Original Article
The correlation between LncRNA-17A expression in peripheral blood mononuclear cells and Wnt/β-catenin signaling pathway and cognitive function in patients with Alzheimer disease

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Abstract: Objective: This study investigated the correlation between LncRNA-17A expression in peripheral blood mononuclear cells (PBMC) and the Wnt/β-catenin signaling pathway and cognitive function in patients sufferer from Alzheimer disease (AD). Methods: 90 cases of AD patients hospitalized during March 2019 to July 2020 were selected into the AD-group, and another 90 healthy volunteers who underwent physical examination during the same period were randomly enrolled as the control-group. The Mini-mental State Examination (MMSE) was applied to measure the cognitive function of the two groups of subjects, and the qRT-PCR was to detect the expressions of LncRNA-17A, Wnt mRNA, Tcf-4 mRNA and β-catenin mRNA in PBMC, and the correlation between LncRNA-17A expression and cognitive function and Wnt/β-catenin signaling pathway was analyzed. Results: MMSE score in AD-group was remarkably lower than that in control-group ($P<0.05$). The relative LncRNA-17A expression in PBMC of AD patients was evidently higher than that of the control-group ($P>0.05$). LncRNA-17A expression in PBMC of AD patients with different severity degree had statistical significance ($P<0.05$); and the relative expression level of LncRNA-17A increased notably with the disease progression ($P<0.05$). The relative expression of Wnt mRNA, Tcf-4 mRNA and β-catenin mRNA in AD-group were apparently superior to those in control-group ($P<0.05$). LncRNA-17A expression in PBMC of AD patients was negatively correlated with MSME score ($P<0.05$), and was positively correlated with Wnt mRNA, Tcf-4 mRNA and β-catenin mRNA ($P<0.05$). Conclusion: LncRNA-17A expression is abnormally reduced in PBMC of AD patients, and is associated with patient's disease progression which is regulated by the activation of Wnt/β-catenin signaling pathway. LncRNA-17A might be the potential molecular markers of AD with diagnostic and prognostic value.

Keywords: Peripheral blood mononuclear cells, LncRNA-17A, Alzheimer disease, Wnt/β-catenin signaling pathway, cognitive function

Introduction

Alzheimer disease (AD) is a degenerative disease of nervous system. The major clinical features of AD patients are memory lapses, cognitive decline, changes in personality and behavior, etc. It seriously affects patients' work and daily life, and bring heavy economic burden to their family and society [1]. The etiology of AD is complex and varied. According to American epidemiological researches, the prevalence of AD in people aged 60-85 is 5-10%, and that in people aged over 85 is 20-50% [2]. The incidence of AD in China has been increas-

ing year by year with the intensification of the population aging process, and the diagnosis and treatment of AD has been a worldwide problem [3, 4]. At present, it is still difficult to diagnose AD in early stage in clinic, and is lack of effective treatment [5]. Therefore, it is of great significance for the in-depth research on the pathogenesis of AD occurrence and development to the early diagnosis and treatment of AD. Long non-coding RNA (LncRAN) can regulate gene expression at both transcriptional and post-transcriptional levels in various diseases. Moreover, LncRNAs can be used as biomarkers and potential therapeutic targets [6].

Table 1. Primer sequences

gene	Primer sequence
LncRNA-17A	Forward primer: 5'-CCACCCTGCAACTGACACAT-3'
	Reverse primer: 5'-GCAAAGGTGCTAATCTTGACTCTTG-3'
Wnt	Forward primer: 5'-TGGATACGTTTCCTTATAAG-3'
	Reverse primer: 5'-GAAATGGAGGCACCCCTTC-3'
Tcf-4	Forward primer: 5'-AGTCAACGGATTTGGTC-3'
	Reverse primer: 5'-AACAGTTGCCGTCCATGAATAG-3'
β-catenin	Forward primer: 5'-CCTCCACATCCTCCCTTCC-3'
	Reverse primer: 5'-GTCGCAGACAGTGATGAACCTC-3'
β-action	Forward primer: 5'-AGTCAACGGATTTGGTCG-3'
	Reverse primer: 5'-CTCGCTCCTGGAAGATGG-3'

LncRNAs are known to play a pivotal role in maintaining cell physiology and a range of human diseases, including neurodegenerative diseases such as AD, cardiovascular pathology and cancer [7]. This study explored and analyzed the correlation between LncRNA-17A expression in PBMC and Wnt/β-catenin signaling pathway and cognitive function in AD patients.

Material and methods

Clinical data

90 cases of AD sufferers hospitalized during March 2019 to July 2020 were selected into the AD group, and another 90 healthy volunteers who underwent physical examination during the same period were randomly recruited as the control-group. The study was launched under the approval of the Ethics Committee of our hospital.

Inclusive and exclusive criteria

Inclusive criteria: (1) Patients who aged ≥60 years old; (2) Those in compliance with AD diagnostic criteria in NINCDS-ADRDA; and (3) The two groups of subjects voluntarily signed informed consent.

Exclusive criteria: (1) Patients with myocardial infarction, stroke, II Diabetes Mellitus, heart failure or autoimmune diseases; (2) Patients with pseudo dementia or associated with vascular dementia or mixed dementia; (3) Patients with moderate or heavy alcohol addiction; (4) Patients with history of severe head trauma; (5) Patients with severe depression or schizophrenia; or (6) Patients who cannot cooperate with the examination of cognitive function.

Assessment of cognitive function

We scored the cognitive function of the two groups by Mini-mental State Examination (MMSE) [8]. The MMSE scale contained 20 clauses including immediate memory, general knowledge, calculation, location orientation, etc. The lower score obtained by the patient referred to the worse of his cognitive function. Dementia criteria: Illiteracy ≤17 points, primary school degree ≤20 points, middle school degree (including technical secondary school) ≤22 points and university degree (including college) ≤23 points. The severity of AD was graded as follows: MMSE≥21 points were classified as mild cognitive dysfunction, MMSE between 10-20 points was moderate cognitive dysfunction, and MMSE≤9 points were severe cognitive dysfunction.

LncRNA-17A expression in PBMC detected by qRT-PCR

We collected the early morning peripheral venous blood from two groups of subjects, and separated the PBMC by Ficoll-hypaque density gradient centrifugation. Extracted the total RNA in PBMC by Trizol reagent, reverse-transcribed into cDNA according to the instructions of kit, and kept for subsequent use. We used β-action as the reference gene. The primer sequences were synthesized by Shanghai Sangon Bioengineering Co., Ltd. as shown in **Table 1**. The reaction system was 10 μL with conditions as follows: pre-denaturation 98°C 30 s, 95°C 5 s, 60°C 5 s, with a total of 40 rounds. The purity of the amplified product was detected by dissolution curve to obtain the CT value. We calculated the relative expression of the target gene by $2^{-\Delta\Delta Ct}$ method and with β-action as the internal reference gene.

Statistical analysis

We applied SPSS 22.0 for data analysis (IBM Corp). The two groups of measurement data were compared by *t*-test, and the three groups of measurement data were compared by analysis of variance; The enumeration data was com-

Table 2. Comparison of clinical data between two groups of subjects

| Group | Number of cases | Gender | | Age (yd, $\overline{x}$±s) | BMI (kg/m^2, $\overline{x}$±s) | Years of Education (y, $\overline{x}$±s) |
		F	M			
AD group	90	52	38	72.38±7.21	22.18±3.10	9.28±2.79
Control group	90	50	40	71.95±8.33	22.09±2.87	9.42±2.86
t/x^2	-	0.091		0.370	0.202	0.332
P	-	0.764		0.712	0.740	0.740

Table 3. Comparison of MMSE scores between two groups of subjects (points, $\overline{x}$±s)

Group	Number of cases	Score of MMSE	t	P
AD group	90	13.10±2.38	26.004	<0.001
Control group	90	28.95±5.27		

Table 4. Comparison of LncRNA-17A expression between two groups of subjects ($\overline{x}$±s)

Group	Number of cases	The relative expression of LncRNA-17A	t	P
AD group	90	1.547±0.318	14.964	<0.001
Control group	90	0.976±0.173		

Table 5. Correlation between the severity of AD and LncRNA-17A expression ($\overline{x}$±s)

Group	Number of cases	The relative expression of LncRNA-17A	F	P
Mild	33	1.122±0.208	27.805	<0.001
Moderate	39	1.498±0.335*		
Severe	18	1.907±0.462*,#		

Note: Compared with mild patients, *$P<0.05$; compared with moderate patients, #$P<0.05$.

pared by x^2, and the correlation was analyzed by Perason. *P<0.05* represented a statistically significant difference. The graphic software was by Graphpad prism9.

Results

Clinical data

There was no statistical significance in gender, age, BMI or years of education between the two groups (*P>0.05*), as shown in **Table 2**.

Comparison of cognitive function

MMSE score in AD group was critically lower than that in control-group [(13.10±2.38) vs. (28.95±5.27)] (*P<0.05*), as shown in **Table 3**.

Comparison of LncRNA-17A expression between two groups of subjects

The relative expression level of LncRNA-17a in PBMC of AD patients was remarkably higher than that of control-group [(1.547±0.318) vs. (0.976±0.173)] (*P>0.05*), as shown in **Table 4**.

Correlation between the severity of AD and LncRNA-17A expression

LncRNA-17A expression in PBMC of AD patients with different severity degree had statistical significance (*P<0.05*); and the relative expression level of LncRNA-17A increased notably with the disease progression [(1.122±0.208) vs. (1.498±0.335) vs. (1.907±0.462)] (*P<0.05*), as shown in **Table 5**.

Comparison of Wnt/β-catenin pathway expression between two groups of subjects

The relative expression degree of Wnt mRNA, Tcf-4 mRNA and β-catenin mRNA in AD-group were apparently higher than those in control-group [(1.851±0.305) vs. (1.194±0.213); (1.922±0.412) vs. (0.908±0.225); (1.721±0.377) vs. (0.932±0.265)] (*P<0.05*) (**Table 6**).

Correlation between LncRNA-17A expression and Wnt/β-catenin pathway expression in AD-sufferers

The LncRNA-17A expression in PBMC of AD patients was negatively correlated with MSME score (r=-0.529, *P<0.05*), and was positively correlated with Wnt mRNA, Tcf-4 mRNA and β-catenin mRNA (r=0.448, 0.429, 0.512; *P<0.05*) (**Figure 1**).

Discussion

AD is the primary cause of Alzheimer disease over the world. Although great efforts have been put to develop drugs for the prevention and treatment of AD, there is lack of available treatment for the disease, which places enor-

Table 6. Comparison of Wnt/β-catenin pathway expression between the two groups ($\overline{x}$±s)

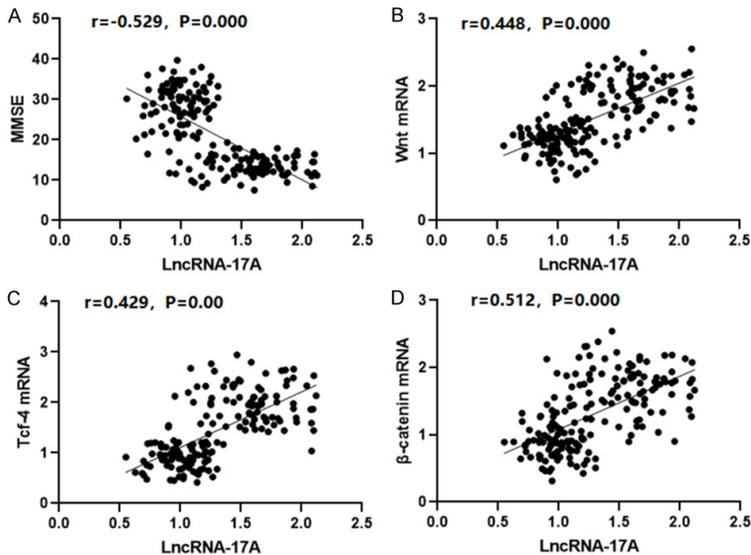| Group	Number of cases	Wnt mRNA	Tcf-4 mRNA	β-catenin mRNA
AD group	90	1.851±0.305	1.922±0.412	1.721±0.377
Control group	90	1.194±0.213	0.908±0.225	0.932±0.265
t	-	16.754	20.492	16.243
P	-	<0.001	<0.001	<0.001

Figure 1. Correlation analysis of LncRNA-17A expression and Wnt/β-catenin pathway expression in AD patients. A: LncRNA-17A and MMSE; B: LncRNA-17A and Wnt mRNA; C: LncRNA-17A and Tcf-4 mRNA; D: LncRNA-17A and β-catenin mRNA.

recent years, however, scholars have found that LncRNA-17A is highly expressed in hippocampus of AD patients and is closely related to clinical symptoms. This suggests that LncRNA-17A is related to the occurrence and progression of AD [17-19]. In this study, we investigated the correlation between LncRNA-17A expression in PBMC and Wnt/β-catenin signaling pathway and cognitive function in patients with AD. The results revealed that the relative LncRNA-17A expression in PBMC of AD patients was obviously higher than that of the control-group, and the relative expression level of LncRNA-17a substantially increased with the aggravation of disease. The result, which is consistent with reports by scholars [20, 21], suggests that LncRNA-17A might play a corresponding regulatory function in the courses of AD. Scholars have shown that LncRNA-17A can construct AD model by regulating autophagy and apoptosis of SH-SY5Y cell line. They discussed its value at the cellular level and believed that LncRNA-17A is a significant molecular mechanism to regulate the progression of AD disease [22, 23].

mous burden on individuals, families and society. Currently, the pathogenesis of AD has not been fully clarified [9, 10]. LncRNAs can regulate gene expression at both transcriptional and post-transcriptional levels in sorts of diseases, and can be used as biomarkers and potential therapeutic targets [11]. LncRNAs are known to play crucial roles in maintaining cell physiology and in a series of human diseases, including neurodegenerative diseases such as AD and cancers [12-14]. In recent years, an increasing number of researches have demonstrated the correlation between LncRNA and the pathogenesis of AD [15, 16].

LncRNA-17A can be specifically expressed in human nerve cells. It primarily participates in the formation of neuronal cell protruding bodies through specific regulatory mechanisms, and is lowly expressed in normal tissues. In

Wnt/β-catenin signaling pathway includes signaling proteins Wnt, transmembrane receptors, and cytoplasmic proteins. The activation of Wnt signaling pathway can promote hematopoiesis, maintain the self-renewal of hepatocytes, and promote development of T/B cells [24]. Wnt signaling pathway T/B plays a considerable role in the process of cell multiplication. According to researches, the overexpression of β-catenin is involved in immune and inflammatory damage. The increase of Wnt/β-catenin may promote the activation of immune response in human bodyand the inflammation and aggravate the immune injury, which may also be another mechanism of AD [25-28]. In our study, the relative expressions of Wnt mRNA, Tcf-4 mRNA

and β-catenin mRNA in AD group were remarkably higher than those in control-group, reflecting that there is activation of Wnt/β-catenin signaling pathway in pathogenesis of AD. In addition, the correlation analysis results demonstrated that LncRNA-17A expression in PBMC of AD patients was negatively correlated with MSME score, and was positively correlated with Wnt mRNA, Tcf-4 mRNA and β-catenin mRNA. These results suggest that LncRNA-17A expression is tightly relevant to the cognitive function in AD sufferers, thus regulating the progression of disease. Meanwhile, LncRNA-17A is related to the activation of Wnt/β-catenin signaling pathway in AD patients, which may promote the occurrence and progression of disease by regulating its signaling pathway.

In conclusion, LncRNA-17A expression in PBMC of AD patients is abnormally reduced, and is associated with patient's disease progression which is regulated by the activation of Wnt/β-catenin signaling pathway. LncRNA-17A might be the potential molecular markers of AD and are worthy of further research and analysis.

Disclosure of conflict of interest

None.

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