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Epigenetic mechanisms underlying the effects of triptolide and tripchlorolide on the expression of neuroligin-1 in the hippocampus of APP/PS1 transgenic mice

Xiaomei Lu[a,b], Baolin Yang[a], Hao Yu[c], Xiaoling Hu[a], Jing Nie[a], Bin Wan[a], Ming Zhang[b] and Cheng Lü[a]

[a]Department of Anatomy, Basic Medical College of Nanchang University, Nanchang, China; [b]Department of Neurology, Second Affiliated Hospital of Nanchang University, Nanchang, China; [c]First Clinical Medical College, Nanchang University, Nanchang, China

ABSTRACT

Context: Neuroligin-1 (NLGN1) is a cell adhesion protein located on the excitatory postsynaptic membrane. β-Amyloid (Aβ)-induced neuroinflammation decreases NLGN1 expression through epigenetic mechanisms. Triptolide (T10) and tripchlorolide (T4) exert protective effects on synapses in Alzheimer's disease (AD) mice, but the mechanisms remain unclear.

Objective: The effects of T10 and T4 on hippocampal NLGN1 expression in AD mice and the epigenetic mechanisms were assessed using chromatin immunoprecipitation and methylated DNA immunoprecipitation.

Materials and methods: Sixty APP/PS1 transgenic mice were randomly divided into an AD model group, a T10-treated group and a T4-treated group ($n = 20$); 20 wild-type littermates served as the control group. APP/PS1 transgenic mice were intraperitoneally injected with T10 (0.1 mg/kg) and T4 (25 μg/kg) once per day for 60 days. NLGN1 expression was examined using western blotting and quantitative PCR.

Results: T10 and T4 increased the levels of the NLGN1 protein and mRNA in hippocampus of AD mice. T10 and T4 inhibited the binding of HDAC2 ($p < 0.01$) and MeCP2 ($p < 0.01$ and $p < 0.05$, respectively) to the NLGN1 promoter, and cytosine methylation ($1.2305 \pm 0.1482 / 1.2554 \pm 0.3570$ vs. 1.6578 ± 0.1818, $p < 0.01$) at the NLGN1 promoter in the hippocampus of AD mice. T10 and T4 increased the level of acetylated histone H3 ($0.7733 \pm 0.1611 / 0.8241 \pm 0.0964$ vs. 0.5587 ± 0.0925, $p < 0.01$) at the NLGN1 promoter in the hippocampus of AD mice.

Conclusions: T10 and T4 may increase hippocampal NLGN1 expression in AD mice through epigenetic mechanisms, providing a new explanation for the mechanism underlying the protective effects of T10 and T4 on synapses.

Introduction

The histopathological changes that are typical of Alzheimer's disease (AD) are neuritic plaques, neurofibrillary tangles, synapse loss and gliosis. The β-amyloid (Aβ) protein is the main component of neuritic plaques. A common hypothesis is that the abnormal metabolism and deposition of Aβ constitute the initial factor and key step inducing the pathogenesis of AD (Reiss et al. 2018). Aβ activates microglia in the brain, which secrete and release various inflammatory mediators (Hansen et al. 2018). Inflammatory mediators inhibit long-term potentiation during synaptic transmission, resulting in structural and functional synaptic impairments (Kelly et al. 2003; Griffin et al. 2006; Jebelli et al. 2014; Liu et al. 2018). However, the specific mechanisms by which inflammatory responses lead to synaptic damage remain unclear. According to Bie et al. (2014), Aβ-induced neuroinflammation increases the binding of histone deacetylase 2 (HDAC2) and methyl-CpG-binding protein 2 (MeCP2) to the neuroligin-1 (NLGN1) promoter and cytosine methylation at the NLGN1 promoter, and inhibits histone H3 acetylation at the latter region. Thus, Aβ-induced neuroinflammation reduces

NLGN1 expression by affecting the epigenetic modification of NLGN1, ultimately damaging the structure and function of synapses. NLGN1 is a cell adhesion protein located on the excitatory postsynaptic membrane that plays an important role in synaptic plasticity (Liu et al. 2016). NLGN1 dysfunction is closely related to the pathogenesis of AD (Tristán-Clavijo et al. 2015).

Epigenetics refers to phenotypic changes that occur without a corresponding alteration in the genetic code. The major epigenetic regulatory mechanisms include DNA methylation, histone modification and microRNA interference. DNA methylation refers to the transfer of a methyl group to the 5th carbon atom of a cytosine and guanine (CpG) dinucleotide, converting cytosine to 5-methylcytosine, a process that is catalyzed by DNA methyltransferases and uses S-adenosylmethionine as a methyl donor. Histone acetylation occurs on a conserved lysine residue at the N-terminal end of the histone and is catalyzed by histone acetyltransferases and histone deacetylases (HDACs) (Kim and Kaang 2017). HDAC2 and MeCP2 are two key regulatory molecules that affect gene transcription through epigenetic modifications. MeCP2 is a chromatin-binding protein that is mainly

CONTACT Cheng Lü ✉ lvcheng119@ncu.edu.cn 📇 Department of Anatomy, Basic Medical College of Nanchang University, 461 Bayi Road, Nanchang, Jiangxi 330006, China; Ming Zhang ✉ zm6861226@126.com 📇 Department of Neurology, Second Affiliated Hospital of Nanchang University, 1 Minde Road, Nanchang, Jiangxi 330006, China

expressed in neurons in the nervous system. HDAC2 is a class I HDAC that is mainly distributed in brain regions associated with learning and memory. MeCP2 contains two characteristic domains; one specifically binds to methylated CpG dinucleotides and the other binds to the corepressor Sin3A and forms a transcriptional inhibitory complex with HDAC2. Thus, DNA methylation recruits HDAC2 to the promoter region through the interaction between MeCP2 and HDAC2, causing the deacetylation of histones at the promoter region and inducing chromatin contraction, thereby blocking gene expression (Guan et al. 2015).

Triptolide (T10) is a diterpene triepoxide isolated from the herb *Tripterygium wilfordii* Hook F. (Celastraceae) that exerts potent anti-inflammatory effects (Chen et al. 2018). Recently, T10 has also been reported to influence epigenetic modification (Hu et al. 2017). Tripchlorolide (T4), a derivative of T10, exerts stronger anti-inflammatory effects and has lower toxicity than its parent compound (Lin et al. 2016). T10 and T4 inhibit neuroinflammation in the brains of AD model animals, ameliorate the behavioural deficits observed in these animals, and exert a clear protective effect on synapses (Pan et al. 2009; Nie et al. 2012; Zeng et al. 2015; Cui et al. 2016). However, the mechanisms by which T10 and T4 protect synapses remain unclear. In this project, we assessed the effects of T10 and T4 on the hippocampal NLGN1 levels in APP/PS1 transgenic mice and explored their epigenetic regulatory mechanisms using western blotting, quantitative PCR (qPCR), chromatin immunoprecipitation (ChIP) and methylated DNA immunoprecipitation (MeDIP), with the aim of further elucidating the target of their synaptic protective effects.

Materials and methods

Reagents

T10 (batch number: 2016091726, purity > 99%) and T4 (batch number: 20180307, purity > 99%) were purchased from Fujian Academy of Medical Sciences. The rabbit monoclonal anti-HDAC2 antibody (ab32117), mouse monoclonal anti-HDAC2 antibody-ChIP Grade (ab12169), rabbit polyclonal anti-MeCP2 antibody-ChIP Grade (ab2828), rabbit polyclonal anti-acetyl-histone H3 antibody-ChIP Grade (ab10812), polyclonal antibody against 5-methylcytosine (ab117133), high sensitivity ChIP kit and methylated DNA immunoprecipitation kit were acquired from Abcam (Cambridge, UK). The rabbit monoclonal anti-NLGN1 antibody (GTX133208) was purchased from GeneTex (Irvine, CA, USA). The mouse monoclonal anti-GAPDH antibody (BM3876), horseradish peroxidase (HRP)-conjugated anti-rabbit immunoglobulin G (IgG) antibody (BA1054) and HRP-conjugated anti-mouse IgG antibody (BA1050) were purchased from BOSTER Biotechnology Co., Ltd. (Wuhan, China). An ultrasensitive enhanced chemiluminescence (ECL) kit was purchased from TIANDZ (Beijing, China). TRIzol reagent was purchased from Invitrogen (Carlsbad, CA). A RevertAid^{TM} HMinus first-strand cDNA synthesis kit was acquired from Thermo Scientific (Waltham, MA, USA), and 2× Easy Taq PCR SuperMix was purchased from QIAGEN (Hilden, Germany).

Animals and drug treatments

Five-month-old female APP/PS1 transgenic mice (strain B6C3-Tg (APPswe.PSEN1dE9) 85Dbo/J) and 5-month-old female non-transgenic wild-type littermates were provided by the Nanjing University Model Animal Research Institute (Certificate Nos. 201800568 and 201801741). Sixty APP/PS1 transgenic mice were randomly divided into an AD model group, a T10-treated group and a T4-treated group ($n = 20$). Twenty wild-type littermates served as the control group. The T10-treated group was intraperitoneally injected with T10 (0.1 mg/kg) and the T4-treated group was intraperitoneally injected with T4 (25 μg/kg) once per day for 60 days, based on previous studies (Cheng et al. 2014; Zeng et al. 2015). T10 and T4 were dissolved in 1% DMSO saline. The other two groups were intraperitoneally injected with the same volume of 1% DMSO saline. Western blotting, qPCR, ChIP and MeDIP were performed after treatment. All experimental procedures involving animals were approved by the Animal Ethics Committee of Nanchang University.

Western blotting

Western blotting was performed as previously described (Wang et al. 2016). The hippocampal tissues were rapidly removed from the rest of the brain and lysed on ice with precooled lysis buffer. After centrifugation, the supernatants were collected and a portion of the homogenates was used to measure total protein concentrations. Then, equal amounts of protein lysates (30 μg) were separated using 8% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. After blocking with 10% non-fat dry milk in TBST containing 0.1% TWEEN 20 for 2 h, the membranes were incubated with the following primary antibodies overnight at 4 °C: rabbit monoclonal anti-NLGN1 (1:3000), rabbit monoclonal anti-HDAC2 (1:5000), rabbit polyclonal anti-MeCP2 (1:2000) and mouse monoclonal anti-GAPDH (1:1000). GAPDH served as a loading control. The membranes were washed with TBST three times and separately incubated with an HRP-conjugated anti-rabbit IgG antibody (1:5000) and HRP-conjugated anti-mouse IgG antibody (1:5000) for 90 min. An ultrasensitive ECL kit was used to detect the immunoreactive signals. Density scanning and image analysis were performed using quantitative software (Image Lab).

qPCR

qPCR was performed using a previously described method (Wang et al. 2016). The hippocampal tissues were rapidly dissected from the rest of the brain. Total RNA was extracted using TRIzol reagent and quantified using a spectrophotometer (Thermo Scientific, Waltham, MA, USA). The RevertAid^{TM} first strand cDNA synthesis kit was used to synthesize cDNA templates from RNA samples. PCR was performed in a PCR thermal cycler (ABI, Vernon, CA, USA) using 2× Easy Taq PCR SuperMix. The mRNA expression level was determined using the $\Delta\Delta$CT method. The following primer sequences were used: NLGN1, 5′-ATCTCTGGCTGGAGCTGGTA-3′, 5′-GCTGATGTGACTGGTGTGGA; HDAC2, 5′-GGTCGTAGGAATGTTGCTGAT-3′, 5′-AAGCCAATGTCCTCAAACAGG-3′; MeCP2, 5′-CCAGGCTTTCTACCCCGTTT-3′, 5′-CTGCCCAGGTCATGGTGATC-3′; and GAPDH 5′-GGTGAAGGTCGGTGTGAACG-3′, 5′-CTCGCTCCTGGAAGATGGTG-3′.

ChIP

The ChIP method used in the present study was similar to a previously described protocol (Bie et al. 2014). The hippocampal

tissues were rapidly dissected from the rest of the brain and crosslinked with 4% formaldehyde for 10 min. Then, the chromatin was dissolved in lysis buffer and sonicated on ice with an ultrasonic cell disruptor (Branson, Danbury, CT, USA) six times for 15 s each to produce fragments of approximately 100–700 bp. The samples were incubated overnight at 4 °C in assay strip wells containing the following antibodies: rabbit polyclonal anti-acetyl-histone H3 antibody-ChIP Grade (0.8 μg/well), mouse monoclonal anti-HDAC2-ChIP Grade (0.8 μg/well) and rabbit polyclonal anti-MeCP2-ChIP Grade (0.8 μg/well). Non-immune IgG (0.8 μg/well) was used as the negative control, and a monoclonal anti-RNA polymerase II antibody (0.8 μg/well) was used as a positive control. The immunocomplexes were washed with a DNA release buffer containing RNase A and proteinase K, and the DNA-histone crosslinks were reversed to obtain a DNA fragment. The purified DNA was subjected to qPCR. The following primer sequences were used: NLGN1, 5′-CCTTTGATCTCCC ACAGACAG-3′, 5′-GGAAAGTGCCATGAAACACC-3′; and GAPDH, 5′-CTCCTGTGTTCTCCCCTCAC-3′, 5′-GTTGAAT TGGAGGAGGCTCA-3′. The ChIP results were normalized to the CT values of the input DNA. The ChIP/input ratio was calculated using the equation $2^{(-\Delta\Delta Ct[\text{normalized ChIP}])}$.

MeDIP

MeDIP was performed as previously described (Han et al. 2016). The hippocampal tissues were rapidly removed from the rest of the brain. DNA was extracted using phenol chloroform. DNA was sonicated with an ultrasonic cell disruptor (Branson, Danbury, CT, USA) on ice three times for 10 s each to produce fragments of approximately 200–1000 bp. Each sample was incubated in assay strip wells containing a polyclonal antibody against 5-methylcytosine (1 μg/well) overnight at 4 °C, and normal mouse IgG was used as a negative control. The immunoprecipitated DNA fragments were eluted with DNA release buffer containing proteinase K. The purified DNA was subjected to qPCR. The following primer sequences were used: NLGN1, 5′-CCTTTGATCTCCCACAGACAG-3′, 5′-GCCTCCATGTGAGTT CAGGT-3′; GAPDH, 5′-CTCCTGTGTTCTCCCCTCAC-3′, 5′-GTTGAATTGGAGGAGGCTCA-3′; and H191CR, 5′-GAGCCGC ACCAGATCTTCAG-3′, 5′-TTGGTGGAACACACTGTGATCA-3′. H191CR served as a positive control (methylated DNA), and GAPDH served as a negative control (unmethylated DNA). The MeDIP results were normalized to the CT values of the input DNA. The MeDIP/input ratio was analyzed as described above.

Statistical analysis

The data are presented as means ± SD. All data were analyzed using one-way analysis of variance with SPSS statistical software (SPSS Inc., Chicago, IL, USA). The least significant difference (LSD) test was used for intergroup comparisons. $p < 0.05$ was considered statistically significant.

Results

T10 and T4 upregulated NLGN1 expression in AD mice

NLGN1 dynamically regulates the plasticity of excitatory synapses and participates in long-term memory formation. We performed western blotting and qPCR to observe the effects of T10 and T4 on NLGN1 expression in the hippocampus of AD mice.

Western blots revealed decreased levels of the NLGN1 protein in AD mice compared with wild-type mice, and the levels of the NLGN1 protein were increased in T10-treated mice and T4-treated mice compared with AD mice (Figure 1). Similarly, qPCR results showed a lower level of the NLGN1 mRNA in AD mice than in wild-type mice and a higher level of the NLGN1 mRNA in T10-treated mice and T4-treated mice than in AD mice (Figure 2). Based on these results, T10 and T4 upregulated the NLGN1 level in AD mice.

T10 and T4 inhibited HDAC2 expression in AD mice

Overexpression of HDAC2 decreases the density of dendritic spines and synaptic plasticity and is implicated in the development of AD (Guan et al. 2009). Therefore, the effects of T10 and T4 on the hippocampal HDAC2 levels in AD mice were determined. Western blots revealed a higher level of the HDAC2 protein in AD mice than in wild-type mice, and the levels in T10-treated mice and T4-treated mice were lower than in AD mice (Figure 1). The qPCR results also revealed a higher level of the HDAC2 mRNA in AD mice than in wild-type mice and a lower level of the HDAC2 mRNA in T10-treated mice and T4-treated mice than in AD mice (Figure 2). Thus, T10 and T4 decreased the HDAC2 level in AD mice.

T10 and T4 inhibited MeCP2 expression in AD mice

MeCP2 influences spine morphogenesis and synaptic transmission and links inflammation with synaptic damage (Tomasoni et al. 2017). Therefore, we examined the effects of T10 and T4 on MeCP2 expression in the hippocampus of AD mice. Western blots showed increased levels of the MeCP2 protein in AD mice compared with wild-type mice and decreased levels of the MeCP2 protein in T10-treated mice and T4-treated mice compared with AD mice (Figure 1). The qPCR assay also revealed an increase in the level of the MeCP2 mRNA in AD mice compared with wild-type mice and a decrease in the level of the MeCP2 mRNA in T10-treated mice and T4-treated mice compared with AD mice (Figure 2). Therefore, T10 and T4 decreased the MeCP2 level in AD mice.

T10 and T4 inhibited the binding of HDAC2 and MeCP2 to the NLGN1 promoter in AD mice

We analyzed the effects of T10 and T4 on the binding of HDAC2 and MeCP2 to the NLGN1 promoter in the hippocampus of AD mice using ChIP assays. The binding of HDAC2 and MeCP2 to the NLGN1 promoter was increased in AD mice compared to wild-type mice, but was reduced in T10-treated mice and T4-treated mice compared to AD mice (Figure 3), suggesting that T10 and T4 inhibited the binding of HDAC2 and MeCP2 to the NLGN1 promoter in AD mice.

T10 and T4 increased the level of acetylated histone H3 at the NLGN1 promoter in AD mice

We used ChIP assays to observe the effects of T10 and T4 on histone H3 acetylation at the NLGN1 promoter in the hippocampus of AD mice. The site to which the antibody used in this experiment binds is lysine 9 of histone H3 (H3K9), a main site
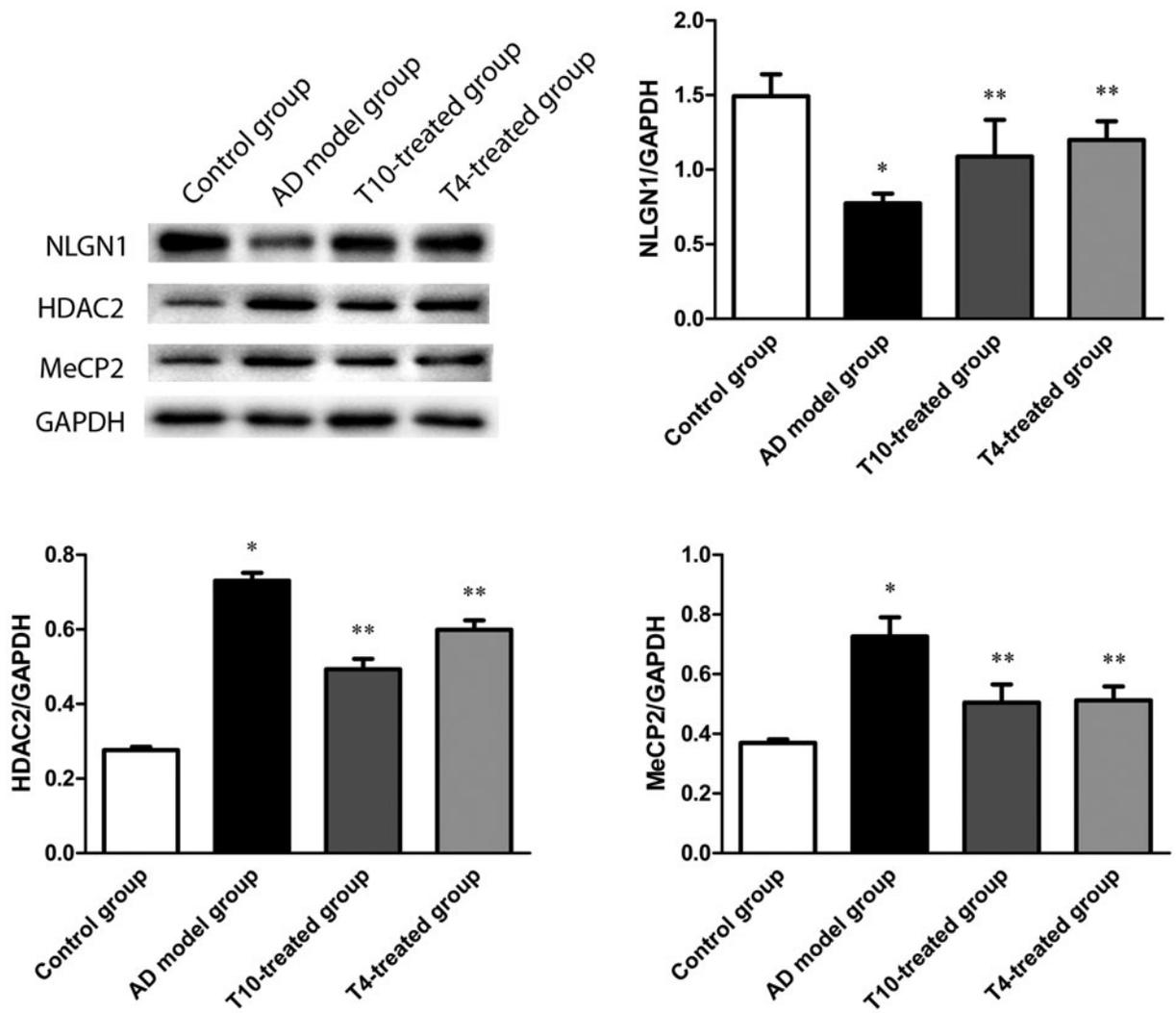
Figure 1. Western blots showed T10- and T4-induced increases in the levels of the NLGN1 protein and decreases in the levels of the HDAC2 and MeCP2 proteins in the hippocampus of AD mice. *$p < 0.01$ compared with the control group and **$p < 0.01$ compared with the AD model group.
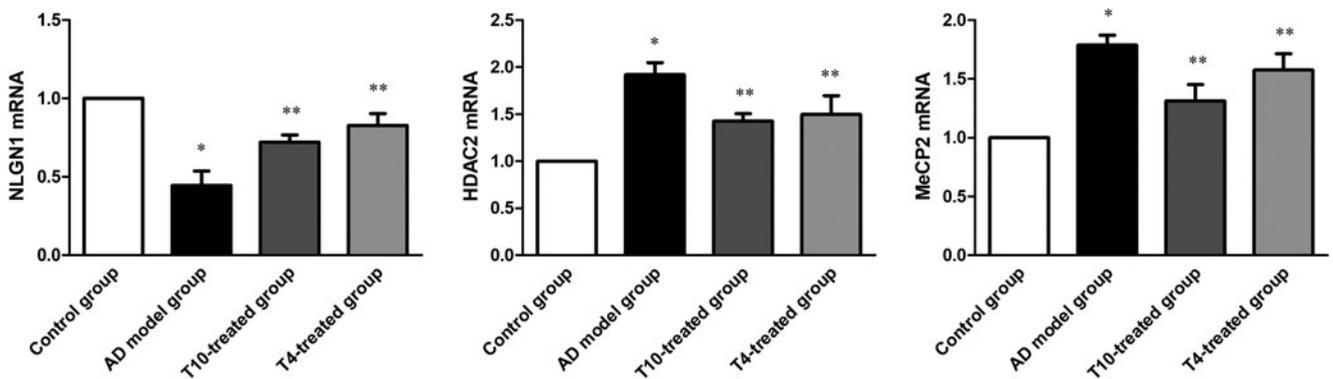

Figure 2. qPCR showed that T10 and T4 increased the expression of the NLGN1 mRNA and inhibited the expression of the HDAC2 and MeCP2 mRNAs in the hippocampus of AD mice. *$p < 0.01$ compared with the control group and **$p < 0.01$ compared with the AD model group.

for histone H3 acetylation. Acetylation of this site promotes the binding of transcription factors to DNA and activates gene transcription (Pokholok et al. 2005). The level of acetylated histone H3 at the NLGN1 promoter was decreased in AD mice compared with wild-type mice and increased in T10-treated mice and T4-treated mice compared with AD mice (Figure 3), suggesting that T10 and T4 increased the level of acetylated histone H3 at the NLGN1 promoter in AD mice.
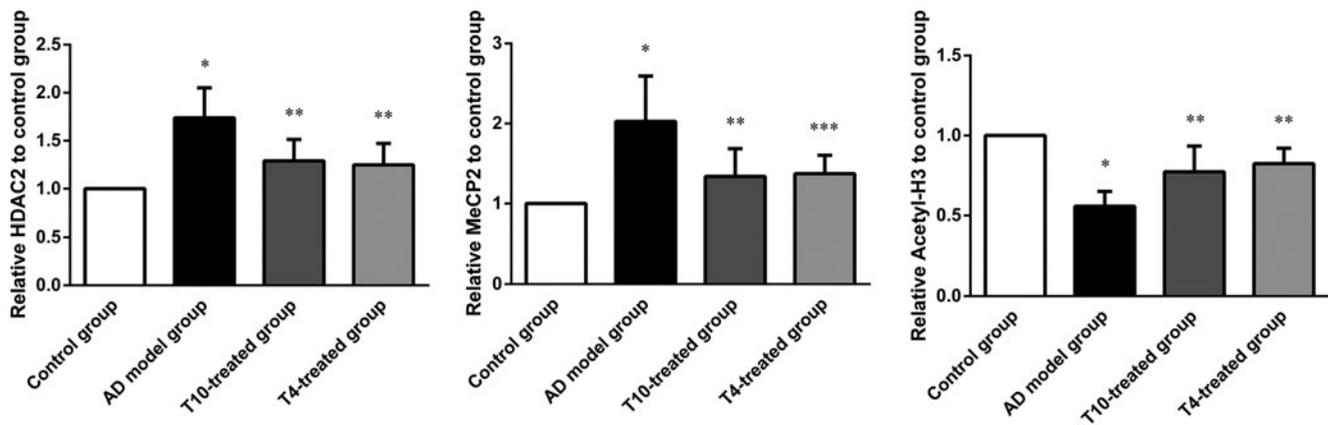
Figure 3. Based on the results of the ChIP assay, T10 and T4 inhibited the binding of HDAC2 and MeCP2 to the NLGN1 promoter and increased the level of acetylated histone H3 at that promoter in the hippocampus of AD mice. $*p < 0.01$ compared with the control group, $***p < 0.05$ compared with the AD model group, and $**p < 0.01$ compared with the AD model group.
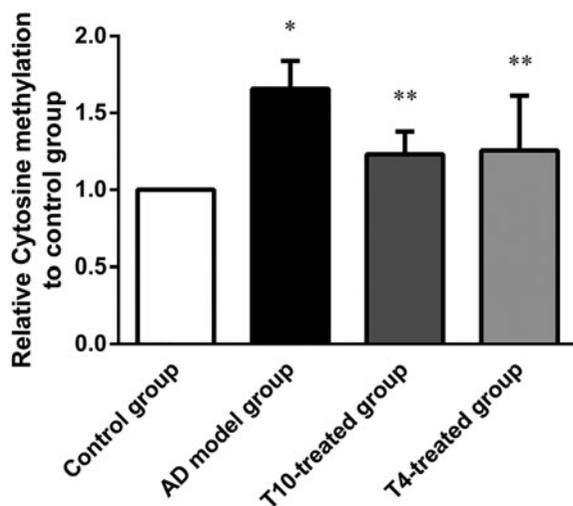

Figure 4. Results from the MeDIP assay showed that T10 and T4 inhibited cytosine methylation at the NLGN1 promoter in the hippocampus of AD mice. $*p < 0.01$ compared with the control group and $**p < 0.01$ compared with the AD model group.

T10 and T4 inhibited cytosine methylation at the NLGN1 promoter in AD mice

MeDIP was used to observe the effects of T10 and T4 on cytosine methylation at the NLGN1 promoter in the hippocampus of AD mice. A higher level of cytosine methylation was observed at the NLGN1 promoter in AD mice than in wild-type mice. However, a lower level of cytosine methylation was observed at the NLGN1 promoter in T10-treated mice and T4-treated mice than in AD mice (Figure 4). Based on these results, T10 and T4 inhibited cytosine methylation at the NLGN1 promoter in AD mice.

Discussion

NLGN1 is located on the excitatory postsynaptic membrane and is a class I transmembrane protein. NLGN1 binds to the neurexin protein on the presynaptic membrane to establish a connection between the presynaptic membrane and the postsynaptic membrane (Xing et al. 2018). Overexpression of NLGN1 increases the density of excitatory synapses and increases the efficiency of presynaptic vesicle release (Chih et al. 2005). An intrahippocampal injection of a small interfering RNA targeting the NLGN1 gene impairs spatial memory and hippocampal long-term potentiation (Bie et al. 2014). Knockout of the NLGN1 gene also decreases the number of newborn neurons and excitatory synapses in mice (Schnell et al. 2014). The formation of glutamatergic synapses is significantly reduced in patients with AD carrying NLGN1 mutations (Tristán-Clavijo et al. 2015). The expression of NLGN1 is significantly decreased in the hippocampus of rats with AD induced by Aβ and APP/PS1 transgenic mice (Bie et al. 2014). Our western blotting and qPCR experiments revealed a decrease in the hippocampal expression of NLGN1 in AD mice compared with wild-type mice. We concluded that NLGN1 regulates synaptic plasticity and that the abnormal expression of NLGN1 influences the pathogenesis of AD.

Synapse loss is one of the pathological characteristics of AD and is closely associated with cognitive dysfunction in patients with AD. Neuroinflammation exerts a detrimental effect on synapses. As shown in the study by Griffin et al. (2006), interleukin-1 released by activated microglia decreases synaptophysin expression in cultured rat cerebral cortical neurons. According to Jebelli et al. (2014), conditioned medium from lipopolysaccharide-stimulated microglia decreases the levels of synaptophysin and drebrin in cultured cerebellar granule neurons. Liu et al. (2018) found that inflammation induced by the injection of lipopolysaccharide into the cerebral ventricles of mice reduces hippocampal synaptophysin and PSD-95 expression. Kelly et al. (2003) observed the inhibition of hippocampal long-term potentiation following the injection of interleukin-1 into the cerebral ventricles of rats. However, the mechanism by which inflammatory responses result in synaptic damage remains controversial. As shown in the study by Bie et al. (2014), inflammation in the brains of subjects with AD reduces NLGN1 expression through epigenetic mechanisms. Our western blotting and qPCR experiments revealed an increase in the hippocampal HDAC2 and MeCP2 expression in AD mice compared with wild-type mice. ChIP and MeDIP assays showed that the binding of HDAC2 and MeCP2 to the NLGN1 promoter and cytosine methylation at the NLGN1 promoter were increased in AD mice compared to wild-type mice, but the level of acetylated histone H3 at the NLGN1 promoter was decreased in AD mice compared to wild-type mice. Taken together, these results support the hypothesis that the decreased NLGN1 expression observed in AD mice may be related to the epigenetic mechanisms induced by inflammation.
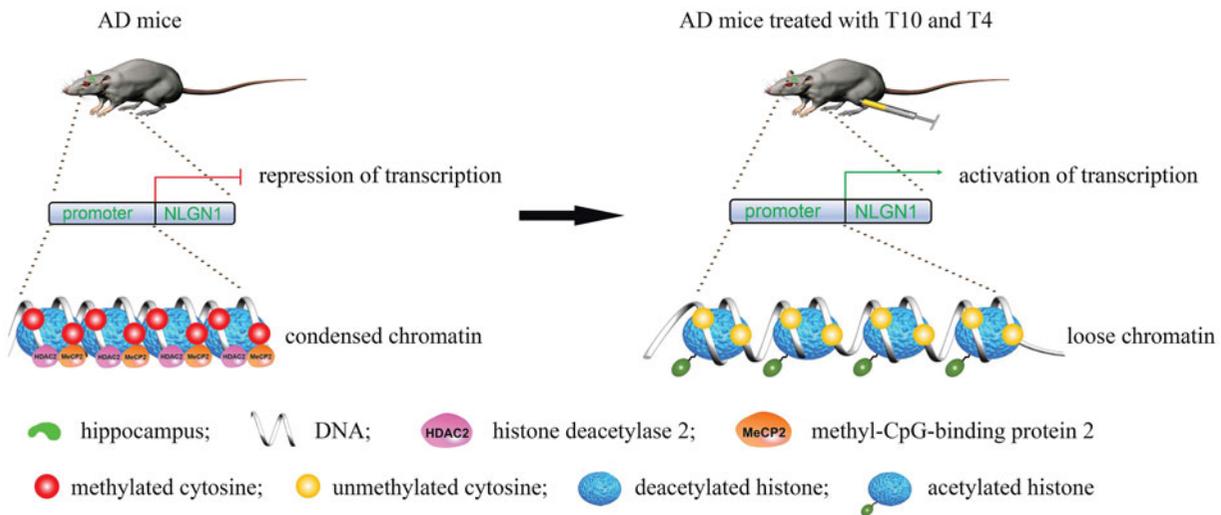
Figure 5. T10 and T4 increase the expression of NLGN1 in the hippocampus of AD mice through epigenetic mechanisms.

In light of the neuroinflammatory pathology in the brains of subjects with AD, T10 and T4 may be promising therapeutic drug candidates for AD. Cui et al. (2016) found that T10 inhibited the inflammatory responses in the brains of AD mice and ameliorate memory impairments in AD mice. Nie et al. (2012) reported a T10-induced increase in synaptophysin levels in an *in vitro* model of AD. Zeng et al. (2015) reported a T4-induced increase in the levels of synaptic proteins in AD mice and improvement in the memory of AD mice. As shown in the study by Pan et al. (2009), T4 inhibits the release of proinflammatory cytokines mediated by Aβ. Thus, T10 and T4 inhibit inflammation in the brains of subjects with AD and exert a definite protective effect on synapses. In our western blotting and qPCR assays, T10 and T4 increased hippocampal NLGN1 expression in AD mice and inhibited hippocampal HDAC2 and MeCP2 expression in AD mice. ChIP and MeDIP assays revealed that T10 and T4 inhibited the binding of HDAC2 and MeCP2 to the NLGN1 promoter and cytosine methylation at the NLGN1 promoter, but increased the level of acetylated histone H3 at that promoter in the hippocampus of AD mice. In summary, T10 and T4 increase the level of acetylated histone H3 at the NLGN1 promoter in the hippocampus of AD mice through epigenetic mechanisms. Increased histone H3 acetylation opens the chromatin structure at the NLGN1 promoter, thereby promoting the transcription of the NLGN1 gene and increasing NLGN1 expression (Figure 5). Our results further support the hypothesis that T10 and T4 protect against synapse loss. However, this study also has some limitations that should be further addressed. Oestrogen plays crucial roles in chromatin modification and memory (Fortress and Frick 2014) and its level varies at different stages of the growth cycle (Fortress et al. 2014). The five-month-old female mice used in this study represent the adult stage of the growth cycle (Fellini et al. 2006) and the oestrogen level was not assessed in this study. The relationship between oestrogen levels and epigenetic mechanisms underlying the effects of T10 and T4 on NLGN1 expression requires further study. In addition, T10 and T4 exert certain toxic and side effects on the reproductive system, digestive system and circulatory system (Xi et al. 2017). Therefore, further studies are also needed to establish different dosages of T10 and T4 to balance the efficacy and toxicity of T10 and T4 and optimize their dosing to protect synapses in subjects with AD.

Conclusions

In the present study, the effects of T10 and T4 on hippocampal NLGN1 levels in AD mice and their epigenetic regulatory mechanisms were investigated. T10 and T4 upregulated hippocampal NLGN1 expression in AD mice and inhibited hippocampal HDAC2 and MeCP2 expression in AD mice. Furthermore, T10 and T4 inhibited the binding of HDAC2 and MeCP2 to the NLGN1 promoter and cytosine methylation at the NLGN1 promoter in the hippocampus of AD mice. However, T10 and T4 increased the levels of acetylated histone H3 at the NLGN1 promoter in the hippocampus of AD mice. Thus, T10 and T4 may increase NLGN1 expression in the hippocampus of AD mice through epigenetic mechanisms, which provide a new explanation for the mechanism underlying the protective effects of T10 and T4 on synapses.

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