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Original Research

Effects of dendrobium polysaccharides on human brain microvascular endothelial cell injury induced by ox-LDL via regulating the miR-378 expression

Lai Chen[1], Shaohui Zhang[2], Xiaoying Liu[1], Jiong Dai[3*], Zhenguo Yan[1], Chunlan Wu[1]

[1] Neurology Department, Shanghai TCM-Integrated Hospital Affiliated to Shanghai University of TCM, Shanghai, 200082, China
[2] Rehabilitation Department, Shanghai Jiangwan Hospital, Shanghai, 200434, China
[3] Neurosurgery Department, Renji Hospital, Shanghai Jiao Tong University, School of Medicine, Shanghai, 200127, China

[*]**Correspondence to:** zg18836@163.com

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Abstract: Brain microvascular endothelial cells are a key part of the blood-brain barrier. This experiment was set up to investigate the effect and molecular mechanism of Dendrobium polysaccharide on oxidized low-density lipoprotein (ox-LDL)-induced damage to the human brain microvascular endothelial cells. For this purpose, human brain microvascular endothelial cells HBMEC were divided into control group (without any treatment), ox-LDL group (50 μg/mL ox-LDL), Dendrobium polysaccharide low, medium and high concentration group (0.1 μg/L, 0.2 μg/L, 0.4 μg/L Dendrobium polysaccharide+50 μg/mL ox-LDL), ox-LDL+miR-NC group (transfection miR-378 mimic negative control+50 μg/mL ox-LDL), ox-LDL+miR-378 group (transfected miR-378 mimics+50 μg/mL ox-LDL), ox-LDL+DP+anti-miR-NC group (transfected miR-378 inhibitor negative control +0.4 μg/L Dendrobium polysaccharide+50 μg/mL ox-LDL), ox-LDL+DP+anti-miR-378 group (transfected miR-378 inhibitor+0.4 μg/L Dendrobium polysaccharide+50 μg/mL ox-LDL). The kit was used to detect the levels of malondialdehyde (MDA) and the activities of superoxide dismutase (SOD) and catalase (CAT); flow cytometry to detect apoptosis; and Western blot to detect B-cell lymph tumor/leukemia-2 (Bcl-2) and Bcl-2 related X (Bax) protein expression; real-time fluorescence quantitative PCR (RT-qPCR) was used to detect the expression of miR-378. Results showed that after treatment with different concentrations of Dendrobium polysaccharides, MDA levels were decreased in ox-LDL-induced human brain microvascular endothelial cells, SOD and CAT activities were increased, apoptosis rate was decreased, Bcl-2 expression was increased, Bax expression was decreased, miR-378 expression was increased, in a dose-dependent manner ($P<0.05$). Overexpression of miR-378 inhibits ox-LDL-induced oxidative stress and apoptosis in human brain microvascular endothelial cells. Inhibition of miR-378 expression reversed the effect of Dendrobium polysaccharide on ox-LDL-induced damage to human brain microvascular endothelial cells. Then dendrobium polysaccharide may inhibit ox-LDL-induced oxidative stress and apoptosis in human brain microvascular endothelial cells by up-regulating the expression of miR-378.

Key words: Dendrobium polysaccharide; miR-378; ox-LDL; Human brain microvascular endothelial cells; Oxidative damage.

Introduction

The blood-brain barrier (BBB) is the area between the extracellular fluid of the brain in the central nervous system and the circulatory system in the body so that if colored substances are injected into the bloodstream, it can be seen that there is no trace of this substance in the brain. This curtain or barrier consists of special capillaries that, unlike the normal structure of capillaries, do not have the usual pores and the intercellular connection in them is of the strong connection type, and as a result, many molecules and micro-molecules as well as bacteria are able to pass through them (via diffusion) and reaching the cerebrospinal fluid is not in the brain. In turn, the endothelial surface of these capillaries is covered with special proteins that allow glucose to enter the brain as a nutrient. Also, gas exchange (oxygen-carbon dioxide) between the circulating blood and the brain of this dam can be done without any problems (1-3). Brain microvascular endothelial cells are an important part of the blood-brain barrier, which plays a role in maintaining normal cerebral vascular function; dysfunction or even death of vascular endothelial cells can cause various vascular diseases (1).

Oxidized low-density lipoprotein (ox-LDL) is an important factor that causes injury to vascular endothelial cells, which plays an important role in cardiovascular disease. Therefore, the protection of the vascular endothelial cell against injury has a close relation to the treatment and prevention of vascular diseases (2). Traditional Chinese medicine polysaccharide is a natural macromolecular compound with a wide range of sources, which has a variety of pharmacological activities and demonstrates immunomodulatory, anti-tumor, antioxidant effects (3). Dendrobium candidum is a traditional valuable Chinese medicinal material and Dendrobium polysaccharides also have antioxidant, bacteriostatic and immunomodulatory effects (4). Studies have shown that dendrobium polysaccharides (DP) have a protective effect on high glucose-induced endothelium-dependent diastolic function (5). Dendrobium polysaccharides can inhibit high glucose-induced

apoptosis of vascular endothelial cells, which prevents diabetic vascular disease to a certain extent (6). Dendrobium polysaccharides also inhibit high glucose-induced oxidative stress injury in human umbilical vein endothelial cells (7). However, the effect and mechanism of Dendrobium polysaccharides on ox-LDL-induced injury to human brain microvascular endothelial cells remain unclear. Studies have reported that miR-378 can alleviate oxygen-sugar deprivation-induced ischemic injury to neuroblastoma N2A cells in mice, and helps to provide a potential therapeutic target for ischemic stroke from the level of miRNAs (8). It indicates that miR-378 may have a certain effect on ischemic brain diseases. Therefore, this paper investigates whether the effect of Dendrobium polysaccharide on ox-LDL-induced injury to human brain microvascular endothelial cells and its mechanism is related to miR-378, with a view to providing strong evidence for the treatment of vascular diseases with Dendrobium polysaccharide and thereby better guiding clinical medication.

Materials and Methods

Materials

Human brain microvascular endothelial cells HBMEC were purchased from Shanghai Kanglang Biotechnology Co., Ltd.; fetal bovine serum and RPMI-1640 medium were purchased from Shenzhen Ziker Biotechnology Co., Ltd.; oxidized low-density lipoprotein (ox-LDL), Dendrobium polysaccharides were purchased from Beijing Chreagen Biological Technology Co., Ltd.; superoxide dismutase (SOD) kit, malonaldehyde (MDA) kit, catalase (CAT) were purchased from Nanjing Jiancheng Bioengineering Institute; Annexin V-FITC/PI apoptosis detection kit was purchased from Beijing Solarbio Science & Technology Co., Ltd.; real-time fluorescence quantitative PCR detection kit, protein extraction kit, and bicinchoninic acid (BCA) kit were purchased from Beijing Biao Laibo Technology Co., Ltd.

Cell processing and grouping

Human brain microvascular endothelial cells HBMEC were cultured in RPMI-1640 medium containing 10% fetal bovine serum. After the cells had grown to 80% confluence, they were digested and passaged. Logarithmic growth phase HBMEC cells were taken and cultured in 50 μg/mL ox-LDL to establish a cell injury model which was recorded as an ox-LDL group; the control group (Con) was added with an equal amount of medium; 0.1 μg/L, 0.2 μg/L. 0.4 μg/L Dendrobium polysaccharide was cultured for 2 h and then added with 50 μg/mL ox-LDL for continued cultivation, which was recorded as low, medium and high-concentration Dendrobium polysaccharide groups.

miR-NC and miR-378 were transfected into HBMEC cells, added with 50 μg/mL ox-LDL for culture and recorded as an ox-LDL+miR-NC group, ox-LDL+miR-378 group; anti-miR-NC, anti-miR-378 were transfected into HBMEC cells, added with 0.4 μg/L Dendrobium polysaccharide and 50 μg/mL ox-LDL for culture, which was recorded as an ox-LDL+DP+anti-miR-NC group, ox-LDL+DP+anti-miR-378 group.

Detection of MDA level and SOD, CAT activity by MDA, SOD and CAT kits

After 48 hours of cell culture, cells of each group were collected and processed according to the kit instructions.

Apoptosis detection by flow cytometry

Cells in each group were cultured for 48h, rinsed twice with pre-chilled PBS, added with 10 μL Annexin V-FITC, and then added with 5 μL PI, mixed well and incubated in the dark for 10 min; flow cytometry was used to detect the apoptosis rate.

Detection of Bcl-2, Bax protein expression by Western Blot

Total protein was extracted and protein concentration was determined by the BCA method. The sample was loaded according to 40μg/well protein. After SDS-PAGE, membrane transfer, blocking, the membrane was cleaned, added with primary antibody (1:500) and incubated at 4°C overnight. The membrane was cleaned with TBST, added with an HRP-labeled secondary antibody (1:1000), incubated at room temperature for 2h on the shaker, followed by membrane cleaning with TBST, ECL color development, and development and fixation of X-ray film in a dark room. Quantity-One software was used to analyze the gray value of protein bands. The relative expression of protein = gray value of the target protein / gray value of internal reference GAPDH.

Detection of miR-378 expression level by real-time fluorescence quantitative PCR (RT-qPCR)

Total cellular RNA was extracted, reverse transcribed into cDNA, and subject to PCR amplification. Each sample was repeated 3 times. The cyclic conditions were 95 ℃ 30 s, 60 ℃ 30 s; 72 ℃ 30 s, a total of 40 cycles. The relative expression level was calculated using the 2-ΔΔCt method. Taking U6 as internal reference, miR-378 upstream primer sequence: 5'-CTCCTGACTCCAGGTCCTGT-3', downstream primer sequence: 5'-GCCTTCTGACTCCAAGTCCA-3'; U6 upstream primer sequence: 5'-CTCGCTTCGGCAGCACA-3', downstream primer sequence: 5'-AACGCTTCACGAATTTGCGT-3'.

Statistical analysis

SPSS 20.0 software was used for statistical analysis. The measurement data were expressed as mean ±standard deviation ($\bar{x}\pm s$), t-test was used for comparison between two groups, one-way analysis of variance was used for comparison between multiple groups, LSD t-test was used for pairwise comparison. $P<0.05$indicates statistically significant differences.

Results

Effect of Dendrobium polysaccharides on ox-LDL-induced oxidative stress in human brain microvascular endothelial cells

Compared with Con group, the ox-LDL group has increased MDA level, decreased SOD and CAT activities ($P<0.05$); compared with the ox-LDL group, low, medium, and high-concentration Dendrobium polysaccharides groups have decreased MDA level, increased

Table 1. Effect of Dendrobium polysaccharides on ox-LDL-induced oxidative stress in human brain microvascular endothelial cells($\bar{x}\pm s$, n=9).

Group	MDA (nmol/mg prot)	SOD (U/mg prot)	CAT (U/mg prot)
Con	3.28±0.28	29.52±2.79	5.80±0.47
ox-LDL	11.81±1.07*	9.32±0.91*	0.85±0.08*
ox-LDL+DP-L	8.89±0.80$^{\#}$	14.58±1.32$^{\#}$	2.07±0.21$^{\#}$
ox-LDL+DP-M	6.55±0.47$^{\#\&}$	18.47±1.21$^{\#\&}$	3.16±0.26$^{\#\&}$
ox-LDL+DP-H	4.51±0.56$^{\#\&\$}$	23.60±2.12$^{\#\&\$}$	4.85±0.43$^{\#\&\$}$
F	220.275	169.168	347.376
P	0.000	0.000	0.000

Note: Compared with Con group, *P<0.05; compared with ox-LDL group, $^{\#}P$<0.05; compared with ox-LDL+DP-L group, $^{\&}P$<0.05; compared with ox-LDL+DP-M group, $^{\$}P$<0.05

SOD and CAT activities in a dose-dependent manner (P<0.05) (Table 1).

Effect of Dendrobium polysaccharides on ox-LDL-induced apoptosis of human brain microvascular endothelial cells

Compared with Con group, the ox-LDL group has increased apoptosis rate, decreased Bcl-2 expression level and increased Bax expression level (P<0.05); compared with ox-LDL group, low, medium, and high-concentration Dendrobium polysaccharides groups have decreased apoptosis rate, increased Bcl-2 expression level and decreased Bax expression level in a dose-dependent manner (P<0.05) (Figure 1, Table 2).

Effect of Dendrobium polysaccharides on the expression of miR-378 in human brain microvascular endothelial cells induced by ox-LDL

Compared with Con group, the ox-LDL group has decreased miR-378 expression level (P<0.05); compared with the ox-LDL group, low, medium, and high-concentration Dendrobium polysaccharides groups have increased miR-378 expression level in a dose-dependent manner (P<0.05) (Table 3).

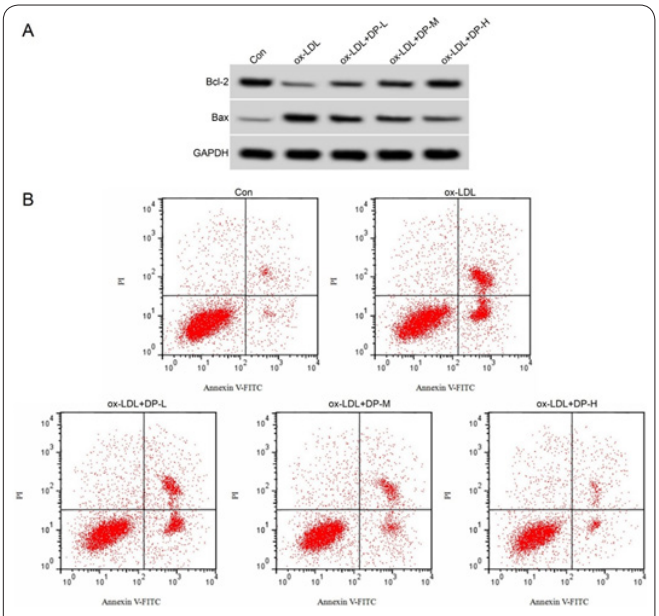

Figure 1. The effect of Dendrobium polysaccharides on ox-LDL-induced apoptosis of human brain microvascular endothelial cells. A: Expression of apoptosis-related proteins; B: Flow chart of apoptosis.

Table 2. Effect of Dendrobium polysaccharides on ox-LDL-induced apoptosis of human brain microvascular endothelial cells($\bar{x}\pm s$,n=9).

Group	apoptosis rate (%)	Bcl-2 protein	Bax protein
Con	6.71±0.67	0.67±0.04	0.15±0.02
ox-LDL	33.34±2.83*	0.24±0.02*	0.55±0.04*
ox-LDL+DP-L	24.41±2.13$^{\#}$	0.35±0.02$^{\#}$	0.42±0.03$^{\#}$
ox-LDL+DP-M	18.71±1.12$^{\#\&}$	0.46±0.04$^{\#\&}$	0.31±0.03$^{\#\&}$
ox-LDL+DP-H	11.61±1.16$^{\#\&\$}$	0.57±0.05$^{\#\&\$}$	0.20±0.02$^{\#\&\$}$
F	317.873	201.946	284.250
P	0.000	0.000	0.000

Note: Compared with Con group, *P<0.05; compared with ox-LDL group, $^{\#}P$<0.05; compared with ox-LDL+DP-L group, $^{\&}P$<0.05; compared with ox-LDL+DP-M group, $^{\$}P$<0.05.

Table 3. Effect of Dendrobium polysaccharides on the expression of miR-378 in human brain microvascular endothelial cells induced by ox-LDL($\bar{x}\pm s$, n=9).

Group	miR-378
Con	1.00±0.06
ox-LDL	0.37±0.04*
ox-LDL+DP-L	0.51±0.05$^{\#}$
ox-LDL+DP-M	0.64±0.06$^{\#\&}$
ox-LDL+DP-H	0.79±0.07$^{\#\&\$}$
F	166.306
P	0.000

Note: Compared with Con group, *P<0.05; compared with ox-LDL group, $^{\#}P$<0.05; compared with ox-LDL+DP-L group, $^{\&}P$<0.05; compared with ox-LDL+DP-M group, $^{\$}P$<0.05.

Table 4. Effect of miR-378 overexpression on ox-LDL-induced oxidative stress in human brain microvascular endothelial cells($x\pm s$, n=9).

Group	miR-378	MDA (nmol/mg prot)	SOD (U/mg prot)	CAT (U/mg prot)
ox-LDL+miR-NC	1.00±0.06	12.23±1.16	8.31±0.78	0.82±0.07
ox-LDL+miR-378	2.96±0.24*	6.55±0.54*	18.71±1.12*	3.89±0.32*
t	23.768	13.317	22.860	28.116
P	0.000	0.000	0.000	0.000

Note: Compared with the ox-LDL+miR-NC group, *P<0.05.

Effect of miR-378 overexpression on ox-LDL-induced oxidative stress in human brain microvascular endothelial cells

Compared with the ox-LDL+miR-NC group, the ox-LDL+miR-378 group has increased miR-378 expression level, decreased MDA expression level, and increased SOD and CAT activities (P<0.05)(Table 4).

Effect of miR-378 overexpression on ox-LDL-induced apoptosis of human brain microvascular endothelial cells

Compared with the ox-LDL+miR-NC group, the ox-LDL+miR-378 group has decreased apoptosis rate, increased Bcl-2 expression level and decreased Bax expression level (P<0.05) (Figure 2, Table 5).

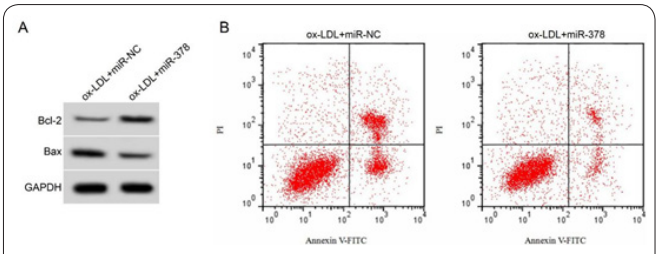

Figure 2. The effect of miR-378 overexpression on ox-LDL-induced apoptosis of human brain microvascular endothelial cells. A: Expression of apoptosis-related proteins; B: Flow chart of apoptosis.

Inhibition of miR-378 expression reverses the effect of Dendrobium polysaccharides (0.4 μg/L) on ox-LDL-induced apoptosis of human brain microvascular endothelial cells

Compared with the ox-LDL+DP+anti-miR-NC group, the ox-LDL+DP+anti-miR-378 group has decreased miR-378 expression level, increased MDA level, decreased SOD and CAT activities, increased cell apoptosis rate, decreased Bcl-2 expression level and increased Bax expression level (P<0.05)(Figure 3, Table 6).

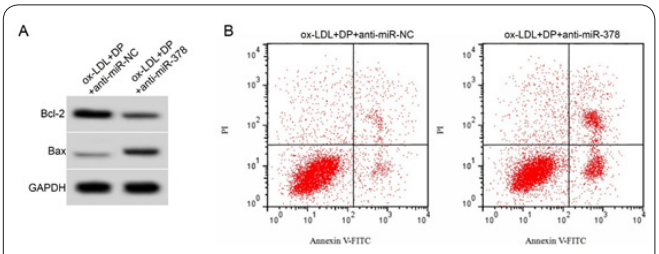

Figure 3. Inhibition of miR-378 expression reverses the effect of Dendrobium polysaccharides on ox-LDL-induced apoptosis of human brain microvascular endothelial cells. A: Expression of apoptosis-related proteins; B: Flow chart of apoptosis.

Discussion

Dendrobium candidum rich in polysaccharides has good hypoglycemic, antioxidant, anti-aging effects. Studying the chemical composition and pharmacological effects of Dendrobium candidum can provide a reference for its further research and clinical application (9). Studies have reported that Dendrobium polysaccharides can inhibit MPP+-induced PC12 cell injury by inhibiting oxidative stress (10). Dendrobium polysaccharides have protective effects against lipopolysac-

Table 5. Effect of miR-378 overexpression on ox-LDL-induced apoptosis of human brain microvascular endothelial cells ($x\pm s$, n=9).

Group	Apoptosis Rate (%)	Bcl-2 protein	Bax protein
ox-LDL+miR-NC	32.00±3.07	0.23±0.02	0.59±0.05
ox-LDL+miR-378	12.53±1.16*	0.53±0.04*	0.29±0.02*
t	17.798	20.125	16.713
P	0.000	0.000	0.000

Note: Compared with the ox-LDL+miR-NC group,*P<0.05.

Table 6. Inhibition of miR-378 expression reversed the effect of Dendrobium polysaccharides on ox-LDL-induced injury to human brain microvascular endothelial cells ($x\pm s$, n=9).

Group	miR-378	MDA (nmol/mg prot)	SOD (U/mg prot)	CAT (U/mg prot)	apoptosis rate (%)	Bcl-2 protein	Bax protein
ox-LDL+DP+anti-miR-NC	1.00±0.07	4.30±0.42	25.14±2.15	5.04±0.47	10.60±1.03	0.60±0.04	0.18±0.02
ox-LDL+DP+anti-miR-378	0.54±0.05*	9.62±0.74*	13.67±1.45*	0.94±0.07*	27.42±2.03*	0.30±0.02*	0.46±0.04*
t	16.042	18.757	12.124	66.903	20.125	20.125	18.783
P	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Note: Compared with the ox-LDL+DP+anti-miR-NC group, *P<0.05.

charide-induced gastric mucosal epithelial GES-1 cell injury (11). Dendrobium polysaccharide has a certain protective effect against hypoxia/reoxygenation injury of H9c2 cells, whose possible mechanism is to increase cell activity, enhance anti-oxidative injury capacity, and inhibit apoptosis (12). Dendrobium polysaccharides reduce malonaldehyde (MDA) levels, increase superoxide dismutase (SOD) activity, inhibit the production of reactive oxygen species (ROS) in cells; and also inhibit apoptosis of H9c2 cells (13-14). The above research indicates that Dendrobium polysaccharides have strong antioxidant and anti-apoptotic effects. In this experiment, to study the effect of Dendrobium polysaccharides on human brain microvascular endothelial cell injury, human brain microvascular endothelial cells were treated with different concentrations of Dendrobium polysaccharides. The result showed that ox-LDL-induced human brain microvascular endothelial cells had decreased MDA levels, increased SOD and catalase (CAT) activities, decreased apoptosis rate, increased Bcl-2 expression level and decreased Bax expression level in a dose-dependent manner. MDA is the final oxidation product of lipid peroxidation, which can indirectly reflect the degree of cell injury; SOD and CAT are antioxidant enzymes that scavenge oxygen free radicals in cells. Hence, this result indicates that Dendrobium polysaccharides can inhibit oxidative stress and apoptosis of human brain microvascular endothelial cells in a dose-dependent manner.

In fact, the blood-brain barrier is a type of support cell that feeds the neurons. And in fact, capillaries are normal, and first, the material enters the neuroglia cell, and then the neuron receives the nutrients it needs, of course, from its backup cell. Blood-Brain Barrier is a strong, interconnected covering of blood vessels in the brain that is responsible for preventing large molecules from entering the brain. This insulation does not allow drugs to enter the brain, just as it protects the brain against germs. One of the main problems for doctors and scientists in the treatment of brain diseases is the blood supply to the brain. Because the blood-brain barrier is a very strong protector against common bacterial infections, the spread of infection in the brain is very rare. However, since antibiotics and antibiotics cannot cross the blood-brain barrier, brain damage It is often very serious and difficult to treat. However, the blood-brain barrier becomes somewhat permeable during brain inflammation, and some antibiotics can enter the brain. There are only two types of bacteria that can cross the blood-brain barrier. One is spiral gram-negative bacteria (spirochetes), such as Borrelia, the cause of Lyme disease, and the other is the bacterium Treponema pallidum, which causes syphilis, which may enter the brain by physically piercing the capillary wall in the brain (1-5).

Studies have reported that propofol-induced miR-378 expression inhibits cell apoptosis and thus plays a protective role against liver ischemia-reperfusion injury (15). miR-378 prevents intestinal ischemia/reperfusion injury by inhibiting intestinal mucosal cell apoptosis (16). Inhibition of lncRNA NEAT1 alleviates hypoxia-induced cardiomyocyte injury by targeting miR-378a-3p (17). miR-378 alleviates ischemic injury by negatively regulating apoptosis-executing molecule caspase-3, and provides a potential therapeutic target for ischemic stroke (18). The results of this experiment suggest that ox-LDL-induced human brain microvascular endothelial cells have decreased miR-378 expression level, and overexpression of miR-378 can inhibit ox-LDL-induced oxidative stress and apoptosis in human brain microvascular endothelial cells. This shows that miR-378 also has a protective effect against injury of human brain microvascular endothelial cells as in other cell injuries. Moreover, this experiment also found that ox-LDL- induced human brain microvascular endothelial cells have increased miR-378 expression level after treatment with Dendrobium polysaccharides; while inhibition of miR-378 expression reverses the effect of Dendrobium polysaccharides on ox-LDL-induced human brain microvascular endothelial cell injury.

Gene expression is the process by which information is used within a gene to produce a functional product. The products of the genes are mainly amino acids, and non-amino acid products include rRNA, tRNA, and snRNA. The process of gene expression is performed by all eukaryotes and prokaryotes (bacteria, etc.). Various steps can be considered for the gene expression process, which generally involves transcription, RNA binding, translation, and post-translational changes of a protein (19-24).

Gene-to-cell regulation makes it possible to control its structure and function, and this is the basis for cellular differences (differentiation), evolution, and the ability of organisms to adapt to new conditions (25-28). Derived cells The differences and distinctions between cells are the results of the expression or non-expression of parts of the gene. Gene regulation can still be considered as one of the sub-layers of evolution because controlling the timing, location, and amount of genes can have important effects on the performance of genes within a cell or the whole of the organelle organism (26-30). In genetics, gene expression is one of the most important fundamental issues that help a genotype to appear as a phenotype. In fact, the genetic code stored in the DNA strands is interpreted by gene expression, and the characteristics and expression of the gene will cause a phenotype in the organism (31-35).

In this research, we investigated the effects of dendrobium polysaccharides on human brain microvascular endothelial cell injury induced by ox-LDL via regulating the miR-378 expression. To sum up, Dendrobium polysaccharide may inhibit ox-LDL-induced oxidative stress and apoptosis in human brain microvascular endothelial cells by up-regulating miR-378 expression.

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