Original Article

LncRNA HOTAIR modulates lipopolysaccharide-induced inflammatory injury in neuronal cell line HT-22

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Abstract

Background: Epilepsy is a neurological disorder of the central nervous system. Inflammation can disrupt the blood-brain barrier, which serves to maintain brain homeostasis. Epilepsy typically occurs in the young and elderly. Further research into epilepsy is needed in order to develop and understand the most appropriate diagnosis and clinical treatment of epilepsy, along with the patho-physiology and mechanism of epilepsy. A well-managed program of research into epilepsy will help to develop a more focused understanding of the occurrence ratio and reasons behind the high prevalence of epilepsy throughout the world. In total, around 50 million people in the world suffer from this neurological disease. The fact is that if the disease is diagnosed properly, sufferers can lead a life seizure-free, as it is estimated that 70% of people with epilepsy recover and lead a normal life. The World Health Organization (WHO) estimates that three quarters of the cases occur in low-income countries. People suffering from epilepsy often face discrimination and stigma in their societies.

Objective: To explore the functional role of long non-coding RNA (lncRNA) Hox transcript antisense RNA (HOTAIR) in hippocampal HT-22 after lipopolysaccharide (LPS) stimulation.

Methods and materials: An *in vitro* model was constructed using LPS treatment. Inflammatory cell injury was monitored through changes in cell viability, cell apoptosis and levels of inflammatory cytokines. The HOTAIR level after LPS stimulation was evaluated using the qRT-PCR method. Afterwards, HOTAIR expression was inhibited via cell transfection. The impact of HOTAIR depletion on LPS-induced cell inflammatory injury, and key kinases of NF-κB and MEK/ERK pathways, were investigated.

Results: The results of the experiment indicated that LPS treatment led to the upregulation of HOTAIR in HT-22 cells, and LPS-induced cell inflammatory injury was reduced by HOTAIR knockdown. Intriguingly, HOTAIR depletion suppressed the phosphorylated levels of crucial kinases of both NF-κB and MEK/ERK pathways.

Conclusions: LPS upregulated HOTAIR, and HOTAIR, can modulate the LPS-induced cell inflammatory injury via NF-κB and MEK/ERK pathways. [*Ethiop. J. Health Dev.* 2020; 34(3): 205-213]

Key words: HOTAIR, LPS, inflammatory injury, neuronal cell, epilepsy, lncRNA, HT-22 cells

Introduction

There is a critical need to quicken health research and strategies. While there have been substantial improvements in different fields of innovation, more significance should be given to research into general wellbeing so that we can devise solutions for those diseases and conditions that are as yet uncurable. Concentrating on experiments will empower us to get increasingly exact data with respect to the hazard factors, disease trends, the study of disease transmission, pathogenicity and findings.

An example of such a disease where a focus on research should be given is epilepsy. Epilepsy is marked by spontaneous recurrent seizures caused by abnormal transmission of electrical signals from the central nervous system (1,2). Epilepsy, one of the most prevalent neurological disorder diseases, affects about 50 million people worldwide (3). The genetic syndrome, brain mass, brain damage, central nervous system tumors and infections are known currently as the risk factors for epilepsy (4). Although the majority of people with epilepsy experience remission via receiving either

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monotherapy or combination therapy with antiepileptic drugs, approximately 5-35% of them still have seizures and are resistant to medication (5). As a result, a more effective treatment strategy is urgently needed to cure epilepsy.

As reported, the blood-brain barrier (BBB) plays an important part in maintaining brain homeostasis by regulating the solute flux (6). Systemic inflammation can disrupt and cause dysfunction in the BBB, which is regarded as a biomarker of epileptogenesis (7). A growing body of evidence attributes the precipitation and recurrence of epilepsy to the inflammation of cerebral parenchyma (8). Inflammation is considered to be an important cause of epilepsy, different from the traditional mechanisms that cause epilepsy, such as the imbalance of excitatory and inhibitory neurotransmitter systems, and hormone disorders (9). Hence, deep exploration into the protection of inflammatory signal interdiction in epileptogenesis and the response of neurons to inflammation are of great significance. 'LncRNAs', which refers to the families of non-coding RNAs (ncRNAs) containing endogenous nucleotides with a length of >200 nucleotides, are emerging as a significant regulator in various human diseases (10). The MEK/ERK usually cataract couples' indication from surface of cell receptors to transcription factors, which eventually regulate the gene expression. Additionally, this cascade controls the movement of many other proteins tangled in apoptosis. Recently, it has been reported in the literature that lncRNAs are actively implicated in the pathogenesis of epilepsy (11-14). LncRNA Hox transcript antisense RNA (HOTAIR) is largely found in the brain, and multivariate analyses indicate that the relative expression level of HOTAIR is related independently to the grades of glioma. ROC curve analysis demonstrates that an expression level of HOTAIR greater than 0.40 represents a moderate value when applying to predict II-IV grades (15). However, the HOTAIR-related mechanistic regulation in epilepsy remains elusive.

In the current study, lipopolysaccharide (LPS) was utilized to construct the *in vitro* inflammation cell model during epilepsy. The possible regulation of HOTAIR and the underlying mechanism were investigated in LPS-treated HT-22 cells.

Materials and methods

Cell culture and treatment: HT-22, the mouse hippocampal neuronal cell line, was procured from Procell Life Science & Technology (Wuhan, China). The H-22 cell line has the capacity to grow in several mouse strains. Its high invasiveness and metastasis make it suitable for use in anti-tumor research. Their unique property has allowed them to be used in anti-tumor research. A study conducted by Zeng *et al.* in 2010 used this unique cell line of H-22, showing how the vaccine from dendritic cells with AFP cDNa fragments (the signal peptide of AFP1, not AFP2) created an extensive anti-tumor immune response. A study done by He *et al.* in 2015 used the H-22 model study hepatic cell carcinoma. A cell sample of HT-22 was allowed to grow in the Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Grand Island, NY, USA) in a humidified incubator filled with 5% CO_2 and 95% air, at 37°C. Ten per cent (v/v) foetal bovine serum (FBS; Gibco) and 1% (v/v) penicillin-streptomycin solution (Invitrogen, Carlsbad, CA, USA) served as the supplements for DMEM. DMEM contains a four-fold higher concentration of amino acids and vitamins than the original Basal Medium Eagle (BME), and is the most commonly used medium for adherent cell phenotypes. The composition of the original DMEM is 1,000 mg/L of glucose. This was first used to culture embryonic mouse cells. Further research proved that with alterations of 4,500 mg/L glucose, it has the capability to provide optimal conditions for the growth of certain cell types. For LPS treatment, the HT-22 cell sample was cultivated in the DMEM, adding 1 μg/ml of LPS (Sigma-Aldrich, St. Louis, MO, USA) for 16 hours.

Cell transfection: For plasmid transfection, the triple HOTAIR-specific short hairpin RNAs (shRNAs) – termed sh-HOTAIR-1, sh-HOTAIR-2 and sh-HOTAIR-3 – were designed and produced by GenePharma Co., Ltd. (Shanghai, China), along with the shRNAs negative control (NC), termed sh-NC. These plasmids were transfected into HT-22 cell samples by application of Lipofectamine™ 3000 Transfection Reagent (Invitrogen). The targeting oligoribonucleotides of HOTAIR-specific shRNAs were as follows: sh-HOTAIR-1, 5'-GCCCCGTCGCGGAAGGTGTGGCGTCAAG-3'; sh-HOTAIR-2, 5'-

ACTGGGGTCTCTACGAACCCAAAGACGA-3'; sh-HOTAIR-3, 5'-TCAATTATAGCGGGCGTTACCAACGCGA-3'; and sh-NC, 5'-CATGCAAACTGAGTATTCGCTGGATCGG-3'.

Cell viability detection: HT-22 cell samples were planted at the density of 1 × 10^4 cells/well in 96-well plates. After LPS treatment, 10 μL of Cell Counting Kit-8 reagent (CCK-8; Dojindo Laboratories, Kumamoto, Japan) was added into each well for two hours at 37°C. Cell viability was monitored by testing the absorbance at 450 nm.

Flow cytometer for cell apoptosis: The cell apoptosis rate was monitored by applying double-staining with Annexin V-fluorescein isothiocyanate (Annexin V-FITC) and propidium iodide (1 × 10^5 cells/well) were treated with LPS, then collected and re-suspended in the binding buffer. Following double-staining as per the protocol of the Annexin V-FITC/PI apoptosis detection kit (BD Biosciences, San Jose, CA, USA), the percentages of apoptotic cell samples were determined by flow cytometer (BD Biosciences).

Western blot: The protein extracted from HT-22 cell samples were acquired by culturing in the radioimmunoprecipitation assay (RIPA) buffer, then quantified and diluted in the loading buffer to the same concentration. RIPA buffer can also be stated as a lysis buffer that can cause lysis of tissues and cells. The composition of RIPA buffer of 1X concentration is 20mM Tris-HCL (pH 7.5), 1mM Na2EDTA, 150 mM NaCl, 1 mM EGTA 1% NP-40 1% Sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1mM Beta-glycerophosphate presence of sodium dodecyl sulfate (SDS) in the composition can cause the protein to denature. Following separation by electrophoresis on 12% SDS-PAGE (polyacrylamide gel electrophoresis), samples were shifted onto the polyvinylidene difluoride (PVDF) membrane and treated with 5% bovine serum albumin (BSA; Beyotime, Shanghai, China). A PVDF membrane has the capacity to bind through hydrophobic interaction with biomolecules. The membrane pores increase the surface area binding and restrict the size of bound proteins. The membrane has a high retention capacity along with a high protein binding capacity. Samples were then probed with the diluted primary antibodies against the internal reference GAPDH (ab8245; Abcam, Cambridge, MA, USA) and Bax (ab32503), Bcl-2 (ab32124), p65 (ab16502), p-p65 (ab86299), IkBα (ab7217), p-IkBα (ab133462), MEK (ab32091), p-MEK (ab96379), ERK (ab184699), p-ERK (ab201015) at 4°C overnight. After washing in the Tris-buffered saline with 0.1% Tween-20 (TBST), membranes were probed with the horse-radish peroxidase (HRP)-tagged secondary antibodies (Abcam) for two hours. HRP is used extensively applications in biochemistry due to its ability to amplify signal along with that increase the detection levels of a target molecule. Samples were finally immersed in the electrochemiluminescence (ECL) luminous liquid (Pierce, Rockford, IL, USA).

Caspase-3 activity detection: The caspase-3 activity kit (Solarbio, Beijing, China) was commercially acquired and employed in line with the user manual. Cell samples of HT-22 in the 96-well plates were prepared for mixing with the reaction buffer and caspase-3 substrate at 37°C for four hours. The reaction mixture was assayed by microplate reader at 405 nm.

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Enzyme-linked immunosorbent assay (ELISA): The culture supernatants were collected from the 24-well plates after treatment or transfection. Levels of tumor necrosis factor-α (TNF-α), interleukin (IL)-10, IL-6 and IL-β in culture supernatants were analyzed using ELISA kits (R&D Systems, Minneapolis, MN, USA) based on the supplier's instruction.

Total RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR): On the basis of recommendations provided by the manufacturer, the total RNAs were extracted from the cultured HT-22 cell samples with TRIzol reagent (Invitrogen) and converted into complementary DNA (cDNA) with PrimeScript RT Reagent Kit (Takara, Otsu, Japan). The expression level of HOTAIR was quantified by qRT-PCRusing SYBR Green PCR Master Mix (Invitrogen) and the Step-One Plus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). HOTAIR expression was standardized to the internal reference GAPDH and calculated via the comparative delta-delta CT method ($2^{-\Delta\Delta Ct}$).

Statistical analysis: Each assay was independently repeated in triplicate. Results were all given as the mean ± standard deviation (SD). Data analysis was processed by Prism 5 software (GraphPad, San Diego, CA, USA). P-value was calculated via Student's t-test or one-way analysis of variance (ANOVA), with the significance level set at less than 0.05.

Results

LPS treatment induced HT-22 cell injury and released inflammatory cytokines: In order to verify whether the *in vitro* inflammatory HT-22 cell model was successfully established, we first assessed the cell viability and cell apoptosis using CCK-8 and flow cytometer assays, then measured the inflammatory cytokines using ELISA. As shown in Figure 1A, the viability of HT-22 cells obviously decreased after treating with LPS ($^{**}p < 0.01$). However, under LPS treatment, the percentage of apoptotic HT-22 cells significantly increased compared to the control group ($^{**}p < 0.01$; see Figure 1B). Likewise, western blot data indicated that the level of anti-apoptosis protein, Bcl-2, was weakened, while the level of pro-apoptotic protein, Bax, was enhanced in the LPS-group (see Figure 1C). The upregulated caspase-3 activity was also detected in the LPS-group, as shown in Figure 1D. We then analyzed the levels of IL-1β, IL-6, IL-10 and TNF-α after treating HT-22 cells with LPS. ELISA results showed that the released protein levels of IL-1β, IL-6, IL-10 and TNF-α into the culture medium were all remarkably elevated after treatment with LPS, compared to the control group (all $^{**}p < 0.01$; see Figure 1E). All of the above results suggest the successful establishment of the cell model with inflammatory injury via LPS stimulation.

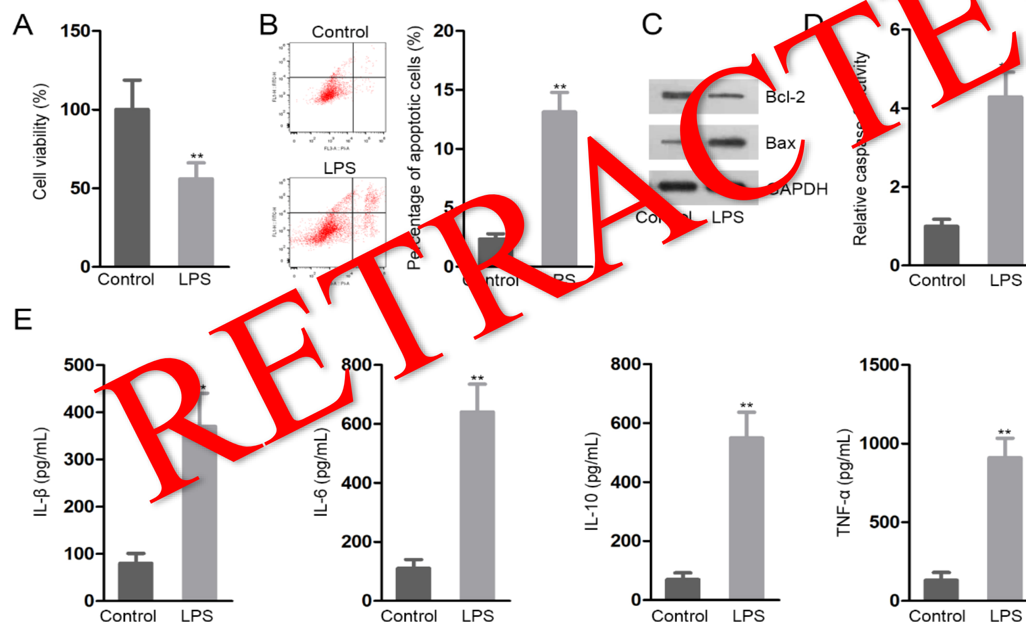

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Figure 1: **LPS treatment induced HT-22 cell injury and released inflammatory cytokines A**

Notes: The cell viability of HT-22 detected by CCK-8 assay; B. The percentage of apoptotic HT-22 cells assayed by flow cytometer; C. Western blot analysis for the levels of apoptosis-associated proteins (Bcl-2 and Bax); D. Caspase-3 activity detection; E. Release of inflammatory cytokines in culture medium were estimated by ELISA; Non-treated cells served as the control; All data were exhibited as the mean ± SD; $^{**}p < 0.01$.

HOTAIR expression was upregulated in LPS-treated HT-22 cells: The expression level of HOTAIR in LPS-treated HT-22 cells were analysed using the qRT-PCR method. The experimental results in Figure 2 show that the HOTAIR level was obviously upregulated in the LPS-group compared to that in the control group ($^{**}p < 0.01$). Data show that the HOTAIR expression level was upregulated after treating HT-22 cells with LPS.

Standard deviation of the replicated data was calculated and percentage error was measured. In all of our experimental study a 5% error was observed.

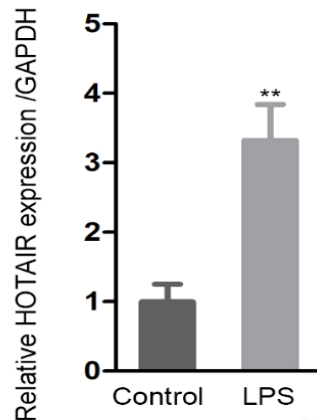

Figure 2: **HOTAIR expression was upregulated in LPS-treated HT-22 cells**

Notes: Expression level of HOTAIR was analyzed using qRT-PCR after treating HT-22 cells with LPS; non-treated cells served as the control; All data were exhibited as the mean ± SD; **p < 0.01.

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HOTAIR depletion ameliorated LPS-induced inflammatory injury of HT-22 cells: First, we estimated the transfection effectiveness of HT-22 cells with the three specific shRNAs to HOTAIR using qRT-PCR. When compared to the sh-NC group, the expression level of HOTAIR in HT-22 cells transfected with sh-HOTAIR-1/2/3 markedly increased, particularly in the sh-HOTAIR-3 group ($^{**}p < 0.01$; see Figure 3A), showing that HOTAIR was expressed non-physiologically after specific transfection. We then implemented assays utilizing the sh-HOTAIR-3. Subsequent CCK-8 assay demonstrated that the decrease of cell viability by LPS was reversed by HOTAIR depletion in comparison with the LPS + sh-NC group (both $^{**}p < 0.01$; see Figure 3B). The increased caspase-3 activity by LPS was reduced by silencing HOTAIR expression in HT-22 cells (both $^{**}p < 0.01$; see Figure 3C). Importantly, the expressions of IL-1β, IL-6, IL-10 and TNF-α were upregulated by LPS, but lowered due to the HOTAIR knockdown when compared with the LPS + sh-NC group (all $^{**}p < 0.01$; see Figure 3D). Taken together, HOTAIR depletion attenuated the LPS-induced inflammatory injury of HT-22 cells. We replicated all the experiments at least three times, and calculated the standard deviation. In each case, less than 5% of error was identified and calculated.

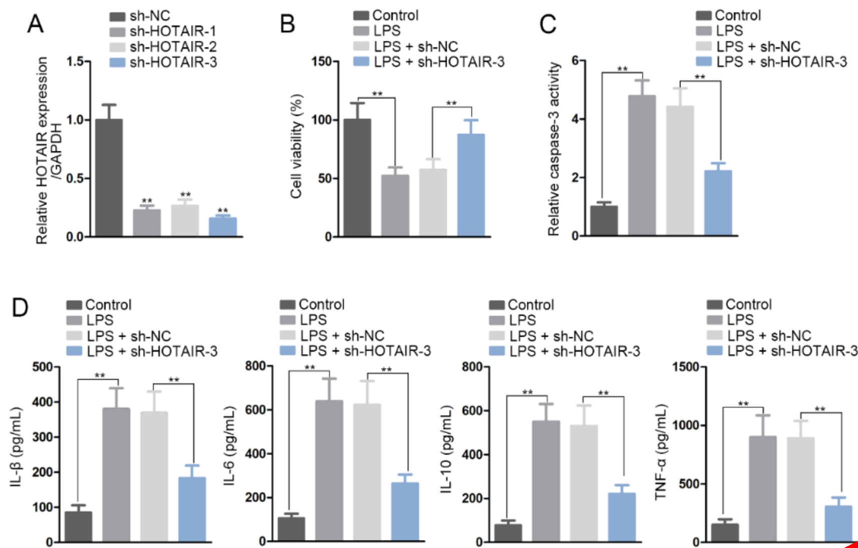

Figure 3: **HOTAIR depletion ameliorated LPS-induced inflammatory injury of HT-22 cells**

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Notes: A. HOTAIR expression level in HT-22 cells transfected with specific shRNAs to HOTAIR, versus sh-NC group; B. Cell viability by CCK-8 assay after inhibiting HOTAIR in LPS-treated HT-22 cells; C. Caspase-3 activity was assessed; D. ELISA was conducted for the levels of inflammatory cytokines in HT-22 cells responding to HOTAIR depletion; Non-treated cells served as the control; All data were exhibited as the mean ± SD; $^{**}p < 0.01$.

HOTAIR depletion inhibited the LPS-induced activation of NF-κB and MEK/ERK pathways in HT-22 cells: To probe into the underlying HOTAIR-related mechanistic regulation, the phosphorylated levels of critical kinases of NF-κB and MEK/ERK pathways were assayed by western blotting. In Figures 4A and 4B, we observe that the levels of phosphorylated p65, IκBα, MEK and ERK were all elevated under LPS treatment. In addition, their upregulations induced by LPS were all weakened in response to the HOTAIR depletion. Data manifested that the activated NF-κB and MEK/ERK pathways by LPS could both be inhibited by repressing HOTAIR expression in HT-22 cells.

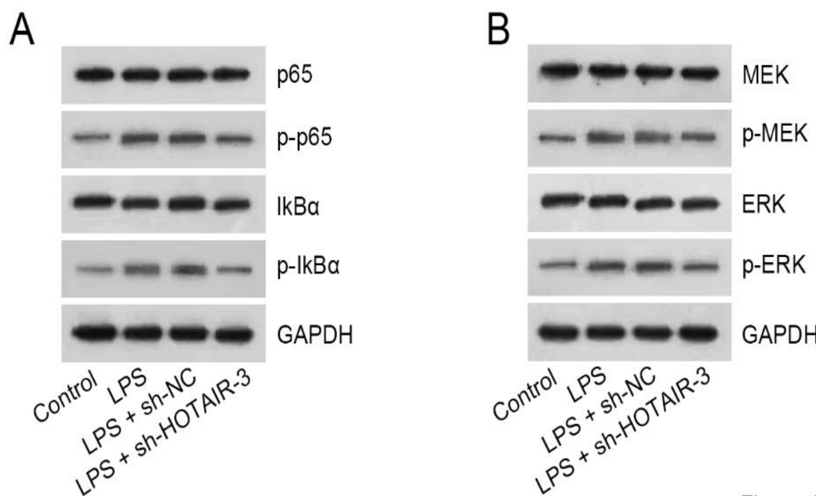

Figure 4: **HOTAIR depletion inhibited the LPS-induced activation of NF-κB and MEK/ERK pathways in HT-22 cells**

Notes: A. The levels of critical kinases of NF-κB pathways was assayed by western blotting in control, LPS, LPS + sh-NC and LPS + sh-HOTAIR-3 groups; B. The levels of key kinases of MEK/ERK pathway in each group; Non-treated cells served as the control; All data were exhibited as the mean ±SD.

Discussion

Of the world's disease burden, epilepsy caused by abnormal neuronal excitability accounts for a considerable proportion. Consequently, the in-depth study of epilepsy has become a hot topic. In our study, HOTAIR was identified to be upregulated in HT-22 cells under LPS stimulation. In addition, we observed that HOTAIR depletion ameliorated the LPS-induced cell inflammatory injury. More importantly, the NF-κB and MEK/ERK pathways activated by LPS treatment were suppressed by silencing HOTAIR expression.

As a primary glycolipid in the outer membrane of Gram-negative bacteria, LPS is widely applied for establishing the *in vivo* or *in vitro* experimental inflammation model (16,17). Moreover, multiple studies concerning the mechanism that contributes to epilepsy development and the potential treatments for epilepsy have been carried out utilizing the murine hippocampal HT-22 cells (18,19). Accordingly, we first constructed the *in vitro* inflammatory HT-22 cell model using LPS stimulation. Cell viability, cell apoptosis and release of inflammatory cytokines were all assayed to ascertain whether the cell model was successfully established. The endotoxin LPS could decrease proliferative cells and increase apoptotic cells in various cell types (20,21). Likewise, proliferative HT-22 cells were markedly reduced, but apoptotic cells were induced by LPS. During cell apoptosis, the Bcl-2 family plays the key role in modulating programmed cell death (22). We found that the anti-apoptotic protein, Bcl-2, was down-regulated, but the pro-apoptotic protein, Bax, was upregulated, following the obvious upregulation of caspase-3 activity. Thus, LPS induced cell injury in mitochondrial- and caspase-dependent ways. More intriguingly, levels of pro-inflammatory cytokines were distinctly enhanced, showing that the inflammation of HT-22 cells was induced successfully.

As mentioned above, HOTAIR was reported to be implicated in the progression of glioma (brain disease), identified as the independent risk factor for II-IV grades. We therefore hypothesized that HOTAIR might be implicated in the development of epilepsy. The HOTAIR expression level in HT-22 cells with inflammatory injury was then analyzed. qRT-PCR data presented the upregulation of HOTAIR expression under LPS treatment, which was consistent with the study of Obaid *et al.*, who report that HOTAIR was over-expressed in LPS-treated macrophages (23). Similarly, Zhang *et al.* report that HOTAIR expression was induced by LPS in human hepatocellular carcinoma cell lines (24). Subsequently, HT-22 cells stimulated with LPS were transfected with HOTAIR-silencing plasmids, followed by detection of cell proliferation, cell apoptosis and levels of pro-inflammatory cytokines. Early studies uncovered that HOTAIR could affect the apoptosis of cardiomyocytes, breast cancer cells and nucleus pulposus cells by affecting apoptotic-related proteins – Bax, Bcl-2, and caspase-3 (25,26). In the present study, LPS-induced inflammatory cell injury was attenuated by HOTAIR depletion. To our knowledge, this report is the first to reveal the role of HOTAIR in LPS-treated HT-22 cells. During LPS-induced inflammation, the ligation of toll-like receptor 4 and LPS causes the activation of NF-κB and MAPK pathways, as well as the inflammatory mediators (27). We thus further analyzed these signaling pathways in HT-22 cells abnormally expressing HOTAIR. Data showed the activation of these two signaling cascades induced by LPS was strongly hindered by HOTAIR depletion, indicating that the regulation of HOTAIR was linked with NF-κB and MEK/ERK pathways. To summarize, HOTAIR expression was strengthened in HT-22 cells by LPS. Depletion of HOTAIR weakened the LPS-induced cell inflammatory injury via NF-κB and MEK/ERK pathways. This study might provide a theoretical basis for in-depth studies of neuronal inflammation-induced epilepsy. This is a major problem in most of the world and it has been addressed in this manuscript from a health development perspective. However, more investigations should be implemented *in vivo* to verify the detailed role of HOTAIR.

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Conclusions

Epilepsy affects the central nervous system with abnormal electric signals coming from the brain which causes abnormal seizures. In this paper, we studied the role of HOTAIR in HT-22 cells. The H-22 cells were induced by LPS and various pathways were studied, and the role of HOTAIR was analyzed. It was established that HOTAIR showed an upregulated expression in H-22 cells that are induced by LPS.

References

1. Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, *et al.* ILAE official report: A practical clinical definition of epilepsy. Epilepsia. 2014;55(4):475-82.
2. Leach JP, Abassi H. Modern management of epilepsy. Clin Med J R Coll Physicians London. 2013;13(1):84-6.
3. Sridharan R. Epidemiology of epilepsy. Curr Sci. 2002;82(6):664-70.
4. Singh A, Trevick S. The epidemiology of global epilepsy. Neurol Clin. 2016;34(4):837-47.
5. Gschwind M, Seeck M. Modern management of seizures and epilepsy. Swiss Med Wkly. 2016;146:w14310.
6. de Wit NM, Vanmol J, Kamermans A, Hendriks JJA, de Vries HE. Inflammation at the blood-brain barrier: The role of liver X receptors. Neurobiol Dis. 2017;107:57-65.
7. Bar-Klein G, Lublinsky S, Kamintsky L, Noyman I, Veksler R, Dalipaj H, *et al.* Imaging blood-brain barrier dysfunction as a biomarker for epileptogenesis. Brain. 2017;140(6):1692-705.
8. Bozzi Y, Caleo M. Epilepsy, seizures, and inflammation: Role of the C-C motif ligand 2 chemokine. DNA Cell Biol. 2016;35(6):257-60.
9. Andrzejczak D, Woldan-Tambor A, Bednarska K, Zawilska JB. The effects of topiramate on lipopolysaccharide (LPS)-induced proinflammatory cytokine release from primary rat microglial cell cultures. Epilepsy Res. 2016;127:352-7.
10. Prensner JR, Chinnaiyan AM. The emergence of lncRNAs in cancer biology. Cancer Discov. 2011;1(5):391-407.
11. Lee D-Y, Moon J, Lee S-T, Jung K-H, Park D-K, Yoo J-S, *et al.* Dysregulation of long non-coding RNAs in mouse models of localization-related epilepsy. Biochem Biophys Res Commun. 2015;462(4):433-40.
12. Geng J-F, Liu X, Zhao H-B, Fan W-F, Geng J-J, Liu X-Z. LncRNA UCA1 inhibits epilepsy and seizure-induced brain injury by regulating miR-495/Nrf2-ARE signal pathway. Int J Biochem Cell Biol. 2018;99:133-9.
13. Jang Y, Moon J, Lee S-T, Jun J-S, Kim T-J, Lim J-A, *et al.* Dysregulated long non-coding RNAs in the temporal lobe epilepsy mouse model. Seizure. 2018;58:110-9.
14. Wu Q, Yi X. Down-regulation of long noncoding RNA MALAT1 protects hippocampal neurons against excessive autophagy and apoptosis via the PI3K/Akt signaling pathway in rats with epilepsy. J Mol Neurosci. 2018;65(2):234-45.
15. Zhao W-H, Yuan H-Y, Ren X-Y, Huang K, Guo Z-Y. Association between expression of HOTAIR and invasiveness of gliomas, and its predictive value. Adv Clin Exp Med. 2019;28(9):1179-83.
16. Uddin MJ, Joe Y, Kim S-K, Jeong S-O, Ryter SW, Pae H-O, *et al.* IRG1 induced by heme oxygenase-1/carbon monoxide inhibits LPS-mediated sepsis and pro-inflammatory cytokine production. Cell Mol Immunol. 2016;13(2):170-9.
17. Jiang W, Luo F, Lu Q, Liu J, Li P, Wang X, *et al.* The protective effect of Trillin LPS-induced acute lung injury by the regulations of inflammation and oxidative state. Chem Biol Interact. 2016;243:127-34.
18. Rahn JJ, Bestman JE, Josey BJ, Inks ES, Stackley KD, Rogers CE, *et al.* Novel vitamin K analogs suppress seizures in zebrafish and mouse models of epilepsy. Neuroscience. 2014;259:142-54.

19. Zheng Y, Huang J, Tao L, Shen Z, Li H, Mo F, *et al.* Corticosterone increases intracellular Zn2+ release in hippocampal HT-22 cells. Neurosci Lett. 2015;588:172-7.
20. Guo C, Yuan L, Wang J-G, Wang F, Yang X-K, Zhang F-H, *et al.* Lipopolysaccharide (LPS) induces the apoptosis and inhibits osteoblast differentiation through JNK pathway in MC3T3-E1 cells. Inflammation. 2014;37(2):621-31.
21. Liu J, Chen S, Ren W, Liu J, Yang P, Chen Z, *et al.* Lipopolysaccharide-induced suppression of periodontal ligament cell proliferation and apoptosis are strengthened under high glucose conditions. Arch Oral Biol. 2017;79:70-6.
22. Ashkenazi A, Fairbrother WJ, Leverson JD, Souers AJ. From basic apoptosis discoveries to advanced selective BCL-2 family inhibitors. Nat Rev Drug Discov. 2017;16(4):273-84.
23. Obaid M, Udden SMN, Deb P, Shihabeddin N, Zaki MH, Mandal SS. LncRNA HOTAIR regulates lipopolysaccharide-induced cytokine expression and inflammatory response in macrophages. Sci Rep. 2018;8(1):15670.
Zhang J, Chen M, Zhai Y, Fu Y. HOTAIR regulates lipopolysaccharide-induced inflammatory response in hepatocytes. J Cell Physiol. 2020;235(5):4247-55.
24. Zhang D, Wang B, Ma M, Yu K, Zhang Q, Zhang X. LncRNA HOTAIR protects myocardial infarction rat by sponging miR-519d-3p. J Cardiovasc Transl Res. 2019;12(3):171-83.
25. Yu Y, Zhang X, Li Z, Kong L, Huang Y. LncRNA HOTAIR suppresses TNF-α induced apoptosis of nucleus pulposus cells by regulating miR-34a/Bcl-2 axis. Biomed Pharmacother. 2018;107:729-37.
26. Yoo M-S, Shin J-S, Choi H-E, Cho Y-W, Bang M-H, Baek N-I, *et al.* Fucosterol isolated from Undaria pinnatifida inhibits lipopolysaccharide-induced production of nitric oxide and pro-inflammatory cytokines via the inactivation of nuclear factor-κB and p38 mitogen-activated protein kinase in RAW264.7 macrophages. Food Chem. 2012;135(3):967-75.

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