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Long non-coding RNA LINC00616 promotes ferroptosis of periodontal ligament stem cells via the microRNA-370 / transferrin receptor axis

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ABSTRACT

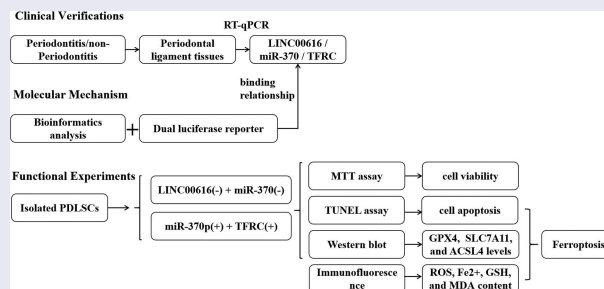
This study was designed to explore the role of lncRNA LINC00616 in the regulation of periodontitis. Cellular functions were measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assays. The content of reactive oxygen species, Fe²⁺, glutathione, and malondialdehyde were measured to determine ferroptosis in *Porphyromonas gingivalis* lipopolysaccharide (LPS-PG) treated periodontal ligament stem cells (PDLSCs), as well as expression of glutathione peroxidase 4 (GPX4), solute carrier family 7 member 11, and acyl-CoA synthetase long-chain family member 4 proteins mRNA and miRNA levels were measured by quantitative reverse-transcription polymerase chain reaction (qRT-PCR). Western blot analysis was performed to assess protein expression. Targeting relationships were predicted using StarBase and TargetScan and verified by a dual luciferase reporter assay. The lncRNA LINC00616 was upregulated in periodontitis ligament tissues of patients with periodontitis and in PDLSCs treated with LPS-PG. Inhibition of LINC00616 promoted cell viability and suppressed ferroptosis of PDLSCs. miR-370 was verified to be a target of LINC00616, and suppressed miR-370 reversed the effects of LINC00616 knockdown on cell viability and ferroptosis in PDLSCs. Additionally, miR-370 targeting the transferrin receptor protein and upregulated transferrin receptor (TFRC) abolished the effects of overexpressed miR-370 on cell viability and ferroptosis of PDLSCs. LINC00616 acted as a competitive endogenous RNA (ceRNA) to promote ferroptosis of PDLSCs via the miR-370/TFRC axis. Therefore, LINC00616 knockdown may be a promising therapeutic strategy for periodontitis.

ARTICLE HISTORY

Received 31 March 2022
Revised 5 May 2022
Accepted 8 May 2022



KEYWORDS

Periodontitis; bone marrow mesenchymal stem cells; LINC00616; ferroptosis; miR-370; TFRC




Highlights

- lncRNA LINC00616 was upregulated in periodontitis.
- Inhibition of LINC00616 promoted cell viability and suppressed ferroptosis of PDLSCs.
- LINC00616 acted as a ceRNA to promote ferroptosis of PDLSCs via miR-370/TFRC axis.
- LINC00616 knockdown may be a promising therapeutic strategy for periodontitis.

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 Supplemental data for this article can be accessed online at <https://doi.org/10.1080/21655979.2022.2076508>

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Introduction

Chronic periodontitis is a common chronic inflammatory disease with oral pathogenic microorganisms as an initiating factor and the formation of the periodontal pocket and resorption of the alveolar bone as the main clinical features [1,2]. Current sequential periodontal therapy and guided tissue regeneration in the treatment of periodontitis have corresponding limitations and cannot achieve physiological and functional regeneration of periodontal tissue [3,4].

Ferroptosis is a regulated cell death process caused by a redox state disorder of the intracellular microenvironment controlled by glutathione peroxidase 4 (GPX4) due to intracellular iron overload [5], which is usually accompanied by an increase in reactive oxygen species (ROS) secretion, iron ions (Fe^{2+} and Fe^{3+}), and malondialdehyde (MDA), and a decrease in glutathione (GSH) secretion [6–8]. As we all know, ferroptosis regulates tumors and brain diseases [9–11]. Recently, researchers found that ferroptosis plays a regulatory role in inflammatory diseases, including periodontitis [12,13]. For example, lipopolysaccharide from *P. gingivalis* (LPS-PG), one major putative pathogen for periodontitis, may elicit mitochondrial dysfunction, high ROS production and oxidative stress [14]. Ferroptosis in periodontal ligament fibroblasts leads to development of periodontitis [13].

Long noncoding RNAs (lncRNAs) play a vital role in the initiation and progression of chronic periodontitis [15]. For example, microarray analysis revealed that 8925 lncRNAs were differentially expressed in periodontitis tissues compared with normal periodontal tissue, indicating that lncRNAs may play key roles in the course of periodontitis [16]. Furthermore, knockdown of lncRNA MEG3 inhibited the osteogenic differentiation of periodontal ligament stem cells (PDLSCs) in periodontitis [17]. Upregulation of lncRNA-01126 promoted the progress of periodontitis through the MEK/ERK signaling pathway [18]. Meanwhile, as one of the largest gene families, miRNAs are ncRNAs with a length of about 19–24 nucleic acids, which are widely found in eukaryotic cells [19]. The potential of miRNAs in biological processes and posttranscription confer regulatory

function in chronic periodontitis [20]. Furthermore, lncRNAs function as a new type of competitive endogenous RNA (ceRNA) regulator through sponging miRNAs [21,22]. LINC00616 is a novel lncRNA identified by microarray analyses between periimplantitis and patients with periodontitis [23].

However, the underlying molecular mechanisms of LINC00616 in PDLSCs are yet to be investigated further. Our study aimed to investigate the role of LINC00616 in the development of periodontitis.

Materials and methods

Patients

Forty patients diagnosed with periodontitis at the Eye Hospital of Hebei and 40 non-periodontitis participants were included in this study with the approval of the Ethics Committee of Eye Hospital of Hebei (No. 2020KY019). This study was carried out in accordance with the Declaration of Helsinki. The inclusion criteria are as follows: 1) Patients with periodontitis need to have teeth extracted or patients without periodontitis need to have teeth extracted by orthodontic surgery; 2) All subjects have signed their informed consent after being fully informed; 3) All participants were over 18 years old. 4. The participants were free of any known systemic diseases.

Periodontal ligament tissue

The tissue around the tooth was disinfected with 75% ethanol (Solarbio, China) and the samples were taken within 2 h after tooth extraction [24]. The root was rinsed 3–5 times with phosphate buffer solution (PBS; Solarbio, China) on a super clean table, and the periodontal ligament tissues were scraped in 1/3 of the root.

Cell culture and establishment of periodontitis cell model

The tissues of the periodontal ligaments were minced into small pieces and treated with collagenase type I digestion (1 mg/mL; Sigma-Aldrich, NJ, USA) at 37°C for 0.25 h. The cell suspensions were then filtered and cultured in DMEM (Gibco, California,

USA) containing 10% FBS (Gibco, California, USA), 2 mM L-glutamine (Gibco, California, USA), and 1% penicillin/streptomycin (Gibco, California, USA) [24]. Cells were digested after 14 days of incubation and single-cell-derived colony cultures were obtained using the limiting dilution technique. PDLSCs were obtained by dilution and passed five times.

The PDLSCs were divided into two groups, the control and LPS-PG groups. The final concentration of 1 µg/mL LPS-PG was added to the LPS-PG group [25–27]. Follow-up experiments were conducted after LPS-PG treatment 24 h later.

Cell transfection

si-LINC00616 (si-LINC00616 1#, si-LINC00616 2#), miR-370 mimic, miR-370 inhibitor, overexpressed transferrin receptor protein plasmids (oe-TFRC), and their negative controls (nc) were purchased from GenePharma (Shanghai, China). The PDLSCs (2×10^5 cells/well) were cultured in six-well plates. Cells were transfected into PDLSCs using Lipofectamine 3000 reagent (Life Technologies, California, USA) following the manufacturer's protocol [28].

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

qRT-PCR was used to detect miRNA and mRNA expression. Firstly, all RNA from tissues as well as PDLSCs were extracted using TRIzol reagent (Thermo Fisher Scientific, USA) and then dissolved in a solution without RNA enzyme. An ultraviolet spectrophotometer (NanoDrop, USA) was used to determine the ratio of A_{260}/A_{280} RNA between 1.8 and 2.0. Next, 1 µg of qualified RNA was used to synthesize cDNA by reverse transcription (SuperScript First-Strand Synthesis Kit; Invitrogen, USA). The SYBR real-time fluorescence quantitative PCR kit (Takara, Japan) was used for fluorescence quantitative PCR analysis, and Primer BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used for primer design [29]. Reaction conditions: 94°C, 4 min 94°C, 30s 56°C, 30s 72°C, 30s, 40 cycles. GAPDH was used as a housekeeping control for LINC00616 and mRNAs. U6 was used as an internal reference for miR-370. Data were analyzed using the $2^{-\Delta\Delta Ct}$

Table 1. Primer sequences.

Gene	Primer
DPP10-A1	5'-CAGCCCTGATAGGCAGTAGAC-3' 5'-TCATTGCCTTTGATCCTGCTGT-3'
RP11-1C8.7	5'-TGTTTTTCCCAAGTGGCAG-3' 5'-AGAGACATATCAGATCCCCAATT-3'
GSE61474_XLOC_022835	5'-ACTTTTATGTGAAGCCAGAGT-3' 5'-TCCTGGTTGTTGCGGCTT-3'
RP11-142A22.4	5'-CTCTCCTGATGGTTGCACGA-3' 5'-TGACAAAGCCAAGGCCAGAA-3'
G009665	5'-AGAACACCCCGATGACGGA-3' 5'-GGCATATTATGTACCCGGAAT-3'
MAFA-AS1	5'-GTGAATGAGGGCAGCTCT-3' 5'-CCCCAACTCCTCCACTTTC-3'
LINC00616	5'-TGCAGCAGGCCACTATTTGA-3' 5'-AGCCACTGAGATCAACTGCC-3'
G083982	5'-AGGTGAAGAGCACTACGAAGA-3' 5'-CTACTATGACGCCACATGCCT-3'
RP4-660H19.1	5'-TAGGACCCTCCAAGCCAGG-3' 5'-TTTCACAGGGAATGGTGCC-3'
TRHDE-AS1	5'-CCTGAGAGCTTACTGCCACA-3' 5'-CAACGCGCTCATCGAGAATG-3'
miR-370	5'-CTCAGCGCTGCTGGGGTG-3' 5'-ACCTCAAGAAGCAGTATTTCCAGG-3'
TFRC	5'-TCGTGAGGCTGGATCTCAAAA-3' 5'-CAATAGCCCAAGTAGCCAATCAT-3'
GAPDH	5'-AGCCACATCGCTCAGACAC-3' 5'-GCCCAATACGACCAATCC-3'
U6	5'-CTCGCTTCGGCAGCACA-3' 5'-AACGCTTACGAATTTGCGT-3'

relative expression method. All primer sequences were listed in Table 1. All experiments were repeated three times.

3-(4,5-dimethylthiazol-2-yl)-

2,5-diphenyltetrazolium bromide (MTT) assay

Cell viability was measured by the MTT assay. The PDLSCs were collected and 100 µL cell suspension (2×10^5 cells/mL) was inoculated into 96-well plates; 20 µL MTT solution (5 mg/mL; Thermo Fisher Scientific, USA) was added to each well and cultured for 4 h (37°C, 5% CO₂). The culture medium was then discarded, 150 µL DMSO was added to each well and vibrated at low speed for 10 min [30]. Finally, the OD490 nm values of each group were detected by a microplate analyzer (NanoDrop, USA). The experiment was repeated three times.

Determination of the content of ROS, Fe²⁺, GSH, and MDA

Levels of intracellular ROS, Fe²⁺, GSH, and MDA were measured separately using an oxidation-

sensitive fluorescent probe DCFH-DA (D6883; Sigma, USA) [31], iron assay kit (ab83366, Abcam, USA), glutathione assay kit (CS0260; Sigma, USA), and lipid peroxidation assay kit (ab118970, Abcam, USA), respectively, according to the manufacturer's instructions.

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay

The death of PDLSC was detected using a TUNEL detection kit (ab66110, Abcam, USA) according to the manufacturer's instructions [32]. PDLSCs were observed under a microscope (Nikon, Japan). Five fields were randomly selected under a 200-fold light microscope and the number of positive cells and total cells were counted.

Western blot assay

The protein expressions of GPX4, solute carrier family 7 member 11 (SLC7A11), and acyl-CoA

synthetase long-chain family member 4 (ACSL4) were detected by western blotting assay. Total protein samples were collected from the PDLSCs of each group and the protein concentration was determined by the BCA method (Sigma, USA). Proteins were resolved on 8% or 15% SDS-PAGE gels (100 V, 1.5–2 h) for separation and transferred to a polyvinylidene fluoride membrane. Primary antibodies (Abcam, USA) were added and incubated successively (overnight at 4°C, 12–16 h), and HRP labeled secondary antibody (at room temperature for 1 h) was added to avoid light for color development (31,460, Invitrogen, USA). The images were collected by an automatic gel imaging analyzer (BioRad, USA). All results were collected using Alpha image analysis software (Alpha Innotech, USA), and the absorbance value (A value) of the target band was analyzed and compared with the gray area value of GAPDH (ab8245, 1: 1000). The ratio of the two represents the relative expression level of the protein in the cell. The primary antibodies were as follows: anti-

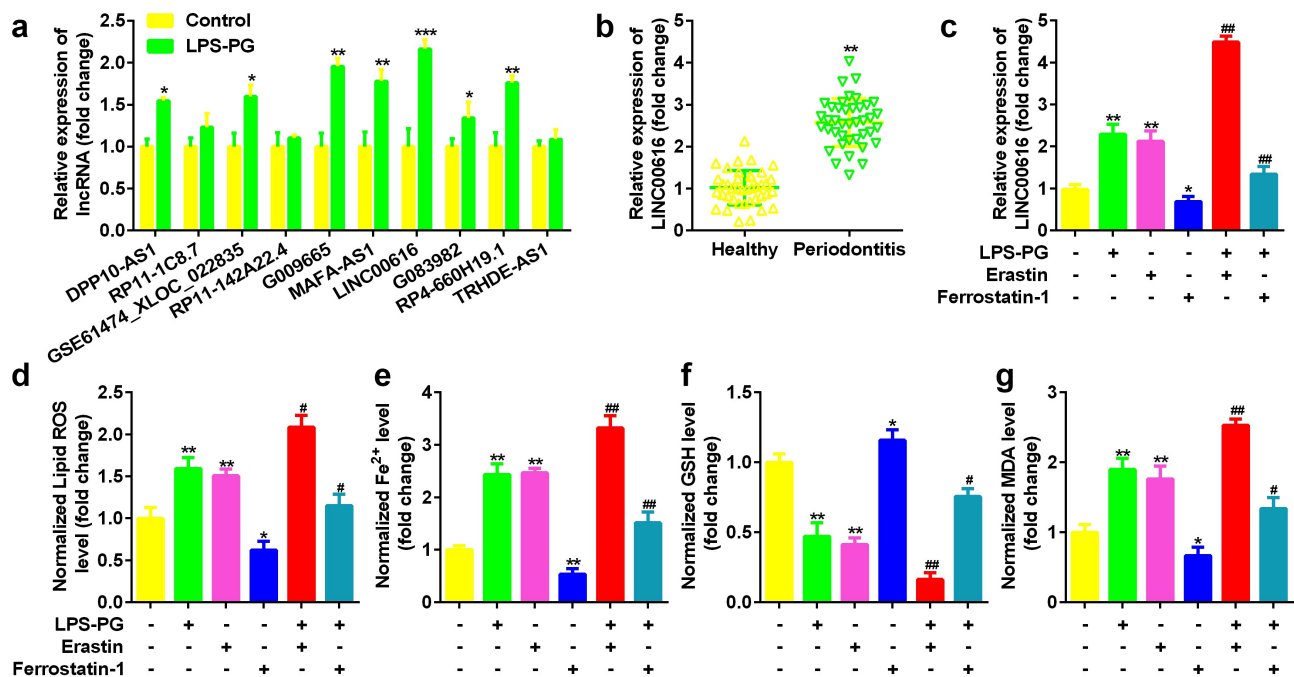


Figure 1. LINC00616 was highly expressed in LPS-PG-treated PDLSCs with ferroptosis. (a) Expression of 10 lncRNAs in PDLSCs before and after LPS-PG treatment measured with qRT-PCR. (b) qRT-PCR analyses of LINC00616 expression in patients with periodontitis. (c) qRT-PCR analyses of LINC00616 expression in PDLSCs. (d-g) Levels of ROS, Fe²⁺, GSH, and MDA in PDLSCs.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared to the control and healthy groups. # $P < 0.05$, ## $P < 0.01$, compared with the LPS-PG group.

GPX4 (ab125066, 1: 1000), anti-SLC7A11 (ab175186, 1: 3000), and anti-ACSL4 (ab205199, 1: 1000).

Dual-luciferase reporter assay

The wild type (wt) and mutant type (mut) of the 3-UTR region of the LINC00616 and TFRC luciferase reporter vectors were designed and synthesized by Guangzhou RiboBio Co., Ltd. (China). The PDLSCs were transfected with miR-370 mimic or nc mimic and wt or mut with LINC00616 or TFRC for 48 h. The PDLSCs were then lysed to detect luciferase activity using a luciferase reporter assay kit (K801-200; BioVision Tech, USA) [17]. Firefly luciferase activity was normalized to Renilla luciferase activity.

Statistical analysis

All statistical analyses were performed using GraphPad Prism version 8.3 software (GraphPad, USA). Data are presented as mean \pm standard deviation from three independent experiments. *P*-values are calculated with Student's *t*-test and one-way analysis of variance followed by Tukey's. A *P*-value less than 0.05 was considered statistically significant.

Results

LINC00616 was highly expressed in LPS-PG treated PDLSCs

Expression of 10 lncRNAs which have been identified to be aberrant expressed in periodontitis were measured in LPS-PG treated PDLSCs, among which LINC00616 was markedly upregulated (Figure 1a) [23]. Meanwhile, LINC00616 was also significantly overexpressed in periodontal ligament tissues in patients with periodontitis compared with normal controls (Figure 1b). Moreover, the expression level of LINC00616 was significantly increased by adding the ferroptosis activator erastin, while dramatically decreasing after treatment with the ferroptosis inhibitor Ferrostatin-1 (Figure 1c).

The level of GSH in LPS-PG and PDLSCs treated with erastin was significantly decreased and increased, respectively, in the Ferrostatin-1 group; however, ROS, Fe²⁺, and MDA levels were dramatically increased by LPS-PG and erastin and reduced by Ferrostatin-1 (Figure 1d-g).

Knockdown of LINC00616 promoted cell viability and inhibited ferroptosis of PDLSCs

Then, we evaluated cellular functions affected by LINC00616 to study the role of LINC00616 in PDLSCs. As shown in Figure 2a, the expression of LINC00616 decreased significantly after transfection of si-LINC00616 into PDLSCs, which was more remarkable in the si-lncLINC00616 1# group. Inhibition of LINC00616 significantly improved cell viability (Figure 2b) and reduced cell death (Figure 2g). Simultaneously, secretion of ROS, Fe²⁺, and MDA was suppressed, while the GSH content increased after LINC00616 was silenced (Figure 2c-f). Furthermore, ferroptosis-related proteins were detected. Treatment with LPS-PG decreased the expression of GPX4 and SLC7A11 and increased the expression of ACSL4, which was dramatically reversed by inhibition of LINC00616 (Figure 2h).

LINC00616 sponged miR-370 in PDLSCs

Next, the potential mechanism was investigated. The binding site between LINC00616 and miR-370 was predicted by the StarBase 3.0 online database (Figure 3a). The luciferase activity of the luciferase-labeled miR-370 mimic and the wild-type LINC00616 cotransfection groups was dramatically decreased compared with that of the nc mimic (Figure 3b). Inhibition of LINC00616 upregulated the expression of miR-370 in PDLSCs (Figure 3c). Furthermore, miR-370 expression was markedly suppressed in periodontal ligament tissues from patients with periodontitis and PDLSCs treated with LPS-PG (Figure 3d-e).

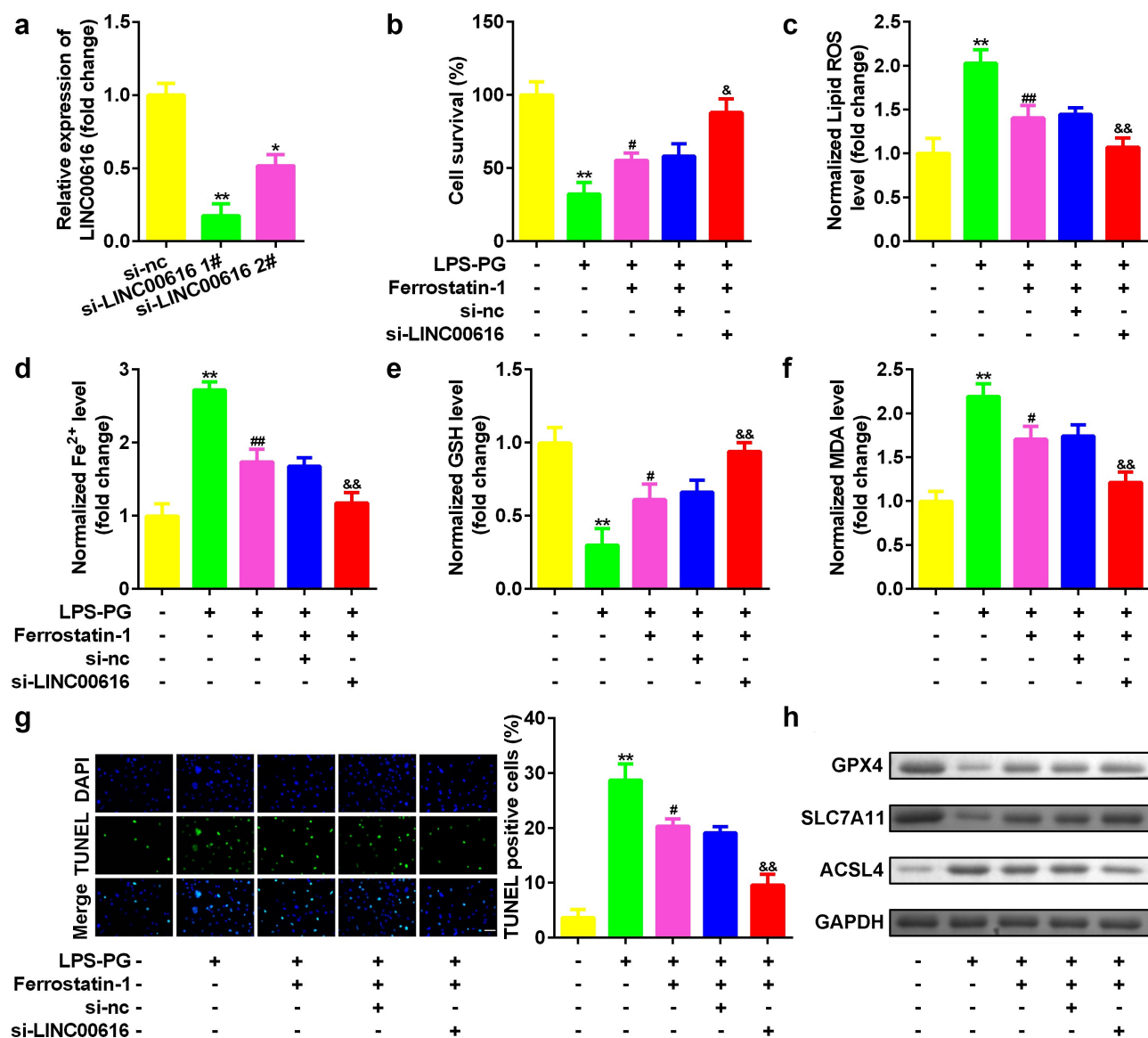


Figure 2. Knockdown of LINC00616 promoted cell viability and inhibited ferroptosis of PDLSCs. (a) LINC00616 expression levels were detected by qRT-PCR in PDLSCs after transfection. (b) Cell viability is measured using an MTT assay after transfection in PDLSCs treated with LPS-PG. (c-f) Levels of ROS, Fe²⁺, GSH, and MDA in PDLSCs treated with LPS-PG after transfection. (g) Images and quantized bar chart of TUNEL stained cells. (h) Expression of GPX4, SLC7A11, and ACSL4 proteins detected by western blotting. * $P < 0.05$, ** $P < 0.01$, compared with si-nc and the control group. # $P < 0.05$, ## $P < 0.01$, compared with the LPS-PG group. & $P < 0.05$, && $P < 0.01$, compared with the LPS-PG + Ferrostatin-1 + si-nc group. The scale length is 200 μm .

Suppression of miR-370 reversed the effects of LINC00616 on cell viability and ferroptosis in PDLSCs treated with LPS-PG

Whether miR-370 affect cellular functions was then discussed. The expression of miR-370 was markedly downregulated by the miR-370 inhibitor and upregulated by the miR-370 mimic, suggesting that the PDLSCs were successfully transfected (Figure 4a).

Knockdown of miR-370 dramatically alleviated the effects of si-LINC00616 by suppressing cell viability (Figure 4b) and promoting cell death (Figure 4g). Furthermore, downregulation of miR-370 reversed the effects of LINC00616 knockdown on the release of ROS, Fe²⁺, and MDA induced by si-LINC00616 (Figure 4c-f) and the expression levels of GPX4, SLC7A11, and ACSL4 proteins (Figure 4h).

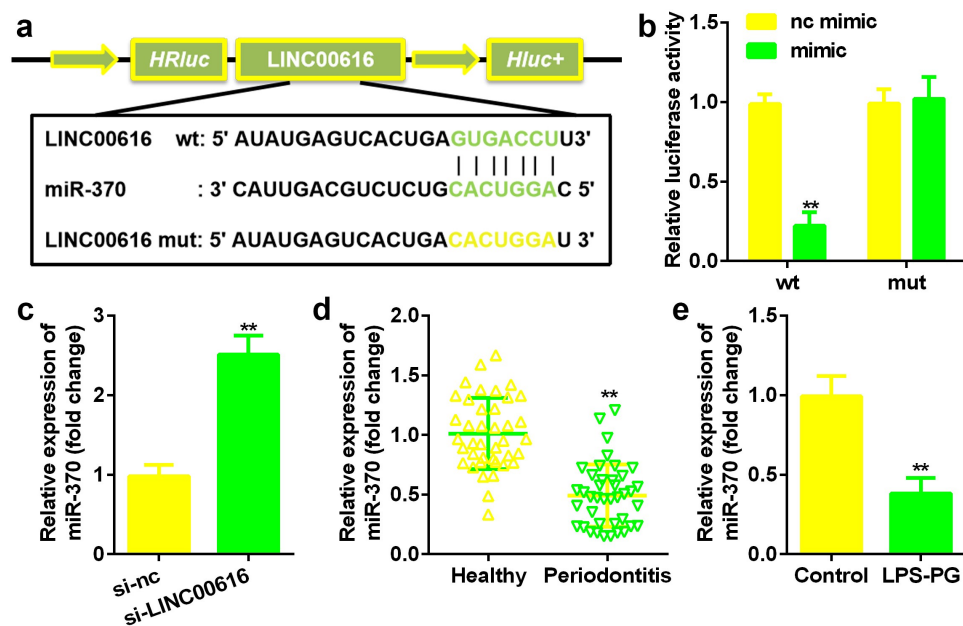


Figure 3. LINC00616 sponged miR-370 in PDLSCs. (a) The binding sites between miR-370 and LINC00616 are predicted by StarBase. (b) The dual luciferase reporter assay confirmed that miR-370 is a target of LINC00616 in PDLSCs. (c-e) qRT-PCR analysis for miR-370 expression in periodontal ligament tissues and PDLSCs.

** $P < 0.01$, compared with the nc mimic, si-nc, healthy group, and control group.

miR-370 directly targeted TFRC in PDLSCs

argetScan 7.2 predicted the binding sites between TFRC and miR-370 (Figure 5a). Luciferase activity was markedly decreased in cells transfected with miR-370 mimic and TFRC 3'-UTR wt (Figure 5b). TFRC expression was markedly reduced by si-LINC00616, which was alleviated by the miR-370 inhibitor (Figure 5c). TFRC was remarkably upregulated in periodontitis ligament tissues of periodontitis patients and LPS-PG treated PDLSCs (Figure 5d-e).

Upregulated TFRC inhibited the effects of downregulated miR-370

Finally, the role of TFRC in regulating PDLSCs was studied. As indicated in Figure 6a, TFRC expression was significantly increased after the oe-TFRC plasmid was transfected into PDLSCs (Figure 6a). Compared with miR-370-overexpressing cells, cotransfection with miR-370 and TFRC vectors significantly inhibited cell viability (Figure 6b) and suppressed ferroptosis (Figure 6c-h).

Discussion

Chronic periodontitis is a common oral chronic inflammatory disease [1,2]. Current periodontitis treatment cannot achieve physiological and functional regeneration of periodontal tissue [3,4]. Therefore, there is an urgent need to identify exact therapeutic targets for periodontitis therapy. Recent evidence suggests that lncRNAs may be associated with periodontitis. For example, the lncRNA AWPPH was overexpressed in plasma from patients with periodontitis compared with healthy controls, which predicts the recurrence of periodontitis [33]. A previous study by Liu et al. suggested that downregulation of the lncRNA MEG3 inhibited the osteogenic differentiation of PDLSCs in periodontitis [17]. Moreover, the lncRNA DCST1-AS1 inhibited the proliferation of PDLSCs in periodontitis [34]. LINC00616 is a newfound lncRNA aberrant expressed in periodontitis; however, the underlying mechanisms remain to be investigated.

Ferroptosis depends on iron and oxidative stress for the regulation of cell death, which differs morphologically, biochemically, and genetically from

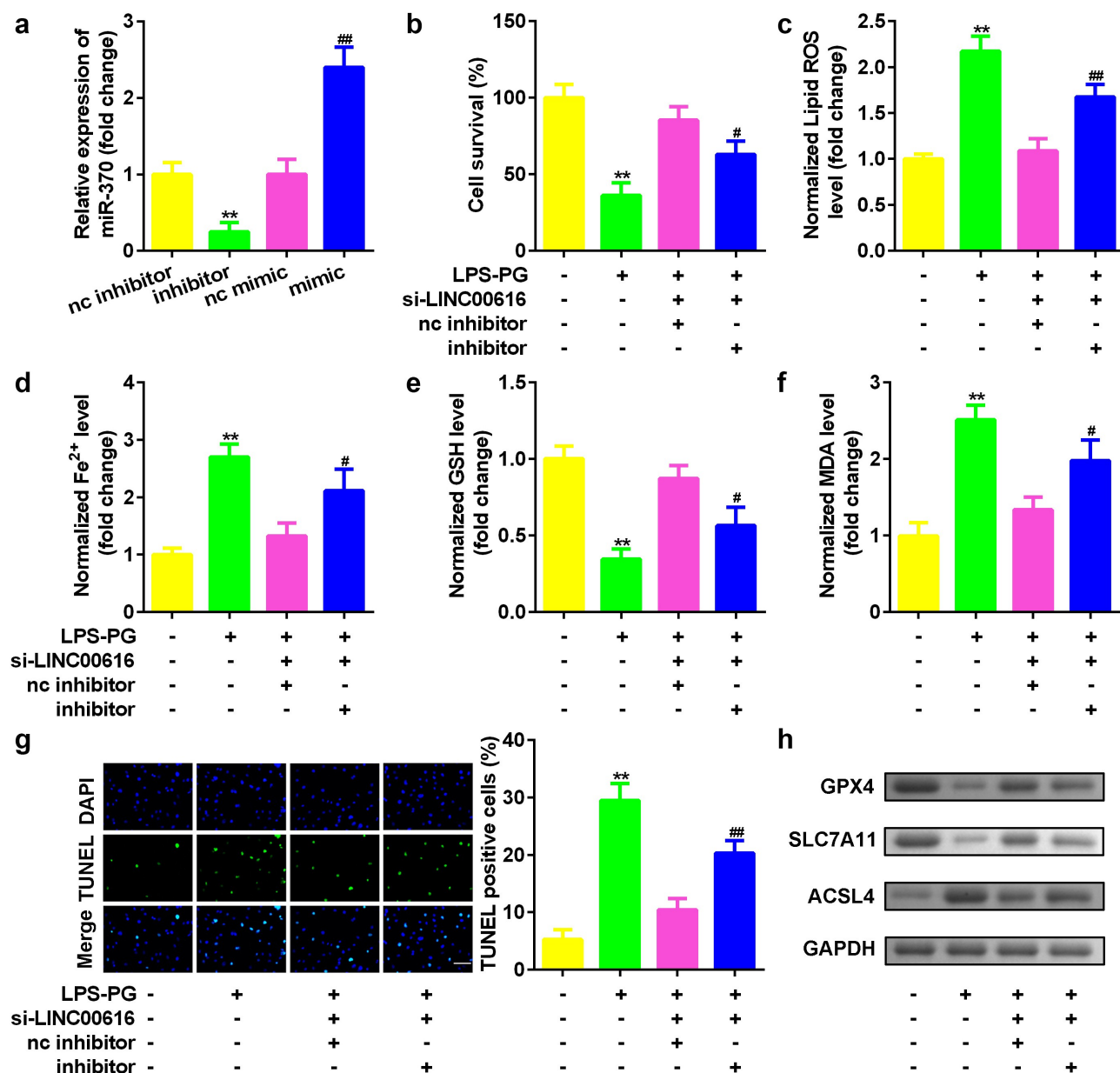


Figure 4. Suppression of miR-370 reversed the effects of LINC00616 on cell viability, and ferroptosis in PDLSCs treated with LPS-PG. (a) qRT-PCR analyses of miR-370 expression levels. (b) Cell viability is measured using an MTT assay after cotransfection in LPS-PG treated PDLSCs. (c-f) Levels of ROS, Fe²⁺, GSH, and MDA in PDLSCs treated with LPS-PG after cotransfection. (g) Images and quantized bar chart of TUNEL stained cells. (h) Expression of GPX4, SLC7A11, and ACSL4 proteins detected by western blotting. ** $P < 0.01$, compared with nc inhibitor and control group. # $P < 0.05$, ## $P < 0.01$, compared with the LPS-PG + si-LINC00616 + nc inhibitor group. The scale length is 200 μm .

apoptosis, necrosis, and autophagy [35]. Ferroptosis is a nonapoptotic, nonnecrotic regulator of cell death without rapid depletion of ATP, and inhibitors of apoptosis, autophagy, or necrosis cannot suppress ferroptosis [35]. Overall, ferroptosis leads to cell death through iron-mediated accumulation of lipid ROS, which interferes with cell integrity and membrane fluidity and

permeability, and these effects may be the main factors that lead to ferroptosis [8–10]. Furthermore, since iron-dependent oxidative stress and lipid peroxidation are common features of ferroptosis and inflammatory diseases, ferroptosis has been intensively studied in various inflammatory diseases [12]. For example, CD8(+)T cells release interferon-gamma (IFN γ) to inhibit the

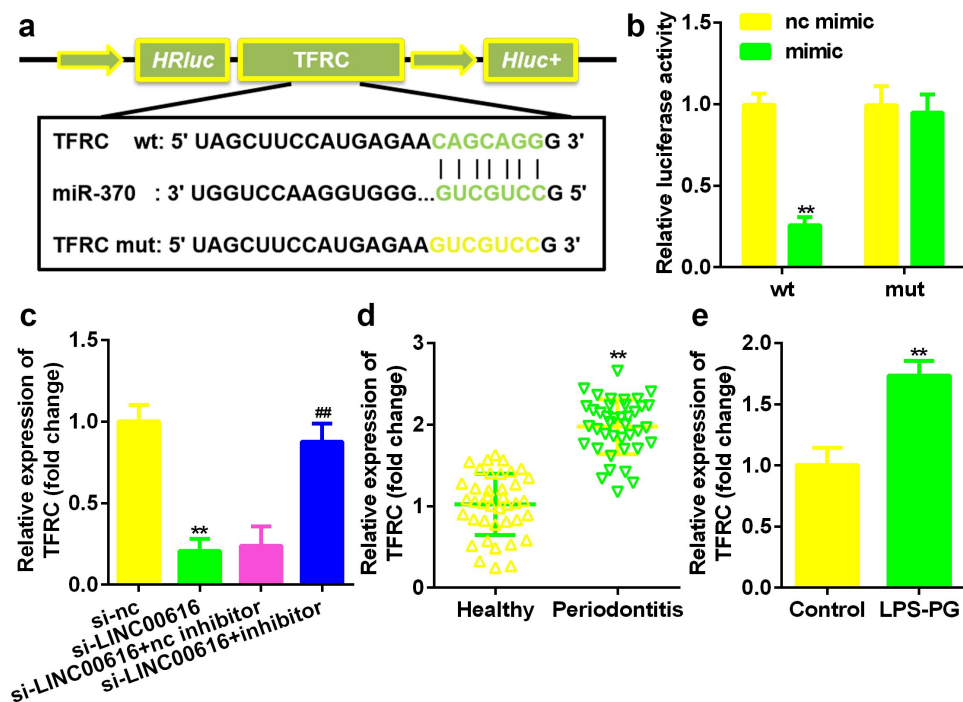


Figure 5. TFRC was the target gene for miR-370. (a) Bioinformatics predicted the binding sites between miR-370 and TFRC. (b) A dual-luciferase reporter assay was performed to confirm the association between TFRC and miR-370. (c-e) qRT-PCR analysis for the expression of TFRC in periodontal ligament tissues and PDLSCs.

** $P < 0.01$, compared with nc mimic, si-nc, Healthy, and control group. ### $P < 0.01$, compared with the nc mimic group.

glutamate-cystine antiporter system xc-, enhance lipid peroxidation, and promote ferroptosis in tumor cells [36]. Ferroptosis also plays a regulatory role in Alzheimer's disease, Parkinson's disease, cerebral hemorrhage, and other diseases [12]. Butyrate disrupted iron homeostasis by activating NCOA4-mediated ferritinophagy, leading to ferroptosis in periodontitis [13]. Therefore, we speculated that inhibiting ferroptosis in the course of periodontitis may be a potential therapeutic direction. In the present study, LPS-PG treatment promoted ferroptosis in PDLSCs. Interestingly, knockdown of LINC00616 accelerated cell viability and inhibited ferroptosis of periodontitis model cells, suggesting that inhibition of LINC00616 may alleviate the process of periodontitis.

LncRNAs have been recognized as sponge miRNAs to regulate downstream genes [37]. Bioinformatic analyses indicated that miR-370 was a target miRNA of LINC00616. miR-370 is differentially expressed in cancers and functions as a proto-oncogene or tumor suppressor in

diverse tumors [38,39]. However, miR-370 has not been investigated in periodontitis. Our data demonstrated that miR-370 expression was decreased in periodontitis patients and periodontitis model cells. Furthermore, inhibition of miR-370 reversed the effects of silenced LINC00616 on cell viability and ferroptosis. In this study, LINC00616 knockdown may protect PDLSCs by binding to miR-370.

TFRC is a gene that encodes a transferrin receptor and plays an important role in the regulation of iron absorption in the body [40]. Transferrin binds to TFRC on the cell surface and forms a complex to transport iron into cells through endocytosis, increasing the concentration of iron in cells and enhancing the sensitivity of cells to the induction of ferroptosis [40-42]. In this research, TFRC was confirmed to be a target gene of miR-370 and upregulated in periodontitis model cells. TFRC overexpression reversed the effect of miR-370 mimic, suppressed cell viability, and accelerated ferroptosis of PDLSCs. These results suggested that

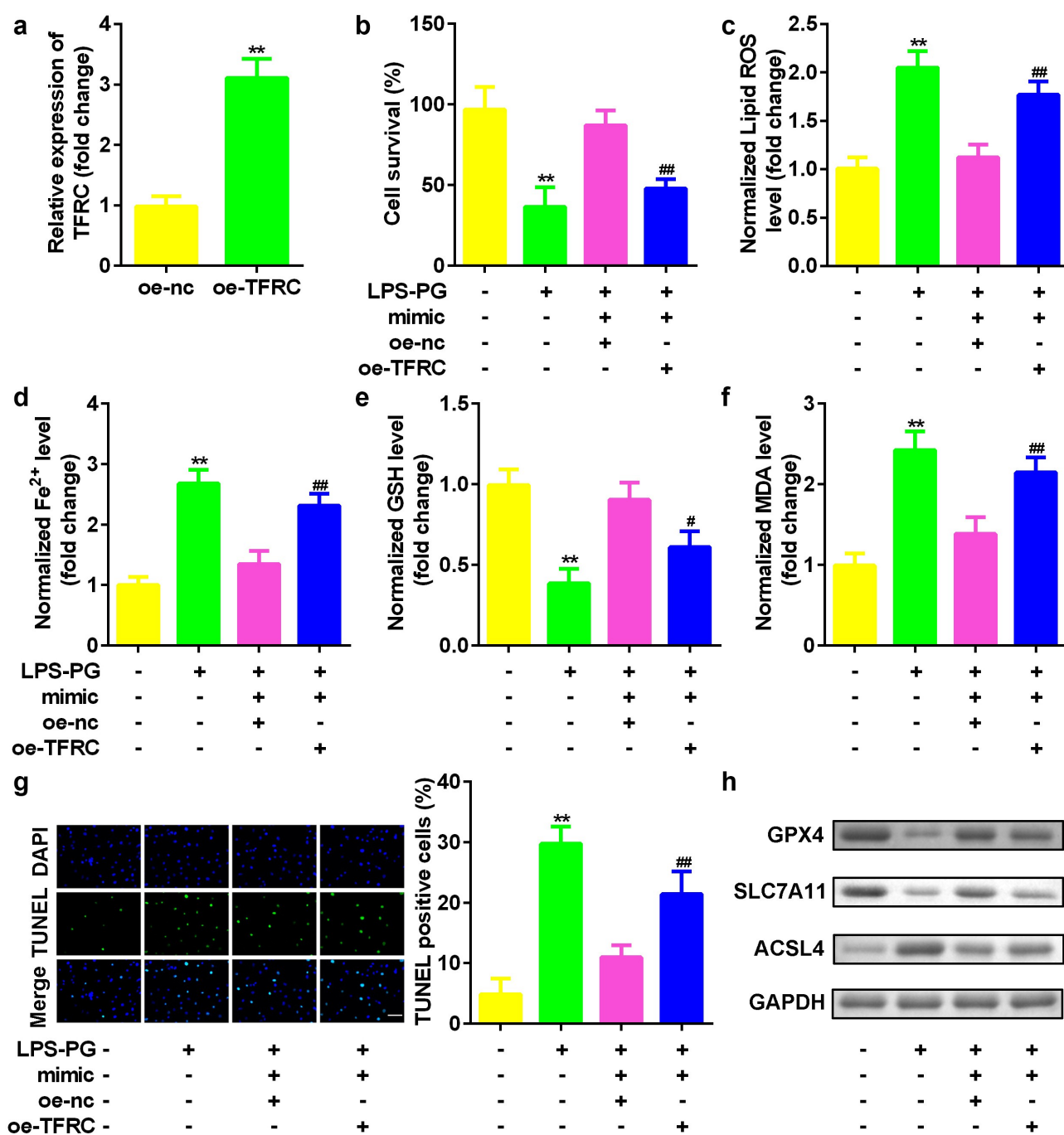


Figure 6. Upregulated TFRC inhibited the effects of downregulated miR-370. (a) qRT-PCR analyses of TFRC expression levels. (b) Cell viability is measured using an MTT assay after cotransfection in PDLSCs treated with LPS-PG. (c-f) Levels of ROS, Fe²⁺, GSH, and MDA in PDLSCs treated with LPS-PG after cotransfection. (g) Images and quantized bar chart of TUNEL stained cells. (h) Expression of GPX4, SLC7A11, and ACSL4 proteins detected by western blotting.

***P* < 0.01, compared with oe-nc and control group. #*P* < 0.05, ##*P* < 0.01, compared with the LPS-PG + mimic + oe-nc group. The scale length is 200 μm.

LINC00616 sponged miR-370 to upregulate TFRC and that TFRC may function as a ferroptosis promoter and degrade the cellular functions of PDLSCs, which is consistent with a previous study [32].

Conclusion

Inhibition of LINC00616 protected PDLSCs by regulating miR-370/TFRC. Knockdown of LINC00616 may be an alternative for the treatment of periodontitis.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This project is supported by Key research and development projects in Xingtai, China (2020ZC348).

Ethical approval

This study protocol was approved by the Ethics Committee of Eye Hospital of Hebei.

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