**ABSTRACT**

Diabetic retinopathy (DR) is the leading cause of decreased vision and blindness globally. The aim of this study was to understand the role of physcion 8-O-β-glucopyranoside (PG) in high glucose (HG)-induced DR and to investigate whether lncRNA NORAD/miR-125/STAT3 signalling was the underlying mechanism involved in DR. To this end, the serum levels of NORAD, miR-125, and STAT3 were determined in patients with DR. The APRE-19 cells were subjected to HG treatment to construct the cell model of DR. HG-disposed APRE-19 cell injury was assessed by detecting cell viability, apoptosis, concentrations of pro-inflammatory cytokines including TNF-α and IL-1β, and ROS generation. Moreover, the effect of PG on HG-disposed APRE-19 cell injury was investigated. NORAD was then overexpressed to investigate the combined effects of NORAD overexpression and PG on HG-disposed APRE-19 cell injury. Furthermore, the regulatory relationship between NORAD and miR-125 as well as miR-125 and STAT3 was investigated. The expression levels of NORAD and STAT3 were significantly increased in the serum of DR patients, while the miR-125 expression was decreased. The HG treatment-induced injury to APRE-19 cells, which were alleviated by PG treatment. Moreover, PG alleviated HG-disposed injury to ARPE-19 cells by decreasing NORAD. NORAD negatively regulated miR-125 expression and the combined effects of NORAD and PG on HG-disposed ARPE-19 cell injury were reversed by miR-125 overexpression. Furthermore, STAT3 was confirmed as a target gene of miR-125. Our results show that PG exerts protective roles in HG-disposed DR via regulating lncRNA NORAD/miR-125/STAT3 signalling. NORAD/miR-125/STAT3 axis may provide a novel perspective for target therapy of DR.

**ARTICLE HISTORY**

Received 1 September 2019
Revised 17 November 2019
Accepted 2 December 2019

**KEYWORDS**

Diabetic retinopathy; physcion 8-O-β-glucopyranoside; NORAD; miR-125; STAT3

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**Introduction**

Diabetic retinopathy (DR) is fast becoming a familiar microvascular complication of diabetes which is characterized by the loss of capillary pericytes and thickening of the basement membrane [1]. DR is the leading cause of decreased vision and blindness worldwide [2]. Current treatments for DR include vitreous surgical techniques and anti-vascular endothelial growth factors such as bevacizumab, and laser photocoagulation [3]. Nevertheless, the treatments are always associated with significant adverse effects and the prognosis of patients with DR remains poor, especially those who are in the advanced stage. To improve the clinical outcomes of DR, it is still imperative to develop effective treatments based on DR pathogenesis.

*Rumex japonicus* Houtt. is a traditional Chinese herbal medicine used for centuries for its antimicrobial and anti-inflammatory properties [4,5]. Physcion 8-O-β-glucopyranoside (PG) is the most active ingredient of this plant and has been shown to display anti-tumour and anti-inflammatory properties. It is effective against various cancers, including breast cancer [6], glioblastoma [7], hepatocellular carcinoma [8], oral squamous cell carcinoma [9], and osteosarcoma [10]. Moreover, PG extracted from *Polygonum cuspidatum* is shown to exert anti-proliferative and anti-inflammatory impacts on MH7A rheumatoid arthritis-derived fibroblast-like synoviocytes [11]. However, it is largely unknown whether PG exerts a protective effect on DR, let alone the underlying mechanism involved.

Long non-coding RNAs (lncRNAs) are RNA transcripts longer than 200 nucleotides which have been pointed out to be key regulators in various physiological and pathological processes [12–14]. Moreover, aberrant expressions of lncRNAs are shown to be pivotal in the DR process [15–19]. Recently, a novel cytoplasmic lncRNA, non-coding RNA activated by DNA damage (NORAD) has been shown to function as an onco-gene in various cancers, for example, gastric cancer [20], pancreatic cancer [21] and oesophageal squamous cell carcinoma [22]. However, the role of NORAD in DR has not been reported. Previously, Yang et al. proved that NORAD/miR-608/STAT3 axis in carcinostasis effects the physcion 8-O-b-glucopyranoside on ovarian cancer cells [23], implying the near correlation between miR-608 and NORAD. Currently, few reports were focussed on miR-608 in DR. To the contrary, miR-125 was found in DR in the HG-disposed condition, and it can be associated with cell apoptosis under HG-disposed condition [24]. Thereby, miR-125 was chosen in this study for deep exploration.
In this study, HG-predisposed APRE-19 cell injury was assessed by detecting cell viability, apoptosis, concentrations of pro-inflammatory cytokines (TNF-α and IL-1β) and ROS generation. Moreover, the impact of PG on HG-disposed APRE-19 cell injury was investigated. NORAD was overexpressed to investigate the combined effects of NORAD overexpression and PG on HG-disposed APRE-19 cell injury. Furthermore, the levels of NORAD, miR-125, and STAT3 in the serum of DR patients, and the regulatory relationships between NORAD and miR-125 as well as miR-125 and STAT3 were investigated to elucidate the regulatory mechanism of PG. Besides, the levels of NORAD, miR-125, and STAT3 in the serum of DR patients were determined. Our findings will provide a novel perspective for target therapy of DR.

### Materials and methods

#### Participants

The clinical study was approved by the local ethics committee and was conducted in accordance with the provisions of the Declaration of Helsinki. Written informed consent was obtained from all participants before enrolment.

The participants were consecutively recruited between May 2017 and May 2018 and were divided into three groups based on the diabetic retinopathy disease severity scale [25]: healthy control (HC), no diabetic retinopathy (NDR), and DR groups. The HC group included healthy participants, 20 males and 12 females, with an average age of 43.5 ± 6.6 years. The NDR group included 19 male and 13 female participants with type 2 diabetes (T1D) patients without DR, with an average age of 45.1 ± 5.6 years; while the DR group included 20 males and 12 females, with an average age of 44.8 ± 6.8 years. Using standardized protocols, anthropometric and biochemical indicators, including body mass index, systolic/diastolic blood pressure, and liver and kidney functions of the three groups were not statistically significant. Individuals with cardiovascular events, renal impairment, hepatic insufficiency, infectious diseases, or other severe systemic diseases, as well as patients during pregnancy and postpartum in the previous three months, were excluded. All individuals underwent ophthalmologic assessments such as visual function, ocular anterior segment, and posterior segment.

#### Serum samples

The peripheral venous blood was collected from participants of the three groups, followed by centrifugation to collect the serum samples. Afterwards, the serum supernatants were aliquoted into RNase-free microcentrifuge tubes and stored at −80 °C.

#### RNA extraction and quantitative PCR (qPCR)

Total RNA was extracted using Trizol Reagent (Life Technologies, Carlsbad, CA, USA) according to manufacturer’s protocol, followed by determination of RNA concentration and purity using a SmartSpec Plus spectrophotometer (Bio-Rad, Hercules, CA, USA). Reverse transcription into cDNA was then performed using the Omniscript RT Kit (Qiagen, Hilden, Germany). Real-time qPCRs for analysing the expression of NORAD and STAT3 were performed using a GoTaq 2-Step RT-qPCR System (Promega, Madison, WI, USA), and β-actin and U6 were used as an internal standard. Real-time qPCRs for detecting miR-125 expression was conducted using miScript II RT Kits (Qiagen, Hilden, Germany), miScript SYBR Green PCR Kits (Qiagen) and miScript Primer Assays, and U6 was used as the reference. All qPCRs were carried out using Mx3005P QPCR System (Stratagene, La Jolla, CA, USA). The relative quantification of gene expression was performed using the 2−ΔΔCT method. Primers used in this study are shown in Table 1.

#### Cell culture and treatment

The human retinal pigment epithelium cell line ARPE-19 (ATCC, Manntissa, VA) was cultured in Dulbecco’s modified essential medium/Ham’s F12 medium (DMEM/F12, Gibco, Grand Island, NY) containing 10% heat-inactivated foetal bovine serum (FBS, Gibco), 100 mg/mL of streptomycin, and 100 U/mL of penicillin, and then maintained at 37 °C in a humidified incubator with 5% CO2. The culture medium was replaced with fresh medium every other day.

To establish the DR cell model, APRE-19 cells were exposed to HG (C2H2O10 Figure 1(A), CAS: 26160-54-8) treatment. Briefly, ARPE-19 cells were seeded in 6-well culture dishes with 5.5 mM d-glucose (normal control) condition until 70–80% confluent. Subsequently, cells were incubated with

### Table 1. Primers used in this study.

| Name       | Sense (5’–3’) | Antisense (5’–3’) |
|------------|---------------|-------------------|
| STAT3      | CTTGGAAGCTGACCCAGGATG | TCCCAATGCTGATCAATGATC |
| STAT3 Mut  | GAAGGCAGGCTCTCTCTTG | AGGCTGCAACGCACCTGACCC |
| STAT2 Wt   | CGGCTAGTAAATTAGGTAATG | CCAAGCTTTTGTGCTGAGAAAGAAG |
| NORAD      | AGGCAAGTCCGAGAAAGCGA | TGGGCTTCATTCCACAGGCAAA |
| Bcl-2      | CTTGCTGAACTCTAGCCAGG | GTAGCACTGACTACAGTG |
| Bax        | ATGGAGGATTCACAGTCGGA | TGGGGCCTGACTACAGTG |
| Caspase-3  | TGCCCTTTATTGGCAAGATG | TGGTTACCTGCTGCTTAC |
| Caspase-9  | GCTCTCCITTGTATTCCCTC | CGGCTTGGGCAACTGCTTAC |
| β-actin    | CTCACACTGGCTGCTTACG | CGGCTTTGACCTCAGGTC |
| miR-125 mimic | UCCUCUGAACCCUACUUGUGA | UUUUCGCAAGGUGUUCGUGT |
| NC mimic   | UUCUCUGAACCCUACUUGUGA | UUUUCGCAAGGUGUUCGUGT |
| miR-125 inhibitor | CACCCCTAAGTCCGAGTCCGGCGGCAGAGTGG | CAGTGCAGGGTCCGAGGT |
| NC inhibitor | CCGGGACCGCTCGTCAGCGGAGAATACTG | AAGGGTTCGAGGAGG |
| miR-125     | GCUCUCUGAGACCUAAC | UUCUCUGACCUAAC |
| U6         | GGCTTACAGAATTGGCTGTCAT | UUCUCUGACCCUACGAG |

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In this study, HG-predisposed APRE-19 cell injury was assessed by detecting cell viability, apoptosis, concentrations of pro-inflammatory cytokines (TNF-α and IL-1β) and ROS generation. Moreover, the impact of PG on HG-disposed APRE-19 cell injury was investigated.
serum-free DMEM/F-12 for 24 h and exposed to different concentrations of D-glucose (10, 30, 50, 70 and 200 mM, respectively) for 48 h in 37°C in a humidified incubator with 5% CO2.

Moreover, to detect whether PG exerted inhibitory effects in DR, APRE-19 cells were treated with various concentrations of PG (0, 0.25, 0.5, 1, 1.5 and 2 µM, respectively, Chengdu Xunchen Biological Technology Co., Ltd., Chengdu, China) for 48 h.

Cell transfection
The pc-NORAD, miR-125 mimics, miR-125 inhibitor or their corresponding controls were synthesized by GenePharma Co. (Shanghai, China). Cell transfections were carried out using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) as per manufacturer’s recommendations.

Cell viability assay
Cell viability was detected with a CCK-8 assay (Roche, Mannheim, Germany). Briefly, APRE-19 cells were plated in 96-well microplates at a density of 2 x 10^3 cells/well. After different treatments, APRE-19 cells were cultured for another 48 h at 37°C. Then CCK-8 solution was added into each well, and the cells were incubated for 3 h at 37°C in a humidified incubator with 5% CO2. The absorbance was measured at 450 nm using a microplate reader (MTP-800; CORONA, Tokyo, Japan) with blank as control.

Apoptosis assay
Cell apoptosis was assessed using an Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kit (Beijing Biosea Biotechnology, Beijing, China). After different treatments, ARPE-19 cells were washed three times with phosphate-buffered saline (PBS, Gibco), and then stained with 5 µL PI/FITC-Annexin V for 30 min under the dark condition. The percentage of apoptotic cells was detected using flow cytometric analysis (Beckman Coulter, Fullerton, CA, USA). The obtained data were analysed using FlowJo software (Treestar, Ashland, OR, USA).

Western blot
After different treatments, ARPE-19 cells were washed with ice-cold PBS and then lysed using a nuclear and cytoplasmic protein extraction kit (Beyotime, Haimen, China). Protein samples were separated with SDS-PAGE and then transferred to a polyvinylidene difluoride membrane (Millipore, USA). After blocking with 5% non-fat dry milk for 1 h at room temperature, primary antibodies against β-actin, Bax, Bcl-2, pro-caspase-3, cleaved-caspase-3, pro-caspase-9, cleaved-caspase-9, and STAT3 (1:1000, Abcam, Cambridge, UK) were used to incubate the membranes overnight at 4°C. After further incubation with the corresponding horseradish peroxidase-linked secondary antibodies (1:5000, Abcam) for 1 h at
room temperature, the protein signals were visualized using a standard enhanced chemiluminescence (ECL) reagent (Amersham Biosciences, NJ, USA).

**Enzyme-linked immunosorbent assay (ELISA)**

After different treatments, cell culture supernatants were collected. Based on the manufacturer's instruction, the concentration of pro-inflammatory cytokines including TNF-α and IL-1β was detected using high-sensitive ELISA kits (eBioscience, CA, USA).

**Reactive oxygen species (ROS) detection**

DCFH-DA (Sigma, St Louis, MO, USA) molecular probes were used to detect the ROS after different treatments. Briefly, ARPE-19 cells were incubated with 10 mM DCFH-DA for 30 min at 37 °C and resuspended in PBS at a density of 1 × 10⁶ cells/mL. At excitation and emission wavelengths of 488 and 525 nm, the cells were monitored using flow cytometry. Untreated cells served as control. The ROS generation was detected using a FACScan flow cytometer and CellQuest software (Becton Dickinson).

**Dual-luciferase reporter assay**

The full-length 3'-UTR segments of STAT3 mRNA containing the miR-125 binding site was amplified using PCR and inserted into the pGL3vector (Promega, WI) to construct the wild-type reporter vectors, namely, pGL3-STAT3-WT. The mutated-type reporter vector, namely, pGL3-STAT3-MUT, was also constructed with point mutations in the seed sequence that was synthesized using a site-directed mutagenesis kit (Stratagene, CA). Then, 1 μg of the above-constructed reporter vectors (pGL3-STAT3-WT or pGL3-STAT3-MUT), 50 pmol of miR-125 mimic (or mimic NC), and 1 μg of a Renilla luciferase expression construct pRL-TK (Promega, WI) were cotransfected into cells using Lipofectamine 2000. At 36 h of transfection, luciferase activity was tested using the dual luciferase assay system (Promega, WI). Renilla luciferase activity was used as a control.

**Statistical analysis**

Each experiment was repeated at least three times. Data were expressed as mean ± standard deviation (SD), and statistical analyses were performed using SPSS version 10.0. The differences between different groups were analysed using one-way ANOVA, followed by the Student–Newman–Keuls test for multiple comparisons. A statistically significant result was obtained when p < .05.

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**Results**

**NORAD/miR-125/STAT3 were abnormally expressed in serum of patients with DR**

To identify the impact of NORAD/miR-125/STAT3 axis in DR, the expression of NORAD, miR-125, and STAT3 in HC, NDR, and DR groups was detected, respectively. Also, NORAD and STAT3 were both significantly increased in the NDR and DR groups than in the HC group, while the miR-125 expression was remarkably decreased (p < .05, Figure 1(B–D)). Moreover, the expression levels of NORAD and STAT3 in the DR group were also dramatically higher than in the NDR group, while miR-125 expression in the DR group was obviously lower than in the NDR group (p < .05, Figure 1(B–D)).

**HG treatment-induced ARPE-19 cells injury**

The APRE-19 cell damages produced from HG treatment were detected to verify whether the DR cell model was successfully established. The result of CCK8 assay uncovered that HG treatment significantly decreased APRE-19 cell viability in a dose-dependent way relative to control (p < .05, Figure 2(A)). Based on this result, 50 mM of α-glucose was used for HG treatment in the following experiments. Consequently, HG disposals resulted in a significant increase in cell apoptosis (p < .01, Figure 2(B)), higher concentrations of pro-inflammatory cytokines including TNF-α and IL-1β (p < .001, Figure 2(C)), and ROS generation (p < .01, Figure 2(D)) in APRE-19 cells. These data indicated that HG treatment caused APRE-19 cell injury.

**PG decreased HG-disposed ARPE-19 cell injury**

To investigate the role of PG in DR, we detected the effects of PG on HG-disposed ARPE-19 cell injury. We first detected the optimal concentration of PG using the CCK8 assay. The results showed that APRE-19 cell viability was gradually decreased with the increase of PG treatments until 1.5 μM and significantly decreased after 2 μM of PG (Figure 3(A)). Thus, 1.5 μM of PG treatment was used for subsequent experiments. Then, the results showed that PG treatment significantly improved HG-disposed ARPE-19 cell injury by increasing cell viability (p < .05, Figure 3(B)) and decreasing cell apoptosis (p < .01, Figure 3(C)), concentrations of pro-inflammatory cytokines including TNF-α and IL-1β (p < .01, Figure 3(D)), and ROS generation (p < .05, Figure 3(E)).

**PG alleviated HG-disposed ARPE-19 cell damage by decreasing NORAD**

The protective mechanism of PG on HG-disposed ARPE-19 cell injury was further investigated. PG treatment dramatically suppressed the NORAD expression in HG-treated ARPE-19 cells in a dose-dependent way (p < .05, Figure 4(A)). Subsequently, the NORAD expression was remarkably overexpressed in ARPE-19 cells by transfection with pc-NORAD compared to transfection with pcDNA3.1 (p < .001, Figure 4(B)). Moreover, in comparison to HG + PG + pcDNA3.1 transfection,
overexpression of NORAD in the HG + PG + pc-NORAD group dramatically changed the influence of PG treatment on HG-disposed ARPE-19 cell injury by decreasing cell viability ($p < .01$, Figure 4(C)) and promoting cell apoptosis ($p < .01$, Figure 4(D)), concentrations of pro-inflammatory cytokines including TNF-α and IL-1β ($p < .01$, Figure 4(E)), and ROS

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**Figure 2.** High glucose (HG) treatment induced ARPE-19 cells injury. (A) Cell viability after HG treatment. (B) Cell apoptosis after HG treatment and the expression levels of apoptosis-related proteins. (C) Concentrations of pro-inflammatory cytokines including TNF-α and IL-1β after HG treatment. (D) ROS generation after HG treatment. All experiments were repeated three times and data are expressed as mean ± SD. *$p < .05$, **$p < .01$, and ***$p < .001$.

**Figure 3.** Physcion 8-O-β-glucopyranoside (PG) decreased HG-disposed ARPE-19 cell injury. (A) Cell viability after different concentration of PG treatment. (B) Cell viability after HG and PG treatments. (C) Cell apoptosis after HG and PG treatments and the expression levels of apoptosis-related proteins. (D) Concentrations of pro-inflammatory cytokines including TNF-α and IL-1β after HG and PG treatments; (E) ROS generation after HG and PG treatments. All experiments were repeated three times and data are expressed as mean ± SD. *$p < .05$, **$p < .01$, and ***$p < .001$. 

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generation \( (p < .01, \text{Figure 4(F)}). \) These data indicated that PG alleviated HG-disposed ARPE-19 cell injury by decreasing NORAD.

**NORAD negatively regulated miR-125 expression and the combined effects of NORAD and PG on HG-disposed ARPE-19 cell injury were reversed by miR-125 overexpression**

Increasing studies revealed that lncRNAs could competitively bind to miRNA binding sites on the identity of competing endogenous RNAs (ceRNAs) or “RNA sponges”, thus modulating the regulatory effect of miRNAs on target mRNAs \([26,27]\). As presented in Figure 5(A), miR-125 expression was significantly decreased in the pc-NORAD group relative to that in the pcDNA3.1 group \( (p < .01) \). Moreover, the miR-125 expression was dramatically decreased in HG-disposed ARPE-19 cells \( (p < .01) \), which was alleviated after PG treatment \( (p < .05) \) (Figure 5(B)). Then, miR-125 was successfully up-expressed/inhibited after transfecting miR-125 mimic/miR-125 inhibitor, respectively, compared with transfection with their respective NC \( (p < .001, \text{Figure 5(C)}) \). In comparison to the HG + PG + pc-NORAD + mimic NG group, overexpression of miR-125 concurrently in the HG + PG + pc-NORAD + miR-125 mimic group significantly reversed the combined effects of NORAD overexpression and PG treatment on HG-disposed ARPE-19 cell injury by promoting cell viability \( (p < .01, \text{Figure 5(D)}) \) and decreasing cell apoptosis \( (p < .01, \text{Figure 5(E)}) \), concentrations of pro-inflammatory cytokines including TNF-\( \alpha \) and IL-1\( \beta \) \( (p < .01, \text{Figure 5(F)}) \), and ROS generation \( (p < .01, \text{Figure 5(G)}) \).

**STAT3 was verified to be targeted by miR-125**

To further investigate the downstream regulatory association between miR-125 and its target, we used TargetScan online tool for further investigation, and STAT3 was identified as a potential target gene of miR-125 (http://www.targetscan.org/cgi-bin/targetscan/vert_71/view, Figure 6(A)). Then, the results showed that the mRNA and protein expression levels of STAT3 were dramatically decreased in the miR-125 group but remarkably enhanced in the miR-125 inhibitor group, than those in the NC group \( (p < .01, \text{Figure 6(B)}) \). Subsequently, the luciferase reporter test revealed that the luciferase activity of STAT3-WT was prominently retrained after cotransfection with miR-125 mimic \( (p < .05, \text{Figure 6(C)}) \). These findings displayed that STAT3 was targeted by miR-125. Moreover, STAT3 level was very high in HG-disposed ARPE-19 cells \( (p < .01) \), which was obviously decreased after PG treatment \( (p < .05) \) (Figure 6(D)).

**Verification test of NORAD/miR-125/STAT3 in regulating DR under PG**

Finally, we overexpressed STAT3 in cells transfected with pc-NORAD, miR-125 mimic and their corresponding control.
vectors to test the discovery of NORAD/miR-125/STAT3 in DR under PG condition (Figure 7). STAT3 was highly expressed in cells with pc-STAT3 transfection than that in the controls \( (p < .001, \text{Figure 7(A)} \) ). As expected, cell viability or apoptosis were respectively decreased or enhanced by the transfected pc-STAT3 in HG-disposed cells transfected with pc-NORAD and miR-125 mimic under PG condition \( (p < .001, \text{Figure 7(B)}, p < .01, \text{Figure 7(C)} \). Also, the flow cytometry results and the apoptotic proteins also performed the corresponding tendency in each group \( (p < .001, \text{Figure 7(D)} \). Besides, we found that cell injury was enhanced after pc-STAT3 transfection along with pc-NORAD and miR-125 overexpression treatments; concentrations of pro-inflammatory cytokines including TNF-\(\alpha\) and IL-1\(\beta\), and ROS generation, which were alleviated by PG treatment. These data suggest that PG may exert protective roles in HG-disposed DR.

Discussion

DR is a chronic and serious eye complication associated with diabetes. Elucidation of key mechanisms underlying DR and the exploration of effective treatments of this disease are needed. In this study, HG treatment-induced ARPE-19 cell injury, as proved by a remarkable decrease in cell viability and significant increases in cell apoptosis, concentrations of pro-inflammatory cytokines including TNF-\(\alpha\) and IL-1\(\beta\), and ROS generation, which were alleviated by PG treatment. Furthermore, STAT3 was verified as a target
gene of miR-125. The expression levels of NORAD and STAT3 were significantly increased in serum of DR patients, while the miR-125 expression was decreased. These data imply the possible protective mechanism of PG against DR and merit further discussion.

NORAD has been shown to promote the development of various cancers [20–22] but the role of NORAD in DR has not been reported. In this study, NORAD and STAT3 were significantly increased in serum of DR patients, and PG alleviated HG-disposed ARPE-19 cell injury by decreasing NORAD. We thus speculate that NORAD may be a drug target of PG to protect against DR. Moreover, the combined effects of NORAD and PG on HG-disposed ARPE-19 cell injury were reversed by miR-125 overexpression. It can, therefore, be speculated that miR-125 may be a downstream target of NORAD to mediate the protective role of PG in DR.

Furthermore, STAT3 was confirmed as a target gene of miR-125 in this study. STAT3 can be assessed not only in serum but also inside the cells. Although intracellular STAT3 was usually used for indexing the significance in diseases, there are many serum STAT3 usages in various kinds of diseases, such as tumour, immune-related disease, child skin disease, and endocrine-related disease. Wang and his colleague analysed the serum level of STAT3 in NSCLC [34] for enhancing its clinical significance, STAT3 signal was found to be abnormal in herpes zoster indexed by the serum STAT3 [35]. Lyu et al. proved that STAT3 signal was pivotal in atopi

![Figure 6](image_url)

**Figure 6.** STAT3 was verified as a target gene of miR-125. (A) The predicted binding sequence between miR-125 and STAT3. (B) The expression levels of p-STAT3/STAT3 mRNA and protein after transfection with miR-125 mimic, miR-125 inhibitor, and their respective NC. (C) Luciferase reporter assay showed that miR-125 could interact with STAT3-WT. (D) The STAT3 expression after HG and PG treatments. All experiments were repeated three times and data are expressed as mean ± SD. *p < .05 and **p < .01.

| Position 1532-1539 of STAT3 3' UTR | Predicted consequent pairing of target region (top) and miRNA (bottom) | Site | Context++ score | Context++ score percentile |
|-----------------------------------|-------------------------------------------------|-----|----------------|--------------------------|
| has-miR-125a-5p                   | 5' ... GGGCTGCGGAGGAUGAAU ... 3'                | 8mer | -0.31          | 96                       |
| has-miR-125b-5p                   | 5' ... GGGCTGCGGAGGAUGAAU ... 3'                | 8mer | -0.34          | 96                       |
dermatitis by detecting the serum STAT3 [36]. Similarly in rheumatoid arthritis, STAT3 in serum was abnormally expressed and thereby acted as an important index for rheumatoid arthritis with different mechanisms [37]. All of these findings proved that the serum STAT3 is also important in indexing many diseases. Plus the primer sequences of STAT3 used in this study (Table 1), we reasonable analysed the expression level of STAT3 in serum for exploring the correlation between miR-125 and STAT3 in DR. It has been shown that STAT3 plays a leading role in tumour inflammation and immunity by regulating numerous oncogenic signalling pathways promoting pro-oncogenic inflammatory pathways, such as nuclear factor-κB and Janus kinase pathways [38]. Moreover, Xu et al. demonstrated that STAT3 is up-regulated in DR-affected tissues and primary retinal vascular endothelial cells under high glucose (HG) conditions [39]. Increasing studies also showed that STAT3 could influence endothelial function in DR [40,41]. The results of our study also revealed that STAT3 expression was significantly increased in HG-treated ARPE-19 cells, which was obviously decreased after PG treatment. Given the key role of STAT3 in DR, we believe that STAT3 may also be a drug target of PG in DR. However, we did not perform more functional experiments to verify the role of STAT3 in HG-disposed ARPE-19 cell injury. Further studies are needed to confirm our findings.

Taken together, our results reveal that PG exerts a protective role in HG-disposed DR via regulating IncRNA NORAD/miR-125/STAT3 signalling. NORAD/miR-125/STAT3 axis may provide a novel perspective for target therapy of DR. Our research team possibly will plan to discover the correlation between miR-608 and IncRNA NORAD in the next work.

Disclosure statement

No potential conflict of interest was reported by the authors.

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