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Descurainia sophia Reduces Podocyte Damage in Diabetic Nephropathy by Down Regulating LncRNA KCNQ1OT1

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ABSTRACT

The objective of this study was to investigate the effects of *Descurainia sophia* (DS) on proliferation, apoptosis and endoplasmic reticulum stress of high glucose (HG)-induced mouse podocytes and its regulation of LncRNA KCNQ1OT1. In vitro cultured immortal mouse podocytes were divided into Con group, HG group, HG+DS-L group, HG+DS-M group, HG+DS-H group. si-NC and si-KCNQ1OT1 were transfected respectively into podocytes, followed by 30 mmol/L glucose intervention for 24 h. pcDNA, pcDNA-KCNQ1OT1 were transfected into podocytes, followed by 200 μg/mLDS and 30 mmol/L glucose intervention for 24 h. CCK-8 method and colony-forming assay were taken to detect cell proliferation ability; flow cytometry was adopted to detect apoptosis rate; qRT-PCR method was used to detect the expression of KCNQ1OT1; Western blot method was used to detect Nephrin, GRP78, GRP94, and Caspase 12 protein expressions. We found that DS enhanced the proliferation of high glucose-induced podocytes (P<0.05), increased the number of clones formed (P<0.05), reduced the apoptosis rate and GRP78, GRP94, Caspase 12 protein levels (P<0.05), and increased Nephrin protein level (P<0.05). High glucose-induced podocytes had increased KCNQ1OT1 expression level (P<0.05). DS reduced KCNQ1OT1 expression level (P<0.05). The inhibition of KCNQ1OT1 expression resulted increase in the survival rate and nephrin protein level of high glucose-induced podocytes (P<0.05), increase in the number of clones formed (P<0.05), reduce in apoptosis rate and GRP78, GRP94, Caspase 12 protein levels (P<0.05). KCNQ1OT1 overexpression reduced DS effect on high glucose-induced podocyte proliferation, apoptosis and endoplasmic reticulum stress. It is concluded that *D. sophia* can promote high glucose-induced podocyte proliferation, inhibit cell apoptosis, endoplasmic reticulum stress by down-regulating the expression of KCNQ1OT1, thereby reducing podocyte damage in diabetic nephropathy.

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SD and NL selected the research samples and carried out cell test. JZ and TW made further medical analysis. All authors conducted the experiments, discussed and analysed the results and wrote the manuscript.

Key words
Descurainia Sophia, LncRNA KCNQ1OT1, Diabetic nephropathy, Podocytes, Proliferation, Apoptosis

INTRODUCTION

Diabetic nephropathy is a major complication of diabetes. Podocyte damage caused by podocyte apoptosis and oxidative stress has relation to proteinuria. Therefore, changing the pathological basis of podocyte damage means great significance for delaying the progression of diabetic nephropathy. At present, traditional Chinese medicine or western medicine is mainly used for treatment in clinical practice, but drugs such as angiotensin converting enzyme inhibitors have limited therapeutic effects on diabetic nephropathy and have no obvious therapeutic effect on diabetic nephropathy patients with persistent proteinuria. If diabetic nephropathy cannot be effectively controlled, the patient's renal function will deteriorate and eventually evolve into end-stage renal disease. Previous studies have shown that Qiditangshen Granules, Astragalus and other traditional Chinese medicine extracts have significant effects in alleviating podocyte damage in diabetic nephropathy (Mao *et al*., 2015; Wang *et al*., 2019; Yang *et al*., 2020; Jiang *et al*., 2017). However, the mechanism by which extracts of traditional Chinese medicine reduces podocyte damage in diabetic nephropathy has not yet been elucidated. *Descurainia sophia* are mature seeds of *Sisymbrium sophia*, a cruciferous plant, which can be used to treat diseases such as heart failure and reduce myocardial cell damage (Kong *et al*., 2019). However, the effect of *D. sophia* on podocyte damage in diabetic nephropathy has not been elucidated. Long-chain non-coding RNA KCNQ1OT1 (LncRNA KCNQ1OT1) has elevated expression level in patients with diabetic nephropathy, which may become a biomarker for predicting the prognosis of patients with diabetic nephropathy (Jiang *et al*., 2017).

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However, the effect of *D. sophia* on the proliferation and apoptosis of podocytes in diabetic nephropathy and the role of KCNQ1OT1 in this process are not yet known. Therefore, using high glucose- induced podocytes to establish a cell injury model, this study investigates whether *D. sophia* can affect the proliferation, apoptosis and endoplasmic reticulum stress of high glucose-induced podocytes by regulating KCNQ1OT1 expression.

MATERIALS AND METHODS

Materials and reagents

D. sophia was purchased from Jiangsu Changjing Seed Industry Co., Ltd. Immortal mouse podocytes were purchased from Beijing Beina Chuanglian Biotechnology Institute. Glucose was purchased from Shanghai Ruji Biotechnology Development Co., Ltd. Lipofectamine 2000 was purchased from Shanghai Regal Biotechnology Co., Ltd. si-NC, si-KCNQ1OT1, pcDNA, and pcDNA-KCNQ1OT1 were purchased from Shanghai Jima Pharmaceutical Technology Co., Ltd. CCK-8 reagents and apoptosis detection kits were purchased from Jiangsu KeyGEN BioTECH Co., Ltd. Trizol, cDNA synthesis, and qRT-PCR kits were purchased from Beijing Tiangen Biochemical Technology Co., Ltd. Rabbit anti-mouse nephrin, GRP78, GRP94, and caspase 12 antibodies were purchased from Santa Cruz, United States. HRP-labeled goat anti-rabbit IgG secondary antibody was purchased from Abcam, United States.

Experiment grouping

For preparation of water extract of *Descurainia sophia* 100 g of *D. sophia* was extracted 3 times in distilled water in a water bath, 1 h each time. The filtrate was concentrated to 100 mL (2 g/mL). sodium chloride 0.9% was used to prepare solutions with concentrations of 50, 100 and 200 μg/mL (Jia *et al*., 2018).

Podocytes were routinely cultured. When the cell growth and confluency reached 80%, they were digested with 0.25% trypsin and counted (2.5×10^5 cells/mL) and then seeded in a 96-well plate (100 μL/well). The culture with 30 mmol/L glucose was cultured for 24 h (HG group). At the same time, the normally cultured cells were labelled as the control group. Different doses (50 μg/mL, 100 μg/mL, 200 μg/mL) of *D. sophia* and 30 mmol/L glucose were used to jointly treat podocytes (Hromadnikova *et al*., 2020) (HG+DS-L group, HG +DS-M group, HG+DS-H group). Podocytes were transfected with si-NC and si-KCNQ1OT1 and culture medium containing 30 mmol/L glucose was added to culture for 24 h, which were recorded as HG+si-NC group and HG+si-KCNQ1OT1 group, respectively. In the subsequent experiments, podocytes were transfected with pcDNA and pcDNA-KCNQ1OT1 respectively, and culture medium containing 200 μg/mL *D. sophia* and 30 mmol/L glucose was added to culture for 24 h, which were respectively recorded as HG+DS-H+ pcDNA group and HG+DS-H+pcDNA-KCNQ1OT1 group.

Cell proliferation detection by CCK-8

Podocytes in the logarithmic growth phase were digested with trypsin and seeded in a 96-well plate (5×10^3 cells/well). After grouping CCK-8 solvent (10 μL/well) was added and incubated for 2 h, detect the optical density value (OD value) of each well, and calculate the cell survival rate (%) = (OD value of the experimental group/ OD value of the control group) × 100%.

Colony-forming assay

Podocytes of each group were seeded in a 6-well plate as 1000 cells in each well and incubated for 14 days for further culture. Culture medium was replaced every 2 d until a macroscopic cell cluster was formed. The culture medium used to wash the cells with PBS and methanol was added as 500 μL/well. The culture plates were placed in a refrigerator at -20°C for 20 min, methanol was discarded and 1% crystal violet staining solution (400 μL/well) was added for 15 min incubation at room temperature. The crystal violet staining solution was removed, the culture plate was washed with distilled water, and the number of colonies formed was observed after drying.

Cell apoptosis rate detection by flow cytometry

Podocytes from each group were washed with PBS. The supernatant was discarded and binding buffer (500 μL) was added to keep the cells in suspension. The apoptosis detection kit instructions were followed to test apoptosis rate of each group.

Detection of KCNQ1OT1 expression level in cells by qRT-PCR

RNA of each group of podocytes was extracted by Trizol method. RNA was reverse transcribed to synthesize cDNA with the help of cDNA synthesis kit. qRT-PCR reaction was carried out with cDNA as a template. The reaction system and reaction conditions followed the kit instructions. KCNQ1OT1 forward primer 5'-CTTACCTGCCTACAACCCCA-3', reverse primer 5'-TCTCTCTGAGGACTCTGGCT-3'; GAPDH forward primer 5'-AACGGATTTGGTCGTATTG-3', reverse primer 5'-GGAAGATGGTGATGGGATT-3'. Primers were designed and synthesized by Sangon Biotech (Shanghai) Co., Ltd. The relative expression of KCNQ1OT1 was detected by ABI 7500 fluorescent quantitative PCR instrument.

Western blot detection of Nephrin, GRP78, GRP94, caspase12 protein expressions

Podocytes of each group was added with 400 μL lysate to extract the total cell protein and the protein concentration was determined by BCA method. The protein was fractionated by SDS-PAGE, transfered to PVDF membrane, blocked with 5% skimmed milk powder for 2 h and then incubated with primary antibody dilutent at 4°C for 24 h. The membranes were washed with TBST to incubate with secondary antibody diluent at room temperature for 1h. ECL was added for development of colour and the gray value of each band was analyzed with ImageJ software.

Statistical analysis

Statistical software SPSS21.0 was used for analysis. Measurement data were expressed as mean± standard deviation (Mean±s). Independent sample *t* test was used for comparison between two groups. One-way analysis of variance was used for comparison between multiple groups $P < 0.05$ indicates statistically significant differences.

RESULTS

Proliferation of high glucose induced podocytes

Compared with control group, HG group had significantly reduced cell survival rate ($P<0.05$) and number of clones ($P<0.05$). Compared with HG all experimental groups had significantly increased cell survival rate ($P<0.05$) and number of clones formed ($P<0.05$) (Table I).

Apoptosis and endoplasmic reticulum stress of high glucose-induced podocyte

Compared with the control group, HG group has significantly increased apoptosis rate and GRP78, GRP94, caspase 12 protein levels ($P<0.05$), and significantly reduced nephrin protein level ($P<0.05$). Compared with the HG group, all experimental groups have significantly reduced apoptosis rate and GRP78, GRP94, caspase 12 protein levels ($P<0.05$) and significantly increased nephrin protein level ($P<0.05$) (Table II).

Table I. Effect of *Descurainia sophia* on the proliferation of high glucose-induced podocytes (±*s*, n=9).

Group	Survival rate (%)	Number of clones formed (pcs)
Control	100.00±7.89	112.00±6.82
HG	37.57±7.20^{a}	38.22±4.79^{a}
HG+DS-L	65.14±9.39^{b}	58.22±5.14^{b}
HG+DS-M	82.00±8.23^{bc}	75.56±3.64^{bc}
HG+DS-H	96.62±7.21^{bcd}	94.22±2.64^{bcd}
F	91.949	326.659
P	0.000	0.000

HG, cultured with 30 mmol/L glucose; HG+DS-L, 30 mmol/L glucose+ *D. sophia* 50 μg/mL; HG +DS-M, 30 mmol/L glucose+ *D. sophia* 100 μg/mL; HG+DS-H, 30 mmol/L glucose+ *D. sophia* 200 μg/mL.

KCNQ1OT1 expression in high glucose-induced podocytes

Compared with the conrol group and HG group, all experimental groups have significantly reduced KCNQ1OT1 expression level ($P<0.05$) (Table III).

Inhibition on the proliferation of high glucose-induced podocytes

Compared with the HG+si-NC group, HG+si-KCNQ1OT1 group has significantly increased cell survival rate ($P<0.05$), and number of clones formed ($P<0.05$)(Table IV).

Table II. Effect of *Descurainia sophia* on apoptosis and endoplasmic reticulum stress of high glucose-induced podocyte (±*s*, n=9).

Group	Apoptosis rate (%)	Nephrin	GRP78	GRP94	Caspase 12
Control	5.43±1.14	0.82±0.05	0.31±0.04	0.26±0.04	0.24±0.05
HG	23.69±2.90^{a}	0.26±0.04^{a}	0.90±0.05^{a}	0.91±0.05^{a}	0.89±0.05^{a}
HG+DS-L	18.82±0.50^{b}	0.49±0.04^{b}	0.70±0.04^{b}	0.73±0.04^{b}	0.72±0.03^{b}
HG+DS-M	16.86±0.52^{bc}	0.60±0.03^{bc}	0.54±0.03^{bc}	0.57±0.03^{bc}	0.57±0.04^{bc}
HG+DS-H	14.78±0.50^{bcd}	0.72±0.03^{bcd}	0.43±0.03^{bcd}	0.44±0.02^{bcd}	0.42±0.03^{bcd}
F	194.305	282.120	320.580	407.379	343.232
P	0.000	0.000	0.000	0.000	0.000

For detials of groups, see Table I.

Table III. Effect of *Descurainia sophia* on KCNQ1OT1 expression in high glucose-induced podocytes(±*s*, n=9).

Group	KCNQ1OT1
Conrol	1.00±0.02
HG	3.49±0.38[a]
HG+DS-L	2.29±0.17[b]
HG+DS-M	1.81±0.09[bc]
HG+DS-H	1.57±0.07[bcd]
F	212.110
P	0.000

For detials of groups, see Table I.

Inhibition of apoptosis and endoplasmic reticulum stress of high glucose-induced podocyte

Compared with HG+si-NC group, HG+si-KCNQ1OT1 group has significantly reduced apoptosis rate and GRP78, GRP94, caspase 12 protein levels (P<0.05), and significantly increased nephrin protein level (*P*<0.05) (Table V).

Table IV. Effect of KCNQ1OT1 expression inhibition on the proliferation of high glucose-induced podocytes (±*s*, n=9).

Group	KCNQ1OT1	Survival rate (%)	Number of clones formed (pcs)
HG+si-NC	1.00±0.03	37.44±6.52	38.11±7.46
HG+si-KC-NQ1OT1	0.36±0.09[a]	80.06±4.80[a]	70.78±3.23[a]
t	20.239	15.792	12.056
P	0.000	0.000	0.000

Note: Compared with HG+si-NC group, [a]*P* <0.05.

KCNQ1OT1 overexpression can reduce the effect of Descurainia sophia *on proliferation, apoptosis and endoplasmic reticulum stress of high glucose-induced podocyte*

Compared with HG+DS-H+pcDNA group, HG+DS-H+pcDNA-KCNQ1OT1 group has significantly reduced survival rate, nephrin protein level (*P*<0.05) and where they have number of clones formed (*P*<0.05), significantly increased apoptosis rate and GRP78, GRP94, caspase 12 protein levels (P<0.05) (Table VI).

Table V. Effect of KCNQ1OT1 expression inhibition on apoptosis and endoplasmic reticulum stress of high glucose-induced podocyte (±*s*, n=9).

Group	Apoptosis rate (%)	Nephrin	GRP78	GRP94	Caspase 12
HG+si-NC	23.99±3.62	0.26±0.05	0.90±0.06	0.90±0.05	0.90±0.04
HG+si-KCNQ1OT1	16.85±0.99[a]	0.65±0.05[a]	0.46±0.06[a]	0.48±0.06[a]	0.41±0.04[a]
t	5.708	16.546	15.556	16.133	25.986
P	0.000	0.000	0.000	0.000	0.000

Note: Compared with HG+si-NC group, [a]*P* <0.05.
HG+si-NC, group that was transfected with si-NC and then treated with high glucose; HG+si-KCNQ1OT1, group that was transfected with si-KCN-Q1OT1 and then treated with high glucose; Nephrin, slit membrane protein; GRP78, glucose-regulated protein 78; GRP94, glucose-regulated protein 94; Caspase12, cysteinyl aspartate-specific protease-12.

Table VI. KCNQ1OT1 overexpression can reduce the effect of *Descurainia sophia* on proliferation, apoptosis and endoplasmic reticulum stress of high glucose-induced podocyte (±*s*, n=9).

Group	KCNQ1OT1	Survival rate (%)	Number of clones formed (pcs)	Apoptosis rate (%)	Nephrin	GRP78	GRP94	Caspase 12
HG+DS-H+pcDNA	1.02±0.03	96.83±2.74	94.44±3.97	14.69±1.12	0.73±0.05	0.43±0.03	0.45±0.04	0.41±0.03
HG+DS-H+pcD-NA-KCNQ1OT1	2.31±0.27[a]	56.95±8.18[a]	59.33±3.54[a]	22.14±2.06[a]	0.49±0.04[a]	0.65±0.06[a]	0.73±0.04[a]	0.69±0.05[a]
t	14.246	13.869	19.802	9.532	11.245	9.839	14.849	14.406
P	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Note: Compared with HG+DS-H+pcDNA group, [a]*P* <0.05.
HG+DS-H+pcDNA, group that was transfected with pcDNA and then treated with DS-H+ and high glucose; HG+DS-H+pcDNA-KCNQ1OT1, group that was transfected with pcDNA-KCNQ1OT1 and then treated with DS-H+ and high glucose; KCNQ1OT1,KCNQ1 overlapping transcript 1.
For other details, see Table V.

DISCUSSION

Early pathological features of diabetic nephropathy include mesangial cell matrix accumulation and glomerular basement membrane thickening. The clinical treatment is mainly to stabilize blood glucose and regulate dyslipidemia. However, patients are prone to certain adverse reactions during western medication, which limits the use of western medicine in clinical practice. Traditional Chinese medicine has certain therapeutic effect on diabetic nephropathy, but the specific mechanism of action is not yet known (Fan *et al*., 2017; Azaman *et al*., 2020; Huang *et al*., 2020). Therefore, it is of great significance to find prescription drugs against diabetic nephropathy from the treasure house of traditional medicine.

D. sophia can improve various indexes of heart failure rats and then improve their heart function (Xiang *et al*., 2018). Flavonoid glycosides, the main component of *D. sophia*, have anti-inflammatory, anti-oxidant and anti-tumor effects (Zhu, 2008; Long *et al*., 2020). The results of this study reveal that high glucose can significantly reduce the proliferation ability of mouse podocytes, while different doses of *D. sophia* can significantly enhance the proliferation ability of high glucose-induced mouse podocytes, and there is significant increase with the increase of drug dosage, suggesting that *D. sophia* can enhance the cell viability of high glucose-induced rats in a dose-dependent manner. The pathological features of podocyte damage mainly include increased podocyte apoptosis, abnormal expression of Nephrin protein and Wilms tumor suppressor gene, while caspase 12 apoptosis pathway mediated by endoplasmic reticulum stress plays an important role in podocyte damage in diabetic nephropathy. Where, GRP78 and GRP94 are the key molecular chaperones of the endoplasmic reticulum. Occurrence of endoplasmic reticulum stress can increase the GRP78 and GRP94 levels and promote the incidence of diseases. Caspase 12 belongs to the caspase family member on the outer membrane of the endoplasmic reticulum. Occurrence of endoplasmic reticulum stress can promote caspase 12 expression and cause the expression of downstream caspase apoptotic molecules and then induce cell apoptosis (Wu *et al*., 2019; Shang *et al*., 2015). The results of this study show that high glucose can increase the apoptosis rate and GRP78, GRP94, caspase 12 protein levels in mouse podocytes, and reduce the Nephrin protein level, while different doses of *D. sophia* can significantly reduce the apoptosis rate, GRP78, GRP94, caspase 12 protein levels and increase nephrin protein levels of high glucose-induced mouse podocytes. It suggests that high glucose stimulation induces endoplasmic reticulum stress in mouse podocytes and promotes cell apoptosis. On the other hand, the GRP78, GRP94, and caspase 12 protein levels were reduced under the action of *D. sophia*, indicating that the endoplasmic reticulum stress-mediated caspase 12 apoptosis pathway can be inhibited by *D. sophia*. In addition, *D. sophia* can promote the expression of the podocyte marker protein Nephrin and reduce the apoptosis of high glucose-induced podocytes, indicating that *D. sophia* can inhibit apoptosis of high glucose-induced mouse podocytes and then reduce podocyte damage in diabetic nephropathy by inhibiting endoplasmic reticulum stress.

Down-regulation of KCNQ1OT1 expression can reduce myocardial ischemia-reperfusion injury after acute myocardial infarction (Li *et al*., 2017). KCNQ1OT1 has elevated expression level in diabetic cardiomyopathy and can promote cardiomyocyte apoptosis (Yang *et al*., 2018b). Silencing the expression of KCNQ1OT1 can inhibit the development of diabetic cardiomyopathy (Yang *et al*., 2018a). The results of this study show that high glucose can increase the expression level of KCNQ1OT1 in mouse podocytes. Different doses of *D. sophia* can significantly reduce the expression level of KCNQ1OT1, and there is significant decrease with the increase of drug dosage, suggesting that *D. sophia* may play a role by reducing KCNQ1OT1 expression level. Further analysis of this study reveals that KCNQ1OT1 expression inhibition can enhance the proliferation of high glucose- induced mouse podocytes and inhibit its cell apoptosis and endoplasmic reticulum stress, suggesting that KCNQ1OT1 expression inhibition can reduce podocyte damage in high glucose-induced mouse. At the same time, high glucose-induced mouse podocytes were jointly treated with *D. sophia* and KCNQ1OT1 overexpression. The results indicate that the cell proliferation ability is significantly reduced, while the apoptosis rate and endoplasmic reticulum stress increase significantly, suggesting that KCNQ1OT1 overexpression can reduce the effect of *D. sophia* on the proliferation, apoptosis and endoplasmic reticulum stress of high glucose-induced mouse podocytes.

In summary, by down-regulating KCNQ1OT1, *D. sophia* enhances the proliferation of high glucose-induced mouse podocytes, inhibits apoptosis, endoplasmic reticulum stress, and thereby reduces podocyte damage in diabetic nephropathy. KCNQ1OT1 may act as a potential target for diabetic nephropathy treatment with *D. sophia*, but the downstream genes and related signaling pathways demand further exploration.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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