the glial fringe from the junction of the nerve root entering the pons to the CNS-PNS interface, according to the localization of glial fringe distinguished by GFAP and DAPI double-labeling staining. We observed that the central length of the CNS-PNS interface has no obvious change in the sham group (Fig. 2A–D) postoperatively, while it gradually extended distally after the trigeminal root mechanical compression operation in the TN animal group (Fig. 2E–H). To better display the dynamic change in the transitional zone, we measured the lengths of the lateral axis (L-axis) and central axis (C-axis) from the trigeminal root–pons junction to the glial fringe (Fig. 3A and Table 1) in the TREZ after the operation. According to our measurements (Fig. 3B and C), the length of the CNS-PNS transitional zone in the TREZ in the TN group after operation was longer than that in the sham operation group ($p < 0.05$), and the interface seemed to gradually migrate distally.

Acetylation of Histone H3 at Lysine 9, 18, and 27 in the TREZ

To assess the epigenetic regulation of acetylated histone H3 in the TREZ, we used immunohistochemistry to investigate the localization pattern of acetylated histone H3K9, H3K18, and H3K27 with an anti-H3 antibody in the cryosections. According to our results, immunoreactive acetylated H3K9 cells distributed on the TREZ in

FIG. 1. Histogram statistics (left) show the behavioral results of the orofacial mechanical stimulus threshold in two TN animal model groups after HDACi or vehicle administration. Asterisks indicate a significant difference ($p < 0.05$). There were significantly more acetylated cells in the TN+HDACi group (A1–F1) than in the TN+Veh group (A–F) on postoperative days 7 and 14. Bar = 100 μm . Figure is available in color online only.

FIG. 2. Merged images illustrating the dynamic changes in the CNS-PNS interface in the TREZ. The glial fringe of the CNS-PNS transitional zone in the TREZ is distinguished by GFAP (red) and DAPI (blue) immunofluorescence staining. Dotted line indicates the junction of the trigeminal root and the pons. The central length of the CNS-PNS interface has no obvious change in the sham operation group (A–D), while it gradually extends distally after the trigeminal root mechanical compression operation in the TN animal group (E–H). Bar = 500 μm . Figure is available in color online only.

FIG. 3. Measuring the length of the TREZ in the rat. **A:** Note the TREZ, where central glia appear in the CNS side (GFAP, red, proximal part of the root) and peripheral glia appear on the PNS side (DAPI, blue, distal part of the root). Dotted line indicates the junction of the trigeminal root and the pons; dashed box indicates sites for the quantification of acetylation of histone H3. The lateral axis (L-axis) and central axis (C-axis; bidirectional arrows) represent the lateral and central axis lengths, respectively, from the trigeminal root–pons junction to the glial fringe of the CNS–PNS transitional zone in the TREZ. Bar = 500 μ m. **B and C:** Average L-axis and C-axis lengths of the TREZ measured on postoperation days in the sham group and TN group, respectively. Asterisks indicate a significant difference ($p < 0.05$). Figure is available in color online only.

both the sham and TN groups (Fig. 4). In the sham group, immunoreactive acetylated H3K9 cells around the CNS–PNS transitional zone decreased from postoperative day 7 to day 21, and few were detected on day 28 (Fig. 4A–D and I and Table 2). In the TN group, the level of histone H3K9 acetylation gradually increased from postoperative day 7 to day 28 (Fig. 4E–I). Merged images showed the positive ratio of immunoreactive acetylated H3K9 cells among the DAPI-positive cells in both groups (Fig. 4A1–H1). According to the H3K9 immunohistochemical staining and DAPI nuclear staining, as well as the nuclear morphological characteristics and arrangement of the GFAP- and P75-immunoreactive cells, oligodendrocytes and astrocytes in the central part of the TREZ and Schwann cells in the peripheral part of the TREZ were found acetylated in the TN animal group on day 21 after mechanical compression (Fig. 4J and K).

The expression tendency of H3K18 acetylation near the CNS–PNS transitional zone of the TREZ (Fig. 5A–H1 and Table 2) was similar to that of H3K9 in that the level of H3K18 acetylation decreased in the sham group while it increased in the TN group after operation, and no obvious acetylated H3K9-positive cells were detected in the sham group on postoperative day 28 (Fig. 5I). The cell types of the acetylated cells included oligodendrocytes, astrocytes, and Schwann cells within the TREZ in the TN group (Fig. 5J and K).

According to the immunofluorescence staining results, the level of histone H3K27 acetylation cells in the sham group (Fig. 6A–D) was relatively lower than that in the TN group (Fig. 6E–H) after operation (Table 2). In the TN group, the amount of immunoreactive acetylated H3K27 cells decreased from postoperative day 7 to day 28. Oligodendrocytes, astrocytes, and Schwann cells within the TREZ were acetylated in the TN group on postoperative day 21 (Fig. 6J and K).

In order to study the role of H3 histone acetylation

in a TN animal model, immunohistochemistry staining of H3K9, H3K18, and H3K27 was also performed after HDACi administration. Compared with the results of histone acetylation in the TN+Veh group (Fig. 1A–F), the immunoreactive acetylated cells of the TN+HDACi group with SAHA administration significantly increased on postoperative day 7 and 14 (Fig. 1A1–F1).

Discussion

In this study, we investigated whether epigenetic mechanisms in the TREZ are associated with the pathogenesis of TN. It is well known that TN is an extremely severe form of facial pain, and the most common etiology of TN is chronic microvascular compression of the TREZ; however, the pathophysiology of TN has not been thoroughly elucidated to date.²¹

Anatomical Physiology of the TREZ

Previous studies have shown that the proximal portion of rootlets in the TREZ contains central nervous tissue

TABLE 1. Lengths of the central part of the CNS–PNS transitional zone in the TREZ

POD	Sham Group		TN Group	
	C-Axis Length	L-Axis Length	C-Axis Length	L-Axis Length
7	1732 $\pm$ 30	1351 $\pm$ 99	1795 $\pm$ 76	1581 $\pm$ 70
14	1723 $\pm$ 13	1396 $\pm$ 132	1931 $\pm$ 33	1559 $\pm$ 79
21	1717 $\pm$ 73	1218 $\pm$ 31	2050 $\pm$ 109	1757 $\pm$ 117
28	1640 $\pm$ 54	1145 $\pm$ 39	2172 $\pm$ 92	1655 $\pm$ 112

POD = postoperative day.

Values expressed as the mean $\pm$ standard deviation in μ m.

FIG. 4. Immunohistochemical detection of histone H3K9 acetylation in the CNS-PNS transitional zone of the TREZ. In the sham group (A–D), immunoreactive acetylated H3K9 cells (green) decreased from postoperative day 7 to day 21, and few were detected on day 28. In the TN group (E–H), acetylated H3K9 cells (green) gradually increased from postoperative day 7 to day 28. Merged images show the positive ratio of immunoreactive acetylated H3K9 cells (green) among the DAPI-positive cells (blue) around the CNS-PNS transitional zone of the TREZ (same area as in the white dashed box in Fig. 3A) in the sham group (A1–D1) and TN group (E1–H1). The ratio of H3K9 acetylated cells in the TN group was higher than that in the sham operation group (I). Asterisks indicate a significant difference ($p < 0.05$). Most of the oligodendrocytes (arrows, J) and astrocytes (triangles) were acetylated in the central part of the TREZ, and Schwann cells (double arrows, K) were acetylated in the peripheral part of the TREZ. Bar = 150 μ m (A–H1) and 50 μ m (J and K). Figure is available in color online only.

TABLE 2. Positive ratio of immunoreactive acetylated H3 cells

POD	Sham Group			TN Group		
	H3K9	H3K18	H3K27	H3K9	H3K18	H3K27
7	52 ± 11	41 ± 7	45 ± 3	74 ± 5	70 ± 7	71 ± 6
14	49 ± 3	44 ± 5	27 ± 6	65 ± 9	68 ± 7	52 ± 8
21	23 ± 2	10 ± 5	4 ± 2	76 ± 6	87 ± 4	50 ± 13
28	3 ± 1	0 ± 0	0 ± 0	69 ± 6	74 ± 5	62 ± 5

Values expressed as the mean ± standard deviation in %.

and the distal portion contains peripheral nervous tissue.^{10–12} Further, several types of glial cells are present in the TREZ, playing different roles in the nervous system. For example, the myelin sheaths in the peripheral portion of the TREZ are produced by Schwann cells, and central myelin sheaths are produced by oligodendrocytes.¹⁰ In addition to oligodendrocytes and Schwann cells, there are also astrocytes and microglia in the TREZ.^{10,11,29} Axon-glia crosstalk and glia-glia crosstalk are very complicated and crucial to the nervous system.²⁷ Axonal integrity depends on the metabolites and neurotrophic factors supplied by glia, and myelinating cells play a key role in preserving axonal connectivity and function.²³ Myelination of oligodendrocytes in the CNS is also stimulated by both axonal activity and astrocytes,²⁸ whereas myelin clearance is modulated by microglia.²³ In the PNS, Schwann cell–axon crosstalk may play a role in trophic and metabolic support to maintain axonal excitability and function, which may also have an impact on peripheral neuropathies.²⁴ Glial dysfunction has been functionally related to neuropathic pain.

As the border of the CNS-PNS junction represents a biological interface characterized by a sharp discontinuity in the variety of glial subtypes, it is more likely to be affected by different types of stimulation on the internal and external environments. In our study, we observed that the glial fringe of the CNS-PNS transitional zone was dramatically altered after a chronic compression injury to the TREZ. This result revealed the occurrence of a plastic change, a reaction to mechanical injury, and/or microenvironmental changes.

Influence of Epigenetic and Environmental Factors on Disease

It has been shown that the epigenome is highly sensitive to environmental factors,¹⁵ such as mechanical stimulation, and that other environmental factors can lead to abnormal changes in epigenetic pathways.^{1,8} Epigenetic regulation of chromatin without changing the DNA sequence plays an important role in the effective regulation of gene expression. Disordered epigenetic gene regulation may lead to human disease.²² The role of epigenetic regulation in the nervous system is a newly emerging field in neurobiology and may link to numerous neurodevelopmental, psychiatric, and neurodegenerative disorders.⁴ Epigenetic studies of axon regeneration after neural injury have been reported in recent years,^{9,26} but studies on the epigenetic regulation of glia are not very common. Chronic microvascular

compression of the trigeminal nerve root is also a kind of mechanical stimulation, but few studies on epigenetic regulation in the TREZ have been reported.

As an important epigenetic marker of chromosomal domains, histone acetylation can acetylate the lysine residues at the N-terminus of histone proteins via HATs to reduce the affinity between histones and DNA. The nucleoprotein structure is highly stabilized through numerous interactions between DNA and histone proteins, while condensed chromatin is transformed into a more relaxed structure by histone acetylation to facilitate gene transcription.^{13,32} Histone acetylation can occur at various lysine residues through different types of stimulation to regulate gene expression. Studies have suggested that histone acetylation may induce the pathological pain state, whereas HDACi has analgesic effects in models of inflammatory pain and reduces mechanical and thermal sensitivity after nerve injury.^{2,6}

Histone H3K9, H3K18, and H3K27 Acetylation in the TREZ in TN

In our study, we investigated for the first time histone H3 acetylation in the TREZ in an animal model of TN that was induced by chronic compression, as previously described.¹⁹ We found that histone acetylation of H3K9, H3K18, and H3K27 was widely distributed in the TREZ in the TN group and that a positive ratio of acetylated histone H3 in the TN group was higher than that in the sham operation group.

Studies have shown that H3K9 is a very important epigenetic marker that correlates with transcriptionally active chromatin and that it has a bidirectional modulation function that can activate genes by acetylation and silence genes by methylation.¹⁷ The HAT proteins GCN5 and PCAF (GCN5/PCAF) and CBP and p300 (CBP/p300) are transcriptional coactivators. It has been shown that CBP/p300-mediated H3K18/27 acetylation is important for recruiting RNA polymerase II to nuclear receptor target gene promoters to initiate transcription, whereas GCN5/PCAF-mediated H3K9 acetylation is dependent on active transcription.¹⁶ Further, it is thought that acetylation of histone H3K27 is a good candidate marker to distinguish between active and poised enhancer states.⁵ It has also been shown that H3K27 acetylation plays an important role in the maintenance of pluripotency and the regulation of key developmental genes in stem cells; however, methylation of H3K27 is usually linked to transcriptional repression.^{20,25}

As there was no agonist of HATs or agonist of histone deacetylase that could be used to intervene in histone acetylation to further study the role of histone acetylation in the TN animal model at present, local administration of HDACi was selected to investigate the relevance between histone acetylation and orofacial mechanical hypersensitivity. In our study, local administration of HDACi upregulated the H3 histone acetylation of the TREZ, which also reduced orofacial mechanical hypersensitivity in the TN animal model.

Therefore, we hypothesize that chronic mechanical compression injury of the trigeminal nerve root in TN patients can alter the plasticity of glia and the microenvironment of the TREZ. Subsequently, the frail microenvi-

FIG. 5. Immunohistochemical detection of histone H3K18 acetylation in the CNS-PNS transitional zone of the TREZ. In the sham group (A–D), immunoreactive acetylated H3K18 cells (green) decreased from postoperative day 7 to day 21, and few were detected on day 28. In the TN group (E–H), acetylated H3K18 cells (green) gradually increased from postoperative day 7 to day 28. Merged images show the positive ratio of immunoreactive acetylated H3K18 cells (green) among the DAPI-positive cells (blue) around the CNS-PNS transitional zone of the TREZ (same area as in the white dashed box in Fig. 3A) in the sham group (A1–D1) and the TN group (E1–H1). The ratio of H3K18 acetylated cells in the TN group was higher than that in the sham operation group (I). Asterisks indicate a significant difference ($p < 0.05$). Most of the oligodendrocytes (arrows, J), astrocytes (triangles), and Schwann cells (double arrows, K) were acetylated in the TREZ. Bar = 150 μ m (A–H1) and 50 μ m (J and K). Figure is available in color online only.

FIG. 6. Immunofluorescence staining of histone H3K27 acetylation in the CNS-PNS transitional zone of the TREZ. The level of histone H3K27 acetylation cells (green) in the sham group (A–D) was relatively lower than that in the TN group after operation. In the TN group (E–H), the amount of immunoreactive acetylated H3K27 cells (green) decreased from postoperative day 7 to day 28. Merged images show the positive ratio of immunoreactive acetylated H3K27 cells (green) among the DAPI-positive cells (blue) around the CNS-PNS transitional zone of the TREZ (same area as in the white dashed box in Fig. 3A) in the sham group (A1–D1) and the TN group (E1–H1). The ratio of H3K27 acetylated cells in the TN group was higher than that in the sham operation group (I). Asterisks indicate a significant difference ($p < 0.05$). Oligodendrocytes (arrows, J), astrocytes (triangles), and Schwann cells (double arrows, K) were acetylated in the TREZ. Bar = 150 μ m (A–H1) and 50 μ m (J and K). Figure is available in color online only.

ronment and further mechanical stimulation may induce epigenetic regulation of glia and dysfunction of glia and axons in the TREZ, which may contribute to the occurrence and development of TN. It was supposed that H3 histone acetylation not only was involved in the induction of neuropathic pain in the early stage of TN, but also was a protective adaptation mechanism for itself to alleviate pain in the chronic stage of TN by maintaining a high level of histone acetylation. Further research is needed to uncover the pathogenesis of TN.

Our study has some limitations. For example, although we used filament to compress the trigeminal nerve root in rats to mimic the common etiology of microvascular compression of the TREZ in a TN patient, the physical properties of the filament may not cause entirely the same physiological characteristics of microvascular compression of the TREZ. How to create a similar microenvironment of microvascular compression to study the interactions between vascular and trigeminal nerve injury is still unresolved. But we consider our animal model to be a close enough representation of the etiology in TN patients so that we can study epigenetic regulation in the pathogenesis of TN in an animal model. There are many different lysine residues of histone H3 and other histones, and determining the role of epigenetic regulation of the acetylation of all histones or DNA methylation in TN would require enormous effort. Moreover, it is generally known that the age of disease onset for most TN patients ranges from middle age to old age, with patients suffering decades of microvascular compression of the trigeminal nerve root. We also know that animal models are not always the same as the actual diseases in patients. Whether a TN animal model with a limited compression injury duration can substitute for the pathophysiology in TN patients with decades of compression injury has not been authoritatively determined.

Conclusions

In summary, our results suggest that a chronic compression injury of the TREZ in rats induces changes in the CNS-PNS transitional interface and stimulates epigenetic regulation of histone H3K9, H3K18, and H3K27 acetylation in the TREZ, which may be involved in the pathogenesis of TN.

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Disclosures

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Author Contributions

Conception and design: D Luo. Acquisition of data: Lin. Analysis and interpretation of data: D Luo. Drafting the article: D Luo. Reviewed submitted version of manuscript: D Luo. Approved the final version of the manuscript on behalf of all authors: D Luo. Statistical analysis: D Luo. Administrative/technical/material support: Lin, L Luo, Gong, Zheng, Wang, Du.

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