Duan, T.; Du, X.; Hu, L.; Chen, J. (2023) The impact of H19 on Aβ treated PC12 cells apoptosis and its mechanism. Revista Internacional de Medicina y Ciencias de la Actividad Física y el Deporte vol. 23 (89) pp. 351-362
DOI: https://doi.org/10.15366/rimcafd2023.89.024

ORIGINAL

IMPACT OF H19 ON AB TREATED PC12 CELLS APOPTOSIS IN YOUNG PLAYERS

Tao Duan[1]#, Xuebei Du[2]#, Lin Hu[3], Jie Chen[1]*

1Affiliated Hospital of North Sichuan Medical College, Nanchong 637000, Sichuan province, China
2Sichuan Academy of Medical SciencesSichuan Provincial People's Hospital, Chengdu 610106, Sichuan Province, China
3Chengdu University, Chengdu 610106, Sichuan Province, China
#: These authors contributed equally to this work
*Corresponding author: Jie Chen
Email: jiec13@163.com

Spanish-English translators:

UNESCO Code / UNESCO Code:
Council of Europe classification / Council of Europe classification:

Recibido 30 de abril de 2020 **Received** April 30, 2020
Aceptado 26 de junio de 2020 **Accepted** June 26, 2020

ABSTRACT

Purpose: To investigate the influence of long-chain non-coding RNA H19 on the apoptosis of Aβ treated PC12 cells and its mechanism. Methods: Exploiting Aβ treated PC12 cells to construct the Alzheimer disease (AD) model, and adopting pcDNA3.1-H19 and si-H19 plasmids to transfect PC12 cells to regulate the expression of H19. Using real-time fluorescence quantitative PCR (qRT-PCR) technology to exam the expressions of H19, miR-29b-5p, BACE1, pro-apoptotic Bax, Caspase3, and apoptosis-inhibiting gene Bcl-2 in PC12 cells. Employing Western blot (WB) to check the expressions of pro-apoptotic Bax, Caspase3, Bcl-2, BACE1 protein, and finally detecting the expression level of Aβ42 via ELISA. Results: Overexpression of H19 boosted the expressions of pro-apoptosis-related genes (i.e., Bax, Caspase3) in Aβ treated PC12 cells, while reducing the expressions of apoptosis-related genes Bcl-2; Simultaneously, miR-29b-5p expression decreased which results in the overexpression of BACE1 and Aβ 42. Inhibiting the H19 expression decreased the expressions of Bax and Caspase3 and increased the expression of Bcl-2; However, the expression of miR-29b-5p reduced which renders the expression level of BACE1 and Aβ42 decreased meaningfully. The above results are

statistically significant. Conclusion: H19 participates in the regulation of AD cell model apoptosis ,which may be relevant to the miR-29b-5p/BACE1/Aβ signal pathway.

KEYWORDS: H19; miR-29b-5p; BACE1; Signal path; PC12 cells

As one of the most common diseases of the elderly, Alzheimer disease (AD) is a nervous system disease. Its incidence rate is increasing year by year, which seriously reduces the quality of life of the elderly. Generally, the diagnosed person will die within 10 years (Scheltens et al., 2016). The pathological characteristics of AD include the formation of neuroinflammatory plaques and neurofibrillary tangles (NFT) in the cerebral cortex and hippocampus. Among them, neuroinflammatory plaques are formed by intracellular deposition of amyloid β (Aβ) produced by the transmembrane protein APP (amyloid precursor protein) cleaved and decomposed by β-secretase (BACE 1) (Hung & Livesey, 2018). However, the basic molecular mechanism of AD is not completely clear. Long non coding RNA (lncRNA) refers to a class of non coding RNA that does not encode proteins and is more than 200 nucleotides (nt) in length. LncRNA produces specific functions at the transcriptional and post transcriptional levels in various diseases, including neurodegenerative diseases, cardiovascular diseases, tumors, etc., and regulates gene expression, so it can be used as a biomarker and a potential therapeutic target (Bhat et al., 2016; Wei, Luo, Zou, & Wu, 2018). Recent research results confirm that lncRNA can affect the occurrence and development of AD (Riva, Ratti, & Venturin, 2016). MicroRNA (miRNA) is a kind of endogenous non coding RNA with a length of about 20-24 nucleotides, and miR-29 is still one of the most interesting miRNA families. It has been found that some miRNAs are specifically expressed or enriched in the human brain, which is related to promoting neuronal differentiation, synaptic plasticity and memory formation (Schratt et al., 2006).

LncRNA H19 is a highly conserved imprinted gene in mammalian cells. In the process of ontogenesis, lncRNA H19 is highly expressed in the embryonic stage, and its expression will be down regulated after birth (Gabory, Jammes, & Dandolo, 2010). However, studies have found that the expression of lncRNA H19 in brain tissue of patients with stroke, epilepsy and Parkinson's syndrome is up-regulated (Voellenkle et al., 2016; Yang et al., 2018). High expression of lncRNA H19 is related to brain damage caused by subarachnoid hemorrhage and status epilepticus (J.-X. Wang et al., 2015), while knockout of H19 will reduce brain damage and brain cell apoptosis (Broderick & Zamore, 2011; Choi, Park, & Park, 2017). Studies have shown that miR-29 may participate in the regulation of APP and BACE1 expression. In patients with sporadic AD, the protein level of BACE1 is abnormally increased, while miR-29 is significantly decreased. miR-29b inhibits the expression level of BACE1 and Aβ in neuronal cells (Hébert et al., 2009;

Hébert et al., 2008). These findings provide theoretical support for the causal relationship between lncRNA H19, miR-29 and AD. We used the biological information software Starbase to predict and analyze that lnc RNA H19 and miR-29b-5p have binding regions. However, there is no relevant research on the impact of the relationship between H19 and miR-29b-5p on the occurrence and development of AD. In summary, this paper mainly studied the effect of lncRNA H19 on cell viability and apoptosis of PC12 cells after Aβ-induced injury and explored the effect of lncRNA H19/miR-29b-5p/BACE1 axis on Aβ protein expression (Chielle, Feltez, & Rossi, 2017).

1. MATERIALS AND METHODS

1.1 Materials

CCK-8 test kit is from Shanghai Biyuntian Biotechnology Co., Ltd; Annexin V-FITC cell apoptosis detection kit was purchased from Tianjin Sanjian Biotechnology Co., Ltd; TRIpure Total RNA Extraction Kit, EntiLink ™ 1st Strand cDNA Kit, EnTurbo ™ SYBR Green PCR kit was purchased from Wuhan Kelu Biotechnology Co., Ltd; Western Blot related reagents were purchased from Beijing Aisben Technology Co., Ltd; The Lipofectamine 2000 (Invitrogen) transfection kit comes from Shanghai Kemin Biotechnology Co., Ltd.

1.2 Methods

1.2.1 Cell culture and modeling

Cell culture: In this study, Aβ25-35 (25μmol/L) was used to induce PC12 cells to construct AD cell model. After 36 hours of culture, the viability level of PC12 cells was tested. Cells in logarithmic phase were randomly divided into eight groups: Normal control group, AD group (pretreated with accumulated Aβ25-35 (25μmol/ L, dissolved in 1% FBS medium) for 36 hours), AD+ PCDNA3.1-NC group (pcDNA3.1 pretreatment followed by Aβ induction treatment), AD+ pcDNA3.1-H19 group (pcDNA3.1-H19 pretreatment and Aβ induction treatment), AD+si-NC group (si-NC pretreatment and Aβ induction treatment), AD+si-H19 group (si-H19 pretreatment and Aβ induction treatment), AD+ smart silencer-H19+ miR-29b-5pinhibitor group (si-H19+miR-29b-5pinhibitor co-transfected cells were treated with Aβ induction) and AD+ smart silencer-H19+ miR-29b-5p mimic group (si-H19+miR-29b-5p mimic co-transfected cells were treated with Aβ induction).

1.2.2 CCK-8 detection of cell activity

First, trypsin was used to digest cells in logarithmic growth phase. Then, according to the relevant procedures in the instructions of CCK-8 test kit, they were divided into experimental group and control group. Finally, 450nm light

absorption value (A value) was measured with the microplate reader, and the survival rate was calculated according to the formula: cell survival rate%=A value of the experimental group/A value of the control group * 100%.

1.2.3 Real time fluorescent quantitative PCR (qRT-PCR)

Each group of cells will extract RNA from each group of cells according to the procedure steps on TRIzol kit and measure its concentration. Then the reverse transcription test kit was used for reverse transcription. After the above operation, qRT-PCR was performed on the assay kit using PCT and the cycle threshold of each group was obtained. Finally, 2-ΔΔCT method was used for quantitative calculation. 1.2.4 Cell transfection

1.2.4 Cell transfection

The well growing cells were cultured in serum-free medium on a six well plate. When the cell fusion degree reaches 60% - 80%, transfect according to the above steps of Lipofectamine 2000 kit. Transfected plasmids were purchased from Guangzhou Ruibo Biotechnology Co., Ltd.

1.2.5 Western Blot (WB)

The total protein of each group was extracted after the cells were lysed with RIPA lysate, and the protein concentration was detected by BCA protein concentration determination kit. After 10% SDS-PAGE separation and electrotransfer, it was sealed with TBST buffer solution at room temperature for 2h, and then used monoclonal antibody for overnight at 4 ℃. The next day, the newly prepared ECL mixture (A:B=1:1) was dripped onto the protein side of the membrane, and then exposed, developed and fixed in the darkroom. AlphaEaseFC software was used for systematic analysis.

1.3 Data analysis

All data were replicated three times to obtain the mean and standard deviation. Data were processed by SPSS22.0 statistical software, and comparison between groups was performed by one-way analysis of variance and t-test. $P < 0.05$ was certified as statistically significant, and GraphPad 8.0 software was used for mapping

2. RESULTS

2.1 Preparation of AD cell model

Compared with the control group, from the analysis of CCK-8 results, the cell activity in the AD cell model group was significantly reduced (Fig. 1A). From the analysis of qRT-PCR results, the expression of apoptosis promoting genes Bax and Caspase3 was up-regulated in AD cell model group, while the

expression of anti apoptosis gene Bcl-2 was decreased (Fig. 1B). From the analysis of flow cytometry apoptosis detection results, the apoptosis rate of AD cell model group was significantly increased (Fig. 1C).

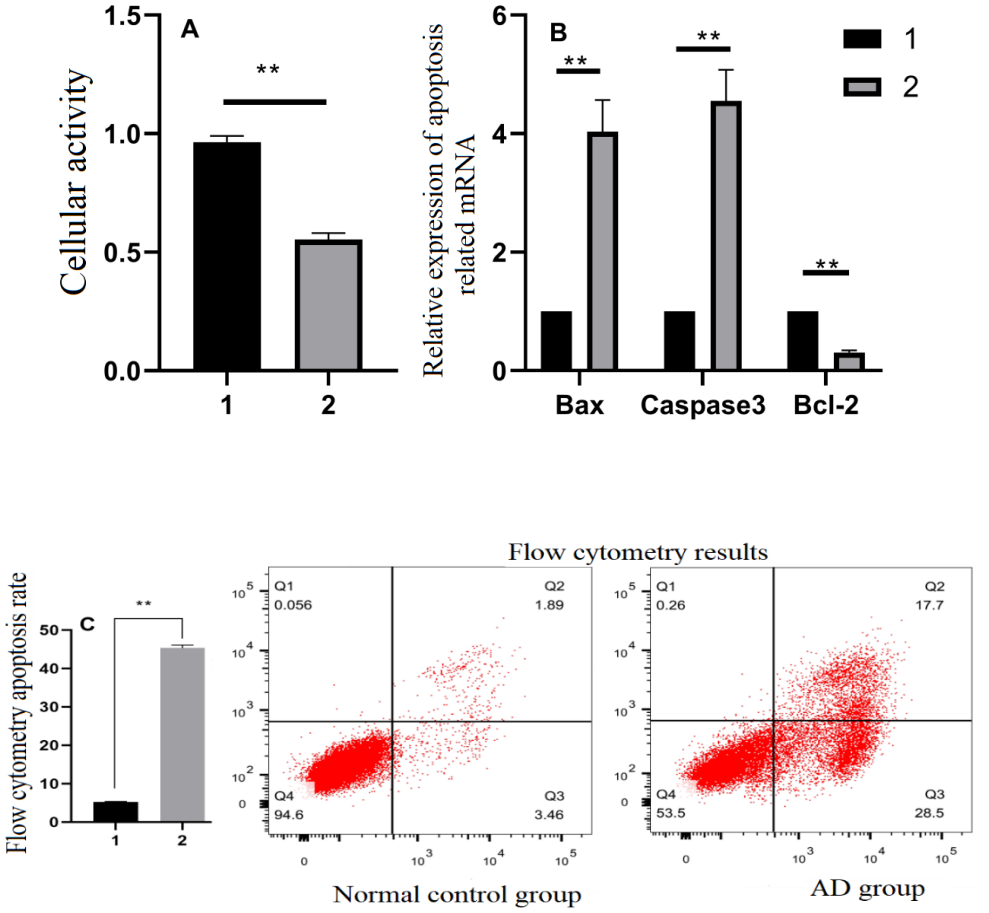


Figure 1. Activity of AD cell model and expression level of apoptosis related genes

A. CCK-8 cell activity test results, * * P ＜ 0.01, n=5; B. Comparison of Bax, Bcl-2, Caspase3 mRNA expression results (qRT-PCR); C. Results of flow cytometry: 1: normal control group, 2: AD group; Compared with the control group, * * P ＜ 0.01, n=3.

2.2 Effect of H19 on AD cell apoptosis

The results of qRT-PCR showed that H19 was overexpressed in AD cell group compared with normal control group (Fig. 2A). In order to study the effect of H19 expression level on apoptosis of PC12 cells induced by Aβ, PC12 cells were transfected with pcDNA3.1-H19 plasmid to make H19 overexpression. The experimental results showed that after transfection of H19 overexpression plasmid, the expression of H19 in AD cell group was significantly increased compared with the control group, and the expression of apoptosis promoting genes Caspase3 and Bax was also further increased, while the expression of anti apoptosis gene Bcl-2 was further reduced (Fig.

2B/C/D). In order to further verify the effect of H19 expression level change on PC12 cell apoptosis, H19 smart silent plasmid was used to transfect PC12 cells to interfere with H19 expression (Fig. A). The results showed that decreasing the expression of H19 could down regulate the expression of proapoptotic gene Bax and Caspase3, and promote the expression of anti apoptotic gene Bcl-2 (Fig. 2B/C/D).

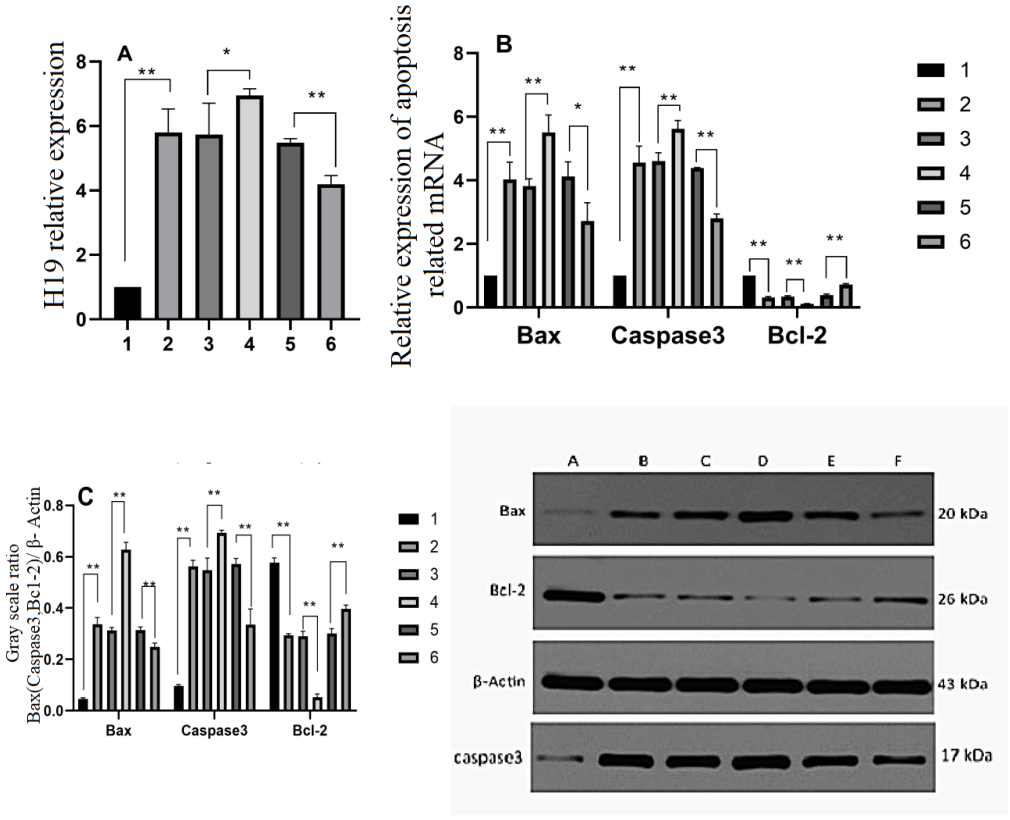


Figure 2. The changes of H19 expression level and its effect on the expression levels of Aβ-induced apoptosis related genes in PC12 cells

1: Normal control group, 2: AD model group, 3: AD+pcDNA3.1-COntrol group, 4: AD+pcDNA3.1-H19 group, 5: AD+si Control group, 6: AD+si-H19 group; A. Phase expression of H19 mRNA (qRT-PCR); B. Phase expression of Caspase, Bax, Bcl-2 mRNA (qRT-PCR); C: Caspase, Bax, Bcl-2 protein expression; D: Western Blot photos of Caspase, Bax and Bcl-2 proteins; ① Relative expression of H19: compared with the normal control group: P<0.01; Compared with pcDNA3.1-COntrol group: P ＜ 0.05; Compared with si Control group: P<0.01.

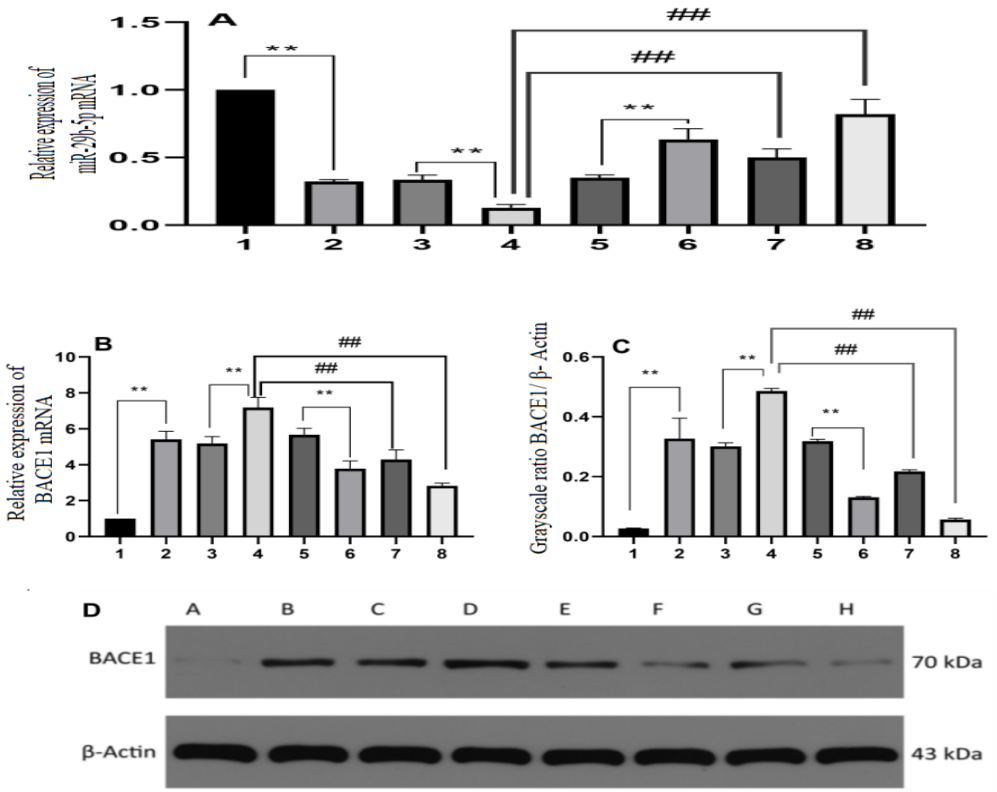
② the relative expression of apoptosis promoting gene Bax mRNA: compared with the normal control group: P<0.01; Compared with pcDNA3.1-COntrol group: P ＜ 0.05; Compared with si Control group: P<0.05;

③ the relative expression of apoptosis promoting gene Caspase3, apoptosis inhibiting gene Bcl-2 mRNA and the expression of Bax, Caspase3 and Bcl-2 protein: compared with the normal control group: P<0.01;

Compared with AD+pcDNA3.1-COntrol group: P<0.05; Compared with AD+si Control group: P<0.05;
*P<0.05， **P<0.01， n=3

2.3 The effect of H19 expression on miR-29b-5p/BACE1 signaling pathway

In order to further explore the downstream regulation mechanism of H19 on PC12 cell apoptosis, the expression levels of each gene in PC12 cells after H19 overexpression were detected. qRT-PCR results showed that the expression of miR-29b-5p mRNA was inhibited, while BACE1 mRNA was up-regulated, BACE1 protein expression level was increased (Figure 3C), and Aβ42 expression level was also increased (Figure 3D) in the AD model group. Compared with the AD+pcDNA3.1-Control group, the expression of miR-29b-5p mRNA was further inhibited, while BACE1 mRNA was further overexpressed. BACE1 protein expression level and Aβ42 expression level were also increased in the AD+ pcDNA3.1-H19 group. Compared with AD+pcDNA3.1-H19 group, the expression of miR-29b-5p mRNA was promoted in AD+si-H19+miR-29b-5pinhibitor group and AD+si-H19+miR-29b-5p mimic group. The expression levels of BACE1 mRNA and BACE1 protein were significantly decreased (both P < 0.01), and the corresponding expression level of Aβ42 was also decreased (both P < 0.01). The above experimental comparison results indicated that H19 could affect the apoptosis and the expression of Aβ42 in Aβ-treated PC12 cells by regulating miR-29b-5p/BACE1 signaling pathway.



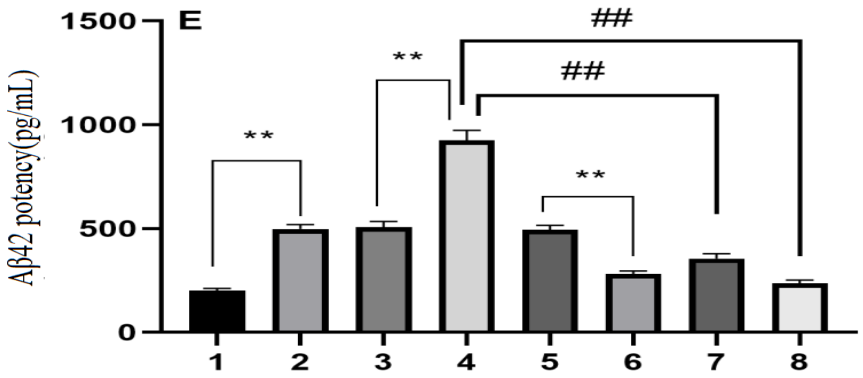


Figure 3. Effect of H19 expression level change on miR-29b-5p/BACE1 signal pathway

*P<0.05， **P<0.01， n=3

1: Normal control group, 2: AD model group, 3: pcDNA3.1-COntrol group, 4: pcDNA3.1-H19 group, 5: si Control group, 6: si-H19 group, 7: Si-H19+miR-29b-5pinhibitor group, 8: si-H19+miR-29b-5p mimic group:

A. Phase expression of miR-29b-5p mRNA (qRT-PCR); B. Phase expression of BACE1 mRNA (qRT-PCR); C: BACE1 protein expression; D: Western Blot photos of BACE1 protein; E: A β 42 comparison of expression results; Compared with the control group, P<0.01; Compared with AD+pcDNA3.1-COntrol group, P<0.01; Compared with AD+pcDNA3.1-H1 group, AD+si-H19+miR-29b-5pinhibitor group and AD+si-H19+miR-29b-5p mimic group, P<0.01

3. DISCUSSION

This study first investigated the effect of lncRNA H19 on the viability and apoptosis of Aβ-induced PC12 cells and whether lncRNA H19 regulates cell apoptosis by activating miR-29b-5p/BACE1 signaling pathway. The results showed that the down-regulation of lncRNA H19 had a protective effect on Aβ-treated PC12 cells, increasing cell viability and inhibiting cell apoptosis. On the contrary, overexpression of lncRNA H19 promoted apoptosis of Aβ-treated PC12 cells and weakened cell viability. It was further found that the expression of lncRNA H19 was significantly up-regulated in Aβ-induced PC12 cells, and overexpression of H19 further inhibited the expression of miR-29b-5p mRNA and increased the expression of BACE1 mRNA and protein. On the contrary, inhibiting the expression of lncRNA H19 can increase the expression of miR-29b-5p mRNA and reduce the expression of BACE1 mRNA and protein, thereby affecting the downstream expression of Aβ42.

As one of the most serious neurodegenerative diseases, the pathogenesis of AD has not been uniformly concluded. Recent studies have found that long non-coding RNA can participate in the development of AD

through a variety of pathways, such as epigenetic, transcriptional and post-transcriptional regulation. Although the research on the relationship between long non-coding RNA and AD is still in its infancy, it has been found that a variety of long non-coding RNA, such as BACE1-AS, BC200, 17A, etc. are specifically expressed in brain tissue and are involved in hippocampal development, synaptic transmission and various transcription-related signaling pathways (Y. Wang, Wang, Chen, & Chu, 2018; Zhang, 2016). Studies at different biological levels have found that long non-coding RNA related to AD may be involved in the formation of Aβ, excessive phosphorylation of tau protein or inflammation. In addition, our experiments confirmed from the cytological level that the high expression of lncRNA H19 further affected the expression of BACE1 and increased the expression of Aβ42 in the AD cell model, which confirmed the involvement of lncRNA H19 in the pathological process of AD. Studies have shown that in cerebral ischemia-reperfusion model, reducing the expression of lncRNA H19 will reduce the size of cerebral infarction, relieve brain edema, and reduce the expression of inflammatory factors in brain tissue (J. Wang et al., 2017). Some studies have also confirmed that the increased apoptosis of cardiomyocytes after hypoxia increases the expression of lncRNA H19 (Sun et al., 2018; J.-X. Wang et al., 2015). Based on the above literature, the expression of H19 has an impact on cell viability and apoptosis. Our study showed that in the AD cell model constructed by Aβ treatment of PC12 cells, the down-regulation of lncRNA H19 would reduce cell apoptosis. Investigation has found that the miRNA expression profile changes in sporadic AD patients. The miR-29a/b-1/c cluster in the brain of AD patients was significantly reduced, and this reduction was related to the increase of BACE1 protein level (Petri & Klinge, 2020; Zong et al., 2011). The expression of miR-29a/b-1 cluster and BACE1 were also found in primary neuronal cultures, which were similar to those in AD patients. Cell studies have confirmed that overexpression of miR-29a and miR-29b1 can cause the decrease of endogenous BACE1 protein expression, while down-regulation of miR-29a can cause the increase of BACE1 expression (Jahangard et al., 2020). It suggests that there is also a potential causal relationship between the expression of miR-29 and amyloid protein at animal level or cell level. This study also reflected that overexpression of miR-29b-5p inhibited the level of BACE1 mRNA and protein and reduced the activation of downstream molecule Aβ42 of BACE1. It shows that miR-29b-5p participates in the expression of BACE1, and miR29 RNA and BACE1 expression are negatively regulated, which means that miR-29 family can be used as potential inhibitors of silencing BACE1 protein expression. The current view is that, in addition to directly affecting the occurrence of diseases, lncRNA can competitively combine with miRNA, indirectly regulate the expression level of target miRNA, and participate in the regulation of its function through signal pathways. In this experiment, we found that the high expression of lncRNA H19 can significantly inhibit the expression of miR-29b-5p. At the same time,

we found that under the condition of further transfection with si-H19+miR-29b-5p mimic or si-H19+miR-29b-5pinnhibitor, BACE1 and downstream Aβ42 are affected, which strongly proves that lncRNA H19 has targeted regulation on miR-29b-5p. It also shows that both miR-29b-5p positive control and si-H19 treatment can effectively inhibit expression level of BACE1 mRNA and protein in Aβ induced PC12 cells, thus reducing the expression of BACE1 downstream molecule Aβ42, which suggests that lncRNA H19 may affect Aβ42 expression by acting on miR-29b-5p/BACE1 signal pathway.

Our study found the expression changes of lncRNA H19 in AD model cells, which confirmed from the cytological level that lncRNA H19 could not only directly affect cell apoptosis, but also target the expression level of miR-29b-5p, thus affecting the downstream expression of BACE1. This study reveals the potential important role of the H19/miR-29b-5p/BACE1 axis in the prevention and treatment of AD, but the detailed biological function and regulatory mechanism need to be further studied.

REFERENCE

Bhat, S. A., Ahmad, S. M., Mumtaz, P. T., Malik, A. A., Dar, M. A., Urwat, U., . . . Ganai, N. A. (2016). Long non-coding RNAs: Mechanism of action and functional utility. *Non-coding RNA research, 1*(1), 43-50.

Broderick, J. A., & Zamore, P. D. (2011). MicroRNA therapeutics. *Gene therapy, 18*(12), 1104-1110.

Chielle, E. O., Feltez, A., & Rossi, E. M. (2017). Influence of obesity on the serum concentration of retinol-binding protein 4 (RBP4) in young adults. *Jornal Brasileiro de Patologia e Medicina Laboratorial, 53*, 81-86.

Choi, G., Park, M., & Park, N. (2017). The Use of Virtual Currencies in Small-value Cross-border Remittances and its Implication. *Remittances Review, 2*(2), 89-119.

Gabory, A., Jammes, H., & Dandolo, L. (2010). The H19 locus: role of an imprinted non-coding RNA in growth and development. *Bioessays, 32*(6), 473-480.

Hébert, S. S., Horré, K., Nicolaï, L., Bergmans, B., Papadopoulou, A. S., Delacourte, A., & De Strooper, B. (2009). MicroRNA regulation of Alzheimer's Amyloid precursor protein expression. *Neurobiology of disease, 33*(3), 422-428.

Hébert, S. S., Horré, K., Nicolaï, L., Papadopoulou, A. S., Mandemakers, W., Silahtaroglu, A. N., . . . De Strooper, B. (2008). Loss of microRNA cluster miR-29a/b-1 in sporadic Alzheimer's disease correlates with increased BACE1/β-secretase expression. *Proceedings of the National Academy of Sciences, 105*(17), 6415-6420.

Hung, C. O., & Livesey, F. J. (2018). Altered γ-secretase processing of APP disrupts lysosome and autophagosome function in monogenic Alzheimer's disease. *Cell reports, 25*(13), 3647-3660. e3642.

Jahangard, Y., Monfared, H., Moradi, A., Zare, M., Mirnajafi-Zadeh, J., & Mowla, S. J. (2020). Therapeutic effects of transplanted exosomes containing miR-29b to a rat model of Alzheimer's disease. *Frontiers in Neuroscience, 14*, 564.

Petri, B. J., & Klinge, C. M. (2020). Regulation of breast cancer metastasis signaling by miRNAs. *Cancer and metastasis reviews, 39*, 837-886.

Riva, P., Ratti, A., & Venturin, M. (2016). The long non-coding RNAs in neurodegenerative diseases: novel mechanisms of pathogenesis. *Current Alzheimer Research, 13*(11), 1219-1231.

Scheltens, P., Blennow, K., Breteler, M. M., De Strooper, B., Frisoni, G. B., Salloway, S., & Van der Flier, W. M. (2016). Alzheimer's disease. *The Lancet, 388*(10043), 505-517.

Schratt, G. M., Tuebing, F., Nigh, E. A., Kane, C. G., Sabatini, M. E., Kiebler, M., & Greenberg, M. E. (2006). A brain-specific microRNA regulates dendritic spine development. *nature, 439*(7074), 283-289.

Sun, Z., Shi, K., Yang, S., Liu, J., Zhou, Q., Wang, G., . . . Yuan, W. (2018). Effect of exosomal miRNA on cancer biology and clinical applications. *Molecular cancer, 17*, 1-19.

Voellenkle, C., Garcia-Manteiga, J., Pedrotti, S., Perfetti, A., De Toma, I., Da Silva, D., . . . Creo, P. (2016). Implication of Long noncoding RNAs in the endothelial cell response to hypoxia revealed by RNA-sequencing. *Scientific reports, 6*(1), 24141.

Wang, J.-X., Zhang, X.-J., Li, Q., Wang, K., Wang, Y., Jiao, J.-Q., . . . Gong, Y. (2015). MicroRNA-103/107 regulate programmed necrosis and myocardial ischemia/reperfusion injury through targeting FADD. *Circulation research, 117*(4), 352-363.

Wang, J., Zhao, H., Fan, Z., Li, G., Ma, Q., Tao, Z., . . . Luo, Y. (2017). Long noncoding RNA H19 promotes neuroinflammation in ischemic stroke by driving histone deacetylase 1–dependent M1 microglial polarization. *Stroke, 48*(8), 2211-2221.

Wang, Y., Wang, L., Chen, C., & Chu, X. (2018). New insights into the regulatory role of microRNA in tumor angiogenesis and clinical implications. *Molecular cancer, 17*, 1-10.

Wei, C.-W., Luo, T., Zou, S.-S., & Wu, A.-S. (2018). The role of long noncoding RNAs in central nervous system and neurodegenerative diseases. *Frontiers in behavioral neuroscience, 12*, 175.

Yang, S., Tang, W., He, Y., Wen, L., Sun, B., & Li, S. (2018). Long non-coding RNA and microRNA-675/let-7a mediates the protective effect of melatonin against early brain injury after subarachnoid hemorrhage via targeting TP53 and neural growth factor. *Cell Death & Disease, 9*(2), 99.

Zhang, Z. (2016). Long non-coding RNAs in Alzheimer's disease. *Current topics in medicinal chemistry, 16*(5), 511-519.

Zong, Y., Wang, H., Dong, W., Quan, X., Zhu, H., Xu, Y., . . . Qin, C. (2011). miR-29c regulates BACE1 protein expression. *Brain research, 1395*, 108-115.