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Saxagliptin reduces the injury of Alzheimer's disease cell model by down-regulating the expression of miR-483-5p

Yiting Hou[1*], Lihua Dong[2], Lihua Cao[2]

[1] Department of Neurology, the First Hospital of Jilin University, Changchun 130021, China
[2] The Affiliated Hospital of Changchun University of Chinese Medicine, Changchun 130021, China

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ABSTRACT

This study aimed to investigate the effect of saxagliptin on the injury of Alzheimer's disease (AD) cell model and its possible mechanism. SK-N-SH cells were cultured in vitro and divided into CON group, AD group, AD+L-SAX group, AD+M-SAX group, AD+H-SAX group, AD+anti-miR-NC group, AD+anti-miR-483-5p group, AD+SAX+miR-NC group and AD+SAX+miR-483-5p group. Then the levels of MDA, SOD and GSH-Px in each group were detected by ELISA method; cell apoptosis was detected by flow cytometry; the protein expression levels of Bax and Bcl-2 were detected by Western Blot; the expression level of miR-483-5p was detected by RT-qPCR. Compared with the control group, MDA content, apoptosis rate, and the expression of Bax protein and miR-483-5p increased in the AD group ($P<0.05$), while the activity of SOD and GSH-Px and the expression of Bcl-2 protein decreased ($P<0.05$). Compared with the AD group, MDA content, apoptosis rate, and the expression of Bax protein and miR-483-5p decreased in the AD+L-SAX group, AD+M-SAX group and AD+H-SAX group ($P<0.05$), while the activity of SOD and GSH-Px and the expression of Bcl-2 protein increased ($P<0.05$). Compared with AD+anti-miR-NC group, MDA content, apoptosis rate, and the expression of Bax protein and miR-483-5p decreased in the AD+anti-miR-483-5p group ($P<0.05$), while the activity of SOD and GSH-Px and the expression of Bcl-2 protein increased ($P<0.05$). Compared with AD+SAX+miR-NC group, MDA content, apoptosis rate, and the expression of Bax protein and miR-483-5p increased in the AD+SAX+miR-483-5p group ($P<0.05$), while the activity of SOD and GSH-Px and the expression of Bcl-2 protein decreased ($P<0.05$). Saxagliptin may reduce the injury of Alzheimer's disease cell model by down-regulating the expression of miR-483-5p.

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Introduction

Alzheimer's disease (AD) is an age-related degenerative disease of the central nervous system, with clinical symptoms mainly manifested as progressive cognitive dysfunction, memory loss, behavioural abnormalities, social impairment, etc. (1). The pathogenesis of AD is complex, and the formation of β-amyloid (Aβ) deposition in the age spots is a typical pathological feature of AD (2). Studies have shown that Aβ can directly or indirectly damage the structure and function of cellular mitochondria, which in turn induces oxidative stress and apoptotic cascade responses, resulting in neuronal cell damage (3). Therefore, reducing or inhibiting Aβ-induced oxidative stress and apoptosis in neuronal cells has a positive effect on the treatment of AD.

Saxagliptin is a potent and selective dipeptidyl peptidase 4 (DPP-4) inhibitor, which inhibits DPP-4 activity and reduces the hydrolysis of glucagon-like peptide 1 by DPP-4, exerting a hypoglycaemic effect, and is commonly used in the treatment of diabetes (4). Studies have shown that saxagliptin can inhibit high glucose-induced endothelial cell injury in the human umbilical vein by down-regulating the expression of lncRNA MALAT1 (5), and saxagliptin can attenuate oxidised low-density lipoprotein (ox-LDL)-induced vascular endothelial cell injury by regulating the miR-590/TLR4/NF-κB pathway (6). However, the effects and mechanisms of saxagliptin on injury in AD cell models are currently unknown. MicroRNAs (miRNAs) are a class of small-molecule non-coding RNAs involved in the regulation of physiological or pathological processes such as apoptosis, oxidative stress, and inflammatory responses, and play an important role in the development of many diseases, including neurodegenerative diseases (7-9). Studies have shown that miR-483-5p expression is up-regulated in the lung tissues of mice with sepsis-induced acute lung injury model, and knockdown of its expression inhibits lipopolysaccharide (LPS)-induced cellular inflammatory factor expression and apoptosis in PMVECs and attenuates lung injury (10). However, the role of miR-483-5p in the development of AD is also currently unknown. Therefore, in this study, we took the neuronal cells SK-N-SH as the research object, and established an AD cell injury model by inducing SK-N-SH cells through $A\beta_{25-35}$, aiming to observe the effect of saxagliptin on AD cell injury and whether it can regulate miR-483-5p expression to play a role in the treatment of AD, with the aim of providing a new way for the treatment of AD.

* Corresponding author. Email: 18844094827@163.com

Materials and Methods

Cells and reagents

SK-N-SH cell line, Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China); foetal bovine serum (FBS), Hycolne (South Logan, UT, USA); RPMI 1640 medium, Beijing Solepol Science and Technology Co. Ltd. (Beijing, China); Lipofectamine™ 2000 kit and TRIzol reagent, Invitrogen (Carlsbad, CA, USA); miR-483-5p inhibitor (anti-miR-483-5p), inhibitor negative sequence (anti-miR-NC), miR-483-5p mimics (mimcs), mimic control sequence (miR-NC), and PCR primers, Shanghai Gemma Pharmaceuticals Technology Co Ltd (Shanghai, China); Malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione reductase (GSH-Px) kit, Nanjing Jianjian Bioengineering Institute (Nanjing, China); Annexin V-FITC/PI apoptosis kit and bicinchoninic acid (BCA) protein assay kit, Shanghai Biyuntian Biotechnology Co Ltd. (Shanghai, China); Rabbit anti-human B-lymphoblastoma-2 (Bcl-2), B-lymphoblastoma-2-associated protein (Bax) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) polyclonal Reverse transcription kit and PCR kit, Shenzhen Jingmei Bioengineering Co. (Shenzhen, China).

Cell culture and transfection

Resuscitated SK-N-SH cells were cultured with Roswell Park Memorial Institute 1640 (RPMI 1640) medium containing 10% FBS. SK-N-SH cells in the logarithmic growth phase were inoculated in 6-well plates at 1×105 cells/well, and transfected with anti-miR-NC, anti-miR-483-5p, miR-NC, miR-483-5p mimics using Lipofectamine™ 2000 liposome assay. The transfection time was 6 h, then the medium was changed. Incubate for another 24 h, and collect the cells for spare.

Cell grouping

Cells were inoculated in 24-well plates at 2.5×104 cells/well. Cells without transfection were divided into the control group (CON group), AD group, AD+L-SAX group, AD+M-SAX group and AD+H-SAX group, in which cells in the CON group were cultured routinely, cells in the AD group were cultured in medium containing 5 μmol/L $A\beta_{25-35}$ (11), and cells in AD+L-SAX, AD+M-SAX and AD+H-SAX groups were cultured in medium containing 0.1, 1, 1 and 1 μmol/L $A\beta_{25-35}$ (11), respectively. were co-cultured with medium containing 0.1, 1.0, 10 μmol/L (5) saxagliptin and Cells transfected with anti-miR-NC and anti-miR-483-5p were co-cultured with medium containing 5 μmol/L L$A\beta_{25-35}$5 and were recorded as AD+anti-miR-NC group and AD+anti-miR-483-5p group, respectively. Cells transfected with miR-NC, miR-483-5p mimics were co-cultured with a medium containing 10 μmol/L saxagliptin and 5 μmol/L $A\beta_{25-35}$5 and were recorded as AD+saxagliptin+miR-NC group, AD+saxagliptin+miR-483-5p group, respectively. The cells in each group were cultured for 24 h. The cells were collected and the relevant indexes were detected.

Enzyme-linked immunosorbent assay for MDA, SOD and GSH-Px in cells

Lysed the cells of each group, centrifuged at 3500 r/min for 5 min and retained the supernatant. Detect the expression of MDA, SOD and GSH-Px in the supernatant by referring to the instructions of MDA, SOD and GSH-Px kits, respectively.

Detection of apoptosis by flow cytometry

The groups were washed twice with PBS, and apoptosis was detected by flow cytometry with reference to Annexin V-FITC/PI kit instructions.

Detection of Bcl-2 and Bax protein expression by Western blot method

Radioimmunoprecipitation assay (RIPA) reagent was used to extract the total protein in the cells, the BCA method was used to determine the protein concentration, and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis was performed with 20 μg/well of protein. After electrophoresis, the membrane was transferred to a polyvinylidene fluoride (PVDF) membrane (Roche, Basel, Switzerland) and closed in 5% skimmed milk powder solution for 1 h. Bcl-2 (1:500) and Bax (1:500) primary antibodies were added respectively, and the membrane was incubated at 4°C overnight. Add goat anti-rabbit secondary antibody (1:2000), and incubate at 37°C for 1 h. Add developing solution, develop the image away from light, and expose the gel imaging system to take pictures.

RT-qPCR detection of miR-483-5p expression

Total RNA was extracted from the cells with TRIzol reagent, reverse transcribed to cDNA and amplified by PCR. Amplification conditions: 95°C for 3 min, 95°C for 10 s, 58°C for 30 s, 72°C for 30 s, a total of 35 cycles. Primer sequences: miR-483-5p upstream 5'-GATGCGTCACA GCTGATCGCCC-3', downstream 5'-GGCACAGCTCCTCAGATCGAT-3'; internal reference U6 upstream 5'-CGCTTCTA CACGTACGCCACGC-3', downstream 5'- TGGGAACCTCTCAACCCTGG-3'. $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression level of miR-483-5p.

Statistical analysis

Statistic Package for Social Science (SPSS) 22.0 software (IBM, Armonk, NY, USA) was used to analyse the experimental data. Measurement data conforming to normal distribution were expressed as mean ± standard deviation ($\bar{x}\pm s$). Comparisons between two groups were made by independent samples t-test; comparisons between multiple groups were made by one-way ANOVA, and further two-by-two comparisons were made by the *SNK-q* test. Differences were expressed as statistically significant at $P<0.05$.

Results

Effect of saxagliptin on oxidative stress in AD cell model

As shown in Table 1, compared with the CON group, MDA content in cells of the AD group was elevated ($P<0.05$) and SOD and GSH-Px activities were decreased ($P<0.05$). Compared with the AD group, the MDA content in the cells of the AD+L-SAX, AD+M-SAX and AD+H-SAX groups was decreased ($P<0.05$), and the activities of SOD and GSH-Px were elevated ($P<0.05$), and the two-by-two comparison of each assay between the three groups showed significant differences ($P<0.05$).

Effects of saxagliptin on apoptosis in AD cell model

As shown in Figure 1 and Table 2, compared with the CON group, the apoptosis rate of AD group was increased (P<0.05), the expression of Bax protein was increased (P<0.05), and the expression of Bcl-2 protein was decreased (P<0.05). Compared with the AD group, the apoptosis rate was decreased in the AD+L-SAX group, AD+M-SAX group and AD+H-SAX group (P<0.05), the Bax protein expression was decreased (P<0.05), and the Bcl-2 protein expression was elevated (P<0.05), and the difference of each test index between the three groups was significant in two-way comparison (P<0.05).

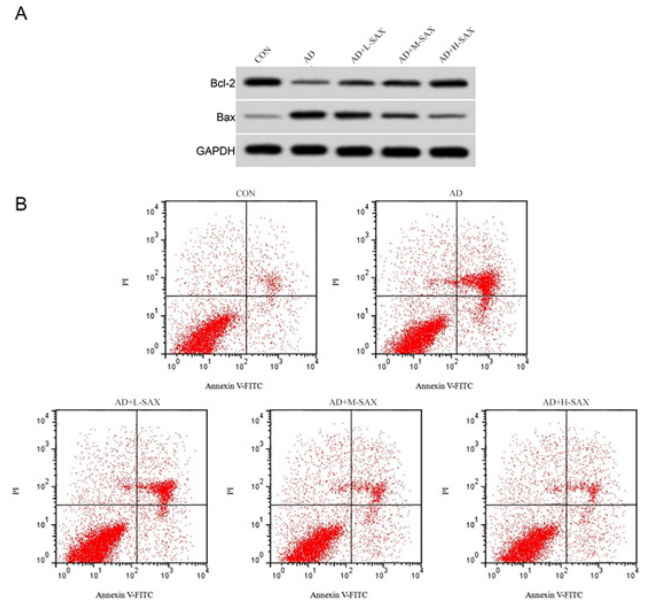

Figure 1. Effect of saxagliptin on apoptosis in AD cell model. Note: A: apoptosis-related protein expression; B: apoptosis flow charts.

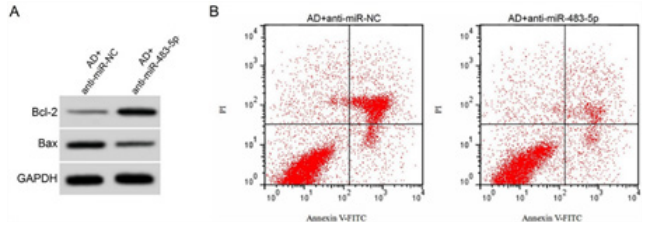

Figure 2. Effect of knockdown of miR-483-5p on apoptosis in AD cell model. Note: A: apoptosis-related protein expression; B: apoptosis flow charts.

Effect of saxagliptin on miR-483-5p expression in AD cell model

As shown in Table 3, miR-483-5p expression was elevated in cells of AD group compared with CON group (P<0.05). Compared with the AD group, miR-483-5p expression was decreased in the cells of the AD+L-SAX group, AD+M-SAX group and AD+H-SAX group (P<0.05), and the difference of miR-483-5p in two-by-two comparisons among the three groups was significant (P<0.05).

Effect of knockdown of miR-483-5p on oxidative stress in AD cell models

As shown in Table 4, compared with the AD+anti-miR-NC group, the MDA content in cells of the AD+anti-miR-483-5p group was decreased (P<0.05), and SOD and GSH-Px activities were elevated (P<0.05).

Effect of knockdown of miR-483-5p on apoptosis in AD cell model

As shown in Figure 2 and Table 5, compared with the AD+anti-miR-NC group, the apoptosis rate of the AD+anti-miR-483-5p group was reduced (P<0.05), the

Table 1. Effect of saxagliptin on oxidative stress in AD cell models ($\bar{x}$±s, n=9).

Group	MDA (μmol/L)	SOD (U/mg)	GSH-Px (U/mg)
CON group	5.59±0.45	64.97±3.68	81.90±4.78
AD group	34.26±2.18[a]	13.89±1.26[a]	31.73±3.07[a]
AD+L-SAX group	26.66±2.29[b]	26.31±2.34[b]	47.88±4.12[b]
AD+M-SAX group	17.13±1.33[bc]	39.39±2.51[bc]	63.91±4.48[bc]
AD+H-SAX group	7.54±0.56[bcd]	56.77±4.12[bcd]	76.21±5.01[bcd]
F	552.613	454.793	202.940
P	0.000	0.000	0.000

Note: [a]P<0.05 compared with CON group; [b]P<0.05 compared with AD group; [c]P<0.05 compared with AD+L-SAX group; [d]P<0.05 compared with AD+M-SAX group.

Table 2. Effect of saxagliptin on apoptosis in AD cell model ($\bar{x}$±s, n=9).

Group	Apoptosis rate (%)	Bcl-2	Bax
CON group	7.89±0.69	0.74±0.04	0.13±0.02
AD group	34.22±2.15[a]	0.22±0.02[a]	0.58±0.04[a]
AD+L-SAX group	26.48±2.45[b]	0.35±0.03[b]	0.44±0.03[b]
AD+M-SAX group	17.71±1.41[c]	0.51±0.03[bc]	0.32±0.02[bc]
AD+H-SAX group	10.70±1.01[bcd]	0.64±0.04[bcd]	0.19±0.02[bcd]
F	383.352	370.583	408.284
P	0.000	0.000	0.000

Note: compared with CON group, [a]P<0.05; compared with AD group, [b]P<0.05; compared with AD+L-SAX group, [c]P<0.05; compared with AD+M-SAX group, [d]P<0.05.

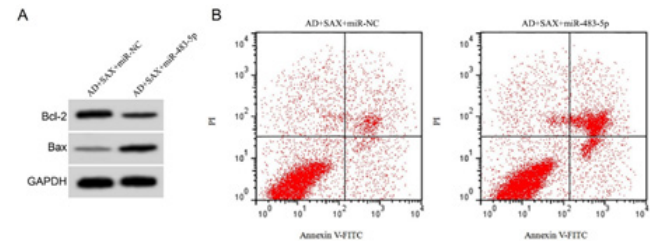

Figure 3. Overexpression of miR-483-5p reverses the effect of saxagliptin on apoptosis in the AD cell model. Note: A: apoptosis-related protein expression; B: apoptosis flow charts.

expression of Bax protein was reduced (P<0.05), and the expression of Bcl-2 protein was elevated (P<0.05).

Overexpression of miR-483-5p reverses the effect of saxagliptin (10 μmol/L) on damage in AD cell model

As shown in Figure 3 and Table 6, compared with the AD+SAX+miR-NC group, the cells in the AD+SAX+miR-483-5p group showed elevated MDA content (P<0.05), decreased SOD and GSH-Px activities (P<0.05), increased apoptosis rate (P<0.05), elevated Bax protein expression (P<0.05) and Bcl-2 protein expression decreased (P<0.05).

Discussion

AD is a neurodegenerative disease with high incidence, which seriously endangers the physical and mental health of the elderly. With the aggravation of the disease, AD patients gradually lose the ability to live independently, causing a heavy mental and economic burden on the family. Currently, most of the treatments for AD are based on Western drugs, which can relieve patients' symptoms in a short period of time, but long-term use will produce a series of side effects and drug resistance (12). Therefore, there is a need to find new therapeutic drugs. The hallmark pathological feature of AD is a large amount of Aβ deposition in the senile plaques of brain tissues, and the neurotoxicity caused by Aβ deposition plays an important role in the development of AD (13).$A\beta_{25\text{-}35}$, one of the species

Table 3. Effect of saxagliptin on miR-483-5p expression in AD cell model ($\bar{x}$±s, n=9).

Group	miR-483-5p
CON group	1.00±0.07
AD group	3.55±0.25[a]
AD+L-SAX group	2.66±0.23[b]
AD+M-SAX group	1.96±0.14[c]
AD+H-SAX group	1.38±0.13[bcd]
F	298.728
P	0.000

Note: Compared with CON group, [a]P<0.05; compared with AD group, [b]P<0.05; compared with AD+L-SAX group, [c]P<0.05; compared with AD+M-SAX group, [d]P<0.05.

Table 4. Effect of knockdown of miR-483-5p on oxidative stress in AD cell model ($\bar{x}$±s, n=9).

Group	miR-483-5p	MDA (μmol/L)	SOD (U/mg)	GSH-Px (U/mg)
AD+anti-miR-NCgroup	1.00±0.06	35.82±2.95	12.96±1.30	30.31±2.34
AD+anti-miR-483-5pgroup	0.25±0.03[a]	12.01±1.20[a]	47.71±3.30[a]	68.47±3.44[a]
t	33.541	22.429	29.392	27.516
P	0.000	0.000	0.000	0.000

Note: aP<0.05 compared with AD+anti-miR-NC group.

Table 5. Effect of knockdown of miR-483-5p on apoptosis in AD cell model ($\bar{x}$±s, n=9).

Group	Apoptosis rate (%)	Bcl-2	Bax
AD+anti-miR-NCgroup	36.49±2.34	0.21±0.02	0.59±0.04
AD+anti-miR-483-5pgroup	14.01±1.04[a]	0.61±0.04[a]	0.24±0.02[a]
t	26.337	26.833	23.479
P	0.000	0.000	0.000

Note: Compared with AD+anti-miR-NC group, [a]P<0.05.

Table 6. Overexpression of miR-483-5p reverses the effect of saxagliptin on injury in the AD cell model ($\bar{x}$±s, n=9).

Group	miR-483-5p	MDA (μmol/L)	SOD (U/mg)	GSH-Px (U/mg)	Apoptosis rate (%)	Bcl-2	Bax
AD+SAX+miR-NC group	1.00±0.05	7.05±0.48	58.78±4.63	77.37±4.76	10.61±1.01	0.66±0.04	0.18±0.02
AD+SAX+miR-483-5pgroup	2.96±0.23[a]	25.27±2.18[a]	23.19±2.21[a]	41.66±3.19[a]	28.89±2.42[a]	0.31±0.03[a]	0.49±0.03[a]
t	24.982	24.487	20.811	18.696	20.913	21.000	25.794
P	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Note: [a]P<0.05 compared to AD+saxagliptin+miR-NC group.

of Aβ, can induce oxidative stress and apoptosis in neuronal cells to damage the neuronal cells, and it is commonly used to establish a model of AD cell injury (14). In this study, $A\beta_{25-35}$ promoted the expression of MDA, decreased SOD and GSH-Px activities, and promoted apoptosis in SK-N-SH cells, which was consistent with the results reported by Shi et al. (15, 16), suggesting that the AD cell injury model was successfully established.

Under normal physiological conditions, oxidation and antioxidation are in dynamic balance in the organism. Whereas, under pathological conditions, when the body produces excessive free radicals or has reduced scavenging capacity, it leads to oxidative stress damage in tissues (17). Studies have shown that the activity of antioxidant enzymes for scavenging free radicals is significantly reduced in brain tissues of AD patients, whereas the indicators of conservation stress injury such as lipid peroxides and carbonylation products such as MDA are significantly elevated (18).SOD and GSH-Px are important antioxidant enzymes in the body, which can scavenge excessive free radicals and reduce the oxidative stress injury of tissues of the organism (19). In the present study, saxagliptin dose-dependently decreased MDA expression and increased SOD and GSH-Px activities in $A\beta_{25-35}$-induced SK-N-SH cells, suggesting that saxagliptin suppressed the level of oxidative stress in $A\beta_{25-35}$-induced SK-N-SH cells.

Neuroapoptosis is an important cause of the development of neurodegenerative diseases such as AD and Parkinson's, and inhibition of neuroapoptosis is important for the alleviation or treatment of neurodegenerative diseases. Apoptosis is a programmed death process that is regulated by a variety of molecules. Bax and Bcl-2 proteins play important regulatory roles in apoptosis, with elevated Bax expression inducing apoptosis and elevated Bcl-2 expression inhibiting apoptosis (20). In this study, saxagliptin dose-dependently reduced the apoptosis rate and Bax protein expression in SK-N-SH cells induced by $A\beta_{25-35}$, while promoting Bcl-2 protein expression, suggesting that saxagliptin inhibited the apoptosis of SK-N-SH cells induced by $A\beta_{25-35}$.

miRNAs are widely found in eukaryotic organisms and are involved in the developmental process of various diseases such as neurodegenerative diseases, cardiovascular and cerebrovascular diseases, and tumours (21-23). Studies have shown that a variety of miRNAs are involved in the developmental process of AD and are potential molecular targets for AD therapy. For example, Miao et al. (24) showed that miR-28-3p expression was elevated in the serum of AD patients and was positively correlated with the degree of inflammation of the disease. Donepezil treatment could reduce the serum miR-28-3p level of the patients and alleviate their symptoms, and miR-28-3p could be used as an indicator for early diagnosis and prognostic assessment of AD patients. Zhao et al. (25) showed that miR-138 expression was elevated in AD models, and overexpression of miR-138 could target and inhibit DEK expression to promote apoptosis in SH-SY5Y cells. Xu et al (26) showed that $A\beta_{1-42}$ treatment decreased miR-107 expression in SH-SY5Y and SK-N-SH cells, whereas overexpression of miR-107 increased $A\beta_{1-42}$-induced apoptosis in SH-SY5Y and SK-N-SH cells. SY5Y and SK-N-SH cell viability and inhibited apoptosis, which may be a potential molecular target for AD therapy.

Studies have shown that miR-483-5p expression was elevated in both plasma and hypoxia-induced cardiomyocytes AC16 in patients with acute myocardial infarction (AMI). Overexpression of miR-483-5p promoted the level of oxidative stress and apoptosis in hypoxia-induced AC16 cells, which may be a potential therapeutic target for the diagnosis and prevention of hypoxic myocardial injury (27-30). However, the effect of miR-483-5p on AD development is unknown. The present study showed that $A\beta_{25-35}$ promoted the expression of miR-483-5p in SK-N-SH cells, whereas knockdown of miR-483-5p inhibited $A\beta_{25-35}$-induced oxidative stress and apoptosis in SK-N-SH cells, suggesting that miR-483-5p may be involved in the process of Alzheimer's disease development and is a potential molecular target for the treatment of this disease. The present study also showed that saxagliptin inhibited the expression of miR-483-5p in $A\beta_{25-35}$-induced SK-N-SH cells, whereas overexpression of miR-483-5p reversed the inhibitory effect of saxagliptin on oxidative stress and apoptosis induced in $A\beta_{25-35}$-induced SK-N-SH cells, suggesting that saxagliptin may attenuate the development and progression of $A\beta_{25-35}$-induced SK-N-SH cells by downregulating the expression of miR-483-5p. β_{25-35}-induced SK-N-SH cell injury.

In conclusion, saxagliptin can effectively inhibit $A\beta_{25-35}$-induced oxidative stress and apoptosis in SK-N-SH cells and attenuate cell injury, and its mechanism of action may be related to the down-regulation of miR-483-5p expression, which provides some experimental basis for its use in the clinical treatment of Alzheimer's disease.

Conflict of Interests

The authors declared no conflict of interest.

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