Article

PRDX6 alleviates lipopolysaccharide-induced inflammation in human gingival fibroblasts by regulation of NRF2

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Abstract: Periodontitis is a progressive and inflammatory oral disease and results in the damage of the supporting tissues of teeth. Peroxiredoxin6 (PRDX6) is an antioxidant enzyme and has been identified as a regulator in redox balance. This study aimed to investigate whether PRDX6 could protect human gingival fibroblasts (HGFs) from lipopolysaccharide (LPS) induced inflammation and its mechanisms. Here, both inflamed and non-inflamed human gingival tissues were collected to assess the expression of PRDX6 and NRF2 by Immunohistochemistry and Western blotting. Furthermore, HGFs were stimulated with LPS, MJ33 (PRDX6 phospholipase A2 inhibitor), or ML385 (NRF2 inhibitor). The expression levels of inflammatory cytokines were measured by RT-qPCR and ELISA, and reactive oxygen species (ROS) was detected using DCFH-DA. PRDX6 was downregulated in inflamed gingival tissues. In HGFs, LPS induced inflammatory cytokines and ROS was upregulated in PRDX6 knockdown cells. Furthermore, co-treatment with MJ33 alleviated LPS-induced inflammatory cytokines and ROS, while inhibiting NRF2 upregulated those in HGFs. Therefore, this study provided a new mechanistic insight that PRDX6, regulated by the NRF2 signaling, alleviates LPS- induced periodontitis in human gingival fibroblasts.

Keywords: PRDX6; inflammation; NRF2; HGFs.

1. Introduction

Periodontitis is a chronic inflammatory disease of the periodontium and results in tooth loss [1, 2]. It affects 35%-50% of the adult population worldwide [3]. It is mainly caused by pathogenic bacteria-induced inflammatory responses and host immune responses [4-6]. Other risk factors of periodontitis include smoking, oxidative stress, systemic diseases, etc. [7]. Therefore, exploring the underlying mechanisms of periodontitis is of great significance. Reactive oxygen species (ROS) are critical in cell physiological processes, such as signal transduction, antimicrobial defense, and cellular transcription [8]. However, the overabundance of ROS production induced by lipopolysaccharide (LPS), a component of periodontal pathogenic bacteria, will cause tissue damage in periodontitis [9-11]. Besides, many reports demonstrated that some antioxidants could ameliorate LPS-induced periodontitis by minimizing oxidative stress [12-15].

Peroxiredoxins (PRDXs) are antioxidant enzymes that control intracellular peroxides level and mediate signal transduction. PRDX6 is an intriguing enzyme involved in many diseases, such as carcinogenesis, inflammation, ocular damage, etc. [16-20]. Unlike other peroxiredoxins, PRDX6 contains single cysteine and mainly acts PL hydroperoxide GPx (PHGPx) activity. Besides, it has two additional catalytic sites with phospholipase A2 activity (PLA2) activity and LPC acyl transferase (LPCAT) activity [21, 22]. Furthermore,

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PRDX6 is regulated by the antioxidant response element (ARE) downstream of the NRF2 (nuclear factor-erythropoietin 2-related factor 2, NRF2) transcription factor [23]. The microarray results showed that PRDX6 was upregulated in periodontitis [24]. However, the detailed function of PRDX6 and NRF2 in LPS-induced periodontitis has not been explored intensively.

This study aimed to explore the role of PRDX6 in modulating the inflammatory response and oxidative stress in periodontitis and its underlying mechanisms. Therefore, our study provided a new sight that PRDX6 plays a protective role in periodontitis through limiting oxidative stress.

2. Materials and Methods

2.1. Reagents and antibodies

E. coli LPS (L8880) was purchased from Solarbio (China) and dissolved in phosphate-buffered solution (PBS). MJ33 (C5326) and ML385 (B8300) were purchased from APEXBIO (USA) and dissolved in H2O and DMSO. PRDX6 monoclonal antibody (13585-1-AP, 1:1000) was purchased from Proteintech (China). NRF2 monoclonal antibody (12721S, 1:1000) was purchased from CST (USA). GAPDH monoclonal antibody (TA-08, 1:1000) was purchased from ZSGB- BIO (China).

2.2. Participant recrument

Gingival biopsies were obtained from healthy volunteers and chronic periodontitis patients at the Stomatology Hospital. The inclusion criteria for all volunteers in this study: (1) age ≥ 21 years and the presence of 20 teeth; (2) volunteers without systemic diseases such as diabetes, rheumatoid arthritis, or coronary heart disease; (3) women without breastfeeding, menstruating, or pregnant; (4) no antibiotics, aspirin, or anticoagulants were used for recent three months; (5) without periodontal treatment within six months; (6) no habits of alcohol and tobacco. For patients with chronic periodontitis, additional criteria include (1) multiple sites with probing depth (PD) ≥ 5mm, (2) attachment loss (AL) ≥ 3mm, (3) bone loss visible on the radiograph. For healthy individuals, additional criteria include (1) without gingival inflammation, (2) without attachment loss, (3) probing depth ≤ 3mm, (4) no bleeding on probing. This study was conducted according to the guidelines approved by the Health Human Research Ethics Committee. All participants signed the written informed consent forms. The characteristics of control volunteers and chronic periodontitis patients are listed in Table 1.

Table 1. Characteristics of the study group.

Parameters	Controls	Patients
Case, n	16	16
Age, n	30.8 ± 5.6	32.3 ± 6.2
Sex, n (%)		
Male	7 (44%)	10 (63%)
Female	9 (56%)	6 (37%)
Probing depth (mm)	2.07 ± 0.29	5.78 ± 1.26
Attachment loss (mm)	0	4.81 ± 1.38
Gingival index	0	2.05 ± 0.67

2.3. Specimen collection

Gingival biopsies were obtained from healthy volunteers (n = 16; mean age 30.8 ± 5.6 years) and chronic periodontitis patients (n = 16; mean age 32.3 ± 6.2 years). Healthy gingival tissues were obtained from patients undergoing crown-lengthening surgery with PD ≤ 3mm and without bleeding on probing. Gingival tissues of chronic periodontitis were collected from patients undergoing periodontal flap surgery with PD ≥ 5mm and positive bleeding on probing. Each gingival tissue was equally divided into two sections.

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One section was used for hematoxylin & eosin (H&E) and Immunohistochemistry (IHC) analysis, and another section was frozen in liquid nitrogen for Western blotting.

2.4. Immunohistochemistry (IHC)

The gingival tissues were collected and fixed in 4% paraformaldehyde overnight. These specimens were embedded in paraffin, serially sectioned (4μm), and stained with H&E. The IHC accessory kit (ZSGB- BIO, China) was used following the protocol provided. The sections were blocked with 0.03% hydrogen peroxide and 2 % bovine serum albumin (BSA). Gingival specimens were incubated with antibodies against NRF2 and PRDX6 at 4℃ for 12h, followed by incubation with secondary antibodies for 1h. Then the samples were counterstained with hematoxylin, dehydrated, and mounted. Morphological changes were observed by light microscopy (Leica, Germany).

2.5. Cell culture

HGFs were isolated from the non-inflamed gingiva of 5 donors who needed extraction of the healthy third molar. The cells were cultured in DMEM medium (Hyclone, USA) supplemented 10% fetal bovine serum (Corning, USA) and 1% penicillin/streptomycin solution (Beyotime, China) at 37 ℃ with 5% CO_2 in a cell incubator. Cells at 4-6 passages were used in the following experiment.

2.6. Cell viability assay

The CCK-8 (MCE, USA) method was used to access cell viability. HGFs were plated at a concentration of 4000/well in 96-well plates. After drug treatment, the fresh medium (100 μL per well) containing CCK-8 solution (10 μL) was added to each well, followed by incubation at 37 °C for 3 h. Cell viability was measured at 450nm using a microplate reader (Bio-Tek, USA).

2.7. Enzyme-linked immunosorbent assay (ELISA) assay

IL-6 and TNF-α levels were detected by ELISA kits (DAKEWE, China) following the protocol provided. Briefly, HGFs were plated in 6-well plates at a concentration of 2×10^5 cells. These cells were supplemented with a fresh medium containing different reagents. After incubation time, HGFs culture supernatants were collected for ELISA assay. The samples were was detected at 450nm using a microplate reader (Bio-Tek, USA).

2.8. Small interfering RNA (siRNA) transfection

2×10^5 HGFs were seeded in 6-well plates before transfection. 24 hours later, cells were treated with siRNA targeting PRDX6 or a negative control siRNA using Lipo8000 (Beyotime, China) as a transfection reagent. The human PRDX6 siRNA (sequence: Sense: 5′-GGAUAUCAAUGCUUACAAUTT-3′;Anti-sense:5′-AUUGUAAGCAUUGAUAUCCTT-3′) was purchased from GenePharma (China). After transfection for 48 h, the efficacy of PRDX6 silencing was analyzed by RT-qPCR and Western blotting.

2.9. Real-time quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA of HGFs was extracted with TRIzol (Invitrogen) and reverse-transcribed with the PrimeScript RT Reagent Kit (Takara, Japan) following the manufacturer's instructions. RT-qPCR was performed with SYBR Green Master Mix (Takara, Japan) on a Stratagene Mx3000P system (Agilent Technologies, USA). The relative gene levels were analyzed using the $2^{-\Delta\Delta CT}$ method. The sequences of examined primer are listed in Table 2.

Table 2. Primer sequences for RT-qPCR.

Gene	Primer sequence
PRDX6	F: 5′-ACCACTGGCAGGAACTTTGATGAG-3′
	R: 5′-GGCTTCTTCTTCAGGGATGGTTGG-3′
NRF2	F: 5′-TCCAAGTCCAGAAGCCAAACTGAC-3′
	R: 5′- GGAGAGGATGCTGCTGAAGGAATC-3′
IL-6	F: 5′- TTCGGTCCAGTTGCCTTCT-3′

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	R: 5′- GGTGAGTGGCTGTCTGTGTG-3′
TNF-α	F: 5′-ATGGCGTGGAGCTGAGAGAT-3′
	R: 5′- TCTGGTAGGAGACGGCGATG -3′
β-Actin	F: 5′- GCCAACACAGTGCTGTCTGG-3′
	R: 5′-CTCAGGAGGAGCAATGATCTTG-3′

2.10. Western blotting

Human gingival tissues and cell samples were lysed in RIPA lysis (Beyotime, China). The total protein concentration was detected by Bradford Protein Assay Kit (Beyotime, China). After that, one-fourth volume of 5× SDS loading buffer was added in protein samples and heated at 99°C for 10 min. Proteins (20 μg/each) were separated by 10% SDS-PAGE and then transferred to PVDF membranes (Millipore, USA). Membranes were blocked with 5% BSA diluted in PBS for 1 h at room temperature and then incubated with primary antibodies against PRDX6, NRF2, and GAPDH overnight at 4°C. After washing with Tris Buffered saline Tween (TBST) three times, membranes were incubated with appropriate HRP-conjugated secondary antibodies for 1 h. After washing three times by TBST, the labeled protein was visualized by imaging system（Alliance Mini 4M; UK）and quantified using ImageJ (Bethesda, USA).

2.11. Determination of ROS generation

Intracellular ROS in HGFs was detected using DCFH-DA (Beyotime, China). HGFs were plated in 6-well plates at a concentration of 1.5×10^5 cells and treated with different test compounds. Cells of each group were harvested and incubated with 500 μL DCFH-DA reagent (10μM) at 37 °C for 30 min. Then cells were washed in PBS, resuspended in 500 μL PBS and analyzed using flow cytometry (CytExpert, USA). Samples can be excited at 488nm, and the data were detectable in the FL1 channel.

2.12. Statistical analysis

Representative data were collected from three or more times independent experiments. All statistics were analyzed using Graphpad Prism 9. Data were displayed as mean ± standard deviation (SD). Statistical significance between the groups was described as follows: $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

3. Results

3.1. The expression of PRDX6 and NRF2 decreased in inflamed gingival tissues

We first determined the effect of periodontitis on the expression levels of PRDX6 and NRF2. H&E staining results showed that the gingival tissues of periodontitis patients exhibited inflammatory cell infiltration, while healthy gingival tissues exhibited healthy architecture (Fig. 1a). The IHC and Western blotting results showed that PRDX6 decreased in periodontitis gingival tissues (Fig. 1b, d). NRF2 is a primary transcription factor that can activate antioxidant genes to defend against oxidative stress. We also assessed the NRF2 expression level in periodontitis and normal gingival tissue. As shown in Fig. 1c and 1d, NRF2 was decreased significantly in inflamed gingival tissues than in control. Therefore, the oxidative capacity was reduced and led to tissue damage in periodontitis.

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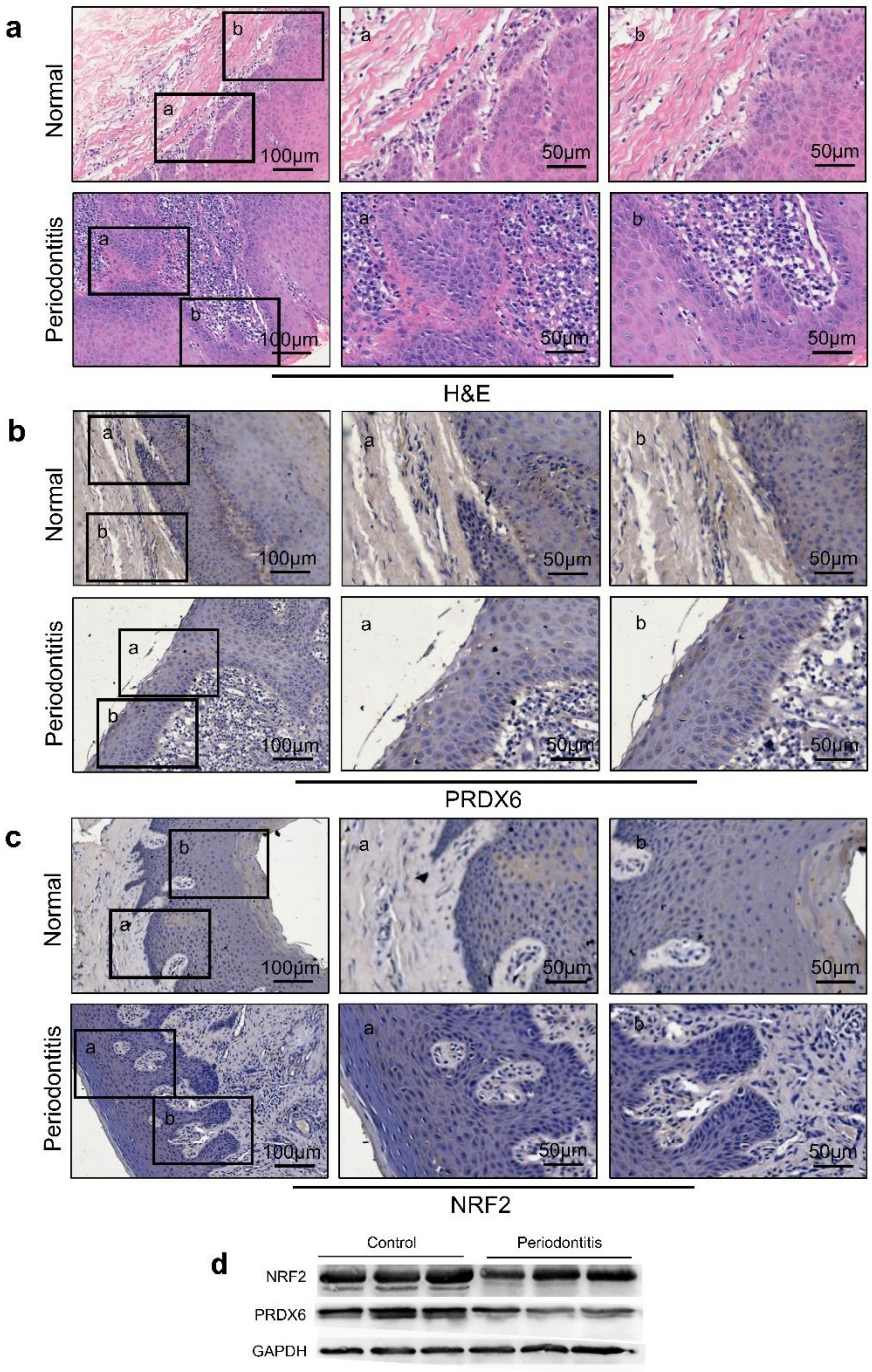

Figure 1. The expression of PRDX6 and NRF2 decreased in inflamed gingival tissues. (**a**) H&E stain in human periodontitis gingival tissues and normal tissues. (**b-d**) The expression of PRDX6 and NRF2 in control and inflammatory gingival tissues was assessed by IHC and Western blotting.

3.2. The expression of PRDX6 and NRF2 in LPS-induced HGFs

Then we investigated the effect of LPS on the expression levels of PRDX6 and NRF2 in HGFs. We first analyzed the consequence of LPS treatment in different concentrations on inflammatory cytokines in HGFs. As shown in Fig. 2a, RT-qPCR results confirmed that the levels of IL-6 and TNF-α were upregulated in a dose-dependent manner and climbed to the top with 10 μg/mL LPS treatment. Then we evaluated the influence of LPS treatment (10 μg/mL) for different hours on inflammatory cytokines in HGFs. As shown in Fig. 2a, IL-6 and TNF-α were elevated significantly after 10 μg/mL LPS treatment for 12 h. We also explored the effect of LPS treatment on the expression of PRDX6 and NRF2 in HGFs. The Western blotting and RT-qPCR results confirmed that the expression levels of PRDX6

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and NRF2 were also increased in a dose-dependent manner under LPS treatment in different concentrations. The up-regulation of PRDX6 and NRF2 was observed from 12 to 24 h, and the down-regulation was observed at 72 h (Fig. 2e and 2f). In brief, the expression of PRDX6 and NRF2 was first increased and eventually decreased under the long-term LPS treatment. Of note, PRDX6 and inflammatory cytokines both significantly increased when HGFs were treated with 10 μg/mL LPS for 12 hours. This treatment was used to guide the subsequent study.

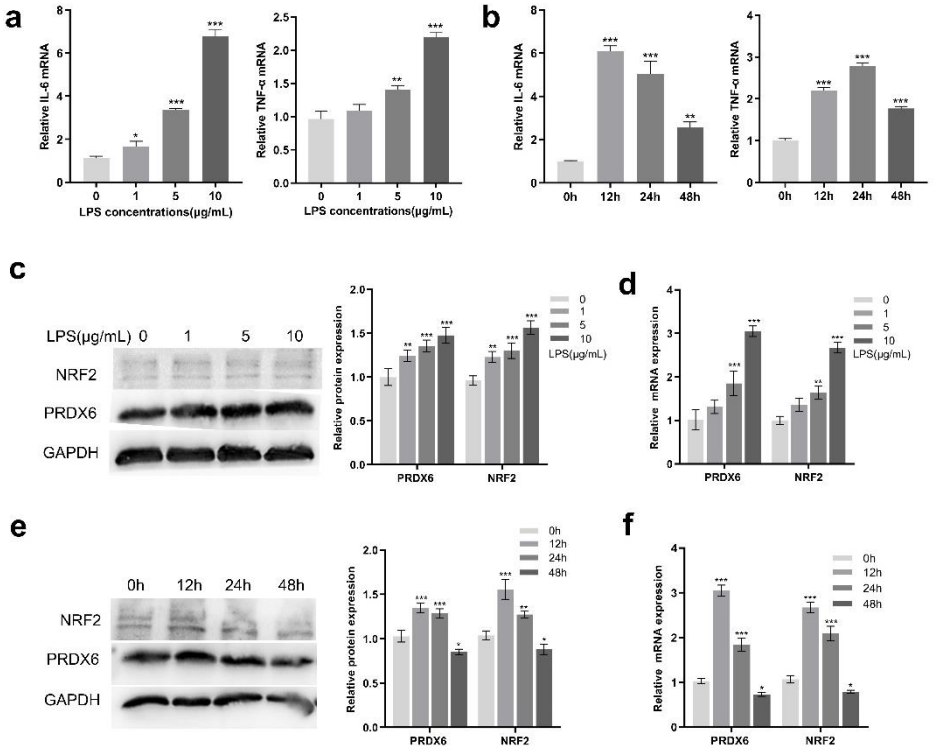

Figure 2. The expression of PRDX6 and NRF2 in LPS-induced HGFs. (**a, b**) HGFs were treated with LPS (0, 1, 5, 10 μg/mL) for 12h or treated with 10 μg/mL LPS for different time (0, 12, 24, and 48 h), then the mRNA expression of IL-6 and TNF-α were assessed by RT-qPCR. (**c, d**) HGFs were treated with LPS (0, 1, 5, 10 μg/mL) for 12h, the protein and mRNA expression of PRDX6 and NRF2 were assessed by Western blotting and RT-qPCR. (**e, f**) HGFs were treated with 10 μg/mL LPS for different time (0, 12, 24, and 48 h), the protein and mRNA expression of PRDX6 and NRF2 were assessed by Western blotting and RT-qPCR. Data were displayed as mean ± SD. *p < 0.05; **p < 0.01; ***p < 0.001.

3.3. PRDX6 protected HGFs from LPS-induced inflammatory cytokines and ROS

To explore the role of PRDX6 in periodontitis, we investigated the levels of inflammatory cytokines and ROS in LPS induced PRDX6 knockdown HGFs. After the transfection of PRDX6 siRNA, PRDX6 expression was significantly decreased (Fig. 3a, b). Subsequently, the cell viability was quantified in PRDX6 knockdown cells with treatment of LPS. The cell viability was reduced in PRDX6 knockdown HGFs compared to control knockdown after LPS treatment. The results of RT-qPCR and ELISA showed that PRDX6 knockdown could not increase the levels of IL-6 and TNF-α than control knockdown. However, it significantly enhanced the expression of inflammatory cytokines in LPS induced HGFs (Fig. 3c, d). Furthermore, PRDX6 knockdown could not upregulate the ROS, while it increased LPS induced ROS in HGFs. Thus, the knockdown of PRDX6 amplifies the LPS induced inflammation and oxidative stress.

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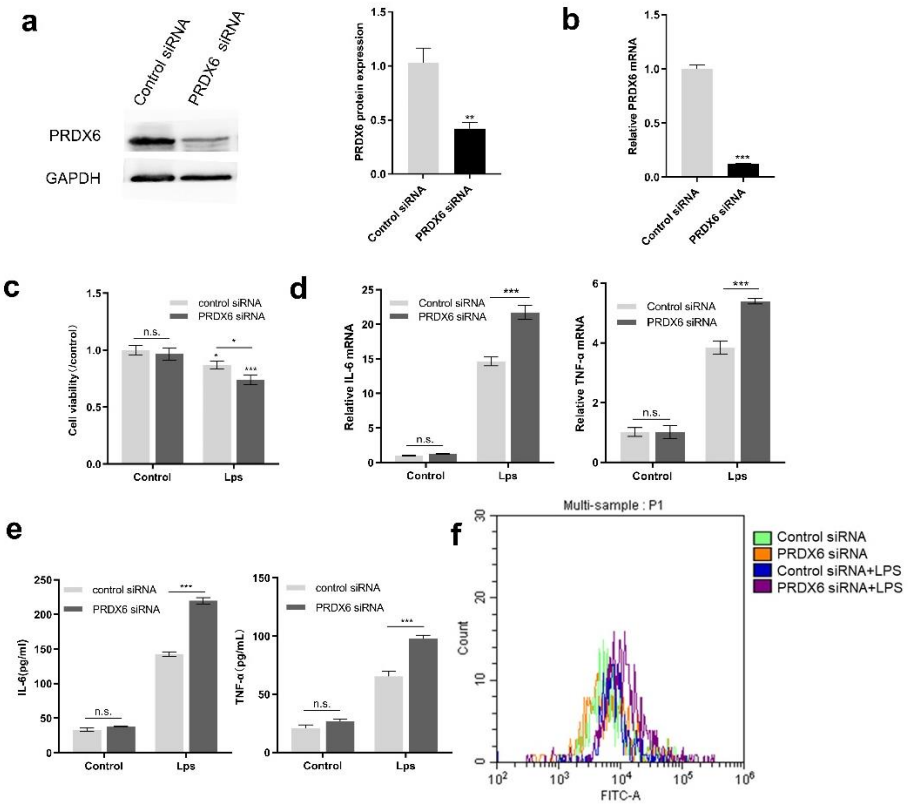

Figure 3. PRDX6 protected HGFs from LPS-induced inflammatory cytokines and ROS. (**a, b**) The mRNA and protein expression levels of PRDX6 were assessed after infection of PRDX6 siRNA for 48 h by RT-qPCR and Western blotting. (**c**) HGFs were stimulated by LPS (10 μg/mL) for 12 h after transfection for 48h, and the cell viability was assayed by CCK8 assay. (**d, e**) The expression levels of IL-6 and TNF-α were detected by RT-qPCR and ELISA. (**f**) ROS production of each group was assayed by flow cytometry. Data were displayed as mean ± SD. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; n.s.: no statistic difference.

3.4. The inhibition of PRDX6-PLA2 alleviated LPS-induced inflammatory cytokines and ROS

Because the specific inhibitors of the PLA2 activity of PRDX6 (MJ33) are commercially available, we mainly focused on whether the PLA2 activity of the multi-tasking enzyme PRDX6 could influence inflammation and oxidative stress. Fig. 4a showed that the cell viability was upregulated in the MJ33+LPS group compared with the LPS group. As shown in Fig. 4b and 4c, MJ33 treatment could not affect the levels of IL-6 and TNF-α in HGFs. However, the LPS-induced inflammatory cytokines were down-regulated by MJ33 co-treatment. In addition, the inhibition of PLA2 activity decreased the production of LPS-induced ROS (Fig. 4d). Therefore, PRDX6 could partially promote inflammation and oxidative stress via PLA2 activity.

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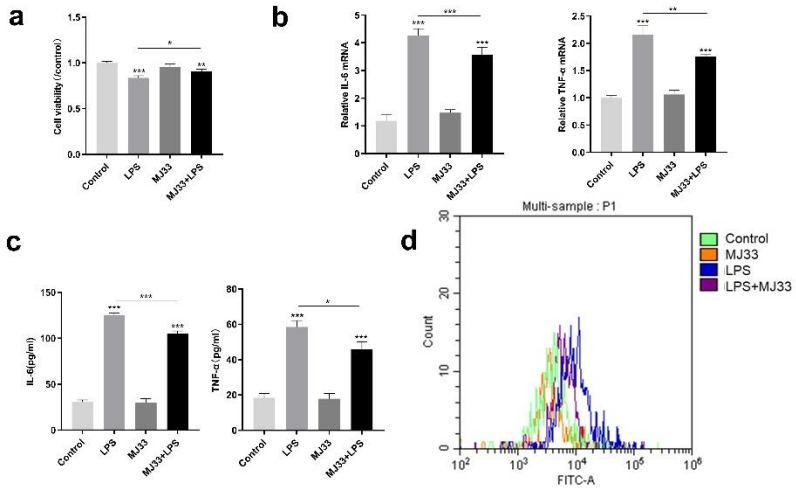

Figure 4. The inhibition of PRDX6-PLA2 alleviated LPS-induced inflammatory cytokines and ROS. (**a**) HGFs were stimulated by LPS (10 μg/mL) with or without MJ33 (5 μM) for 12h, the cell viability was assayed by CCK8 assay. (**b, c**) The expression of IL-6 and TNF-α of each group was detected by RT-qPCR and ELISA. (**d**) ROS production of each group was assayed by flow cytometry. Data were displayed as mean ± SD. *p < 0.05; **p < 0.01; ***p < 0.001; n.s.: no statistic difference.

3.5. PRDX6 regulated by NRF2 ameliorated LPS-induced inflammatory cytokines and ROS

PRDX6 served as a downstream target gene of NRF2. To further confirm the effect of NRF2 regulation on the expression of PRDX6, we used NRF2 inhibitor ML385 to inhibit the NRF2. The cell viability under the co-treatment of ML385 and LPS could be further decreased than cells only treated with LPS (Fig. 5a). The RT-qPCR results confirmed that the expression levels of NRF2 and PRDX6 were decreased with the ML385 treatment than control cells. The co-treatment of LPS and ML385 reduced the expression of NRF2 and PRDX6 compared with the LPS group (Fig. 5b). As shown in Fig. 5c and 5d, ML385 treatment could not initialize the expression levels of IL-6 and TNF-α, while it could enhance LPS-induced inflammatory cytokines in HGFs. Furthermore, LPS-induced ROS were improved in the meantime by ML385 co-treatment (Fig. 5e). Therefore, these results indicated that NRF2 could regulate the expression of PRDX6 to protect HGFs against inflammation and oxidative stress.

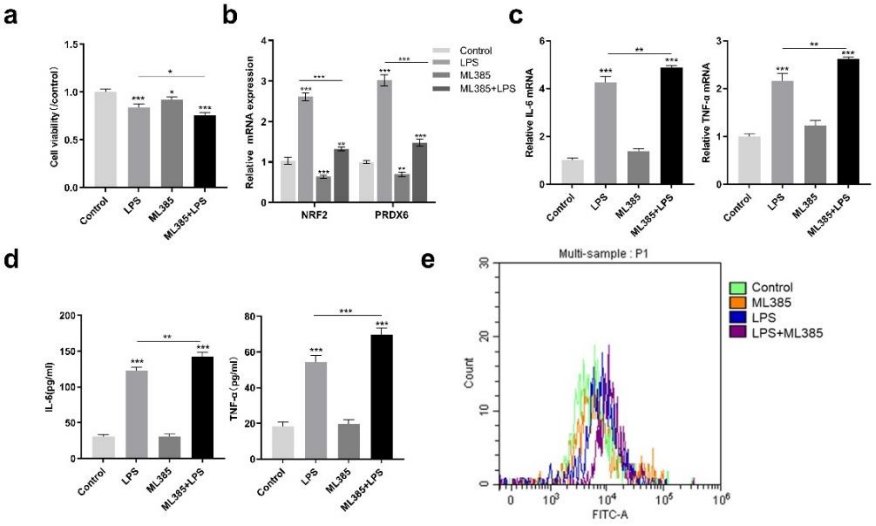

Figure 5. PRDX6 regulated by NRF2 ameliorates LPS-induced inflammatory cytokines and ROS. (a) HGFs were stimulated by LPS (10 μg/mL) with or without ML385 (5 μM) for 12 h, and the cell viability was assayed by CCK8 assay. (**b**) The mRNA expression of NRF2 and PRDX6 of each group was assessed by RT-qPCR. (**c, d**) The expression levels of IL-6 and TNF-α of each group were

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detected by RT-qPCR and ELISA. (**e**) ROS production of each group was assayed by flow cytometry. Data were displayed as mean ± SD. *p < 0.05; **p < 0.01; ***p < 0.001.

4. Discussion

Even though the LPS-induced periodontitis model has been established for years and is widely used in pre-clinical studies, the precise mechanisms remain unclear. It is reported that HGFs can be activated by LPS and release significant inflammatory factors, which contribute to periodontal tissue destruction [25, 26]. What's more, there are still exit oxidative stress injuries because of the accumulation of oxidation products[27]. It has been reported that PRDX6 is multifunctional to scavenge peroxides and generate ROS via PLA2 activity[28]. Therefore, PRDX6 is a regulator of the cellular redox of biological systems. In this study, we mainly discussed the role of PRDX6 in LPS-induce inflammation and oxidative stress in HGFs.

PRDX6 holds an essential position in inflammatory diseases and hasn't been reported in periodontitis. We found that the expression level of PRDX6 was decreased in inflamed gingival tissues due to severe oxidative damage in chronic periodontitis. HGFs are the most abundant cells in periodontal tissue and the critical barrier against bacterial infection. In HGFs, the expression of PRDX6 was increased considerably to defend against the LPS-induced oxidative damage at first, while it eventually decreased and lost the protective effect.

Interestingly, PRDX6 has been recently identified as the regulator of redox balance. We then identified the inflammatory cytokines and ROS in PRDX6-silenced cells. PRDX6 knockdown elevated the levels of inflammatory cytokines released by HGFs, which indicated that PRDX6 plays a protective role in LPS-induced inflammation. Furthermore, more ROS production could be observed in silencing HGFs treated with LPS and participates in the onset and amplification of inflammation. PRDX6 could play peroxidase activity to act as an antioxidant reducing short-chain hydroperoxide. Thus, PRDX6 limits oxidative stress in LPS-induced HGFs. It has been reported that PRDX6 plays a paradoxical role in inflammatory and immune responses. For example, in the LPS-induced acute kidney injury model, PRDX6-overexpressed mice showed less renal apoptosis and leukocyte infiltration than wild mice [29]. Conversely, PRDX6 lost its antioxidant activity in aortic lesions or aneurysms, characterized by high inflammation and oxidative stress [30]. Our results showed that PRDX6 could alleviate ROS and has a protective effect on periodontitis. However, we only demonstrated this mechanism in vitro experiments, and the vivo model was necessary to analyze the essential role of PRDX6 in the future.

Notably, PRDX6 is a peroxiredoxin that owns multiple enzyme activities, peroxidase, PLA2, and LPCAT. It has been reported that blocking the PLA2 activity of PRDX6 by MJ33 enhanced ROS [31]. In our study, the inhibition of PRDX6-PLA2 activity down-regulated the production of LPS-induced ROS and inflammatory cytokines. The potential mechanism for MJ33 to alleviate the inflammation and oxidative stress through the inhibition of the PLA2 activity to block NOX2 activation. NOX2 is the enzyme responsible for ROS generation associated with inflammation [32]. It has been reported that enhanced NOX2 activity is most likely one of the ROS sources in periodontal tissues [33]. PRDX6-PLA2 could play a protective role in reducing phospholipid hydroperoxides and repairing peroxidized cell membranes. However, it is less than the protective role of PHGPx activity and LPCAT activity. This result was in line with the study that blocking PRDX6-PLA2 activity could attenuate lungs damage, presumably by inhibiting oxidative stress and preventing the amplification of lungs inflammation [32]. Therefore, inhibiting PRDX6-PLA2 activity alleviated the LPS-induced inflammation and oxidative injury in HGFs.

Furthermore, the NRF2 is a vital regulator of the cellular antioxidant response, as many of its downstream genes prevent oxidative stress [34]. The activated NRF2 can be transported to the nucleus and subsequently bind to ARE to improve the transcription of some antioxidant genes, such as HO-1 and NQO1[35, 36]. In our study, the

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downregulation of NRF2 was observed in inflammatory gingival tissues compared to the normal gingival tissues. What's more, NRF2 was intensely activated to promote HGFs at first and eventually was decreased with long-term LPS treatment, which is in line with the result of PRDX6. With the treatment of NRF2 inhibitor ML385, the expression of PRDX6 was down-regulated. In addition, the levels of inflammatory cytokines and ROS were increased as ML385 eliminated the protective role of NRF2. Therefore, the NRF2 could play a protective role in LPS induced-HGFs. However, this study only explored the primary mechanism between PRDX6 and NRF2 (Fig. 6), and the deep mechanism still needs to be further explored.

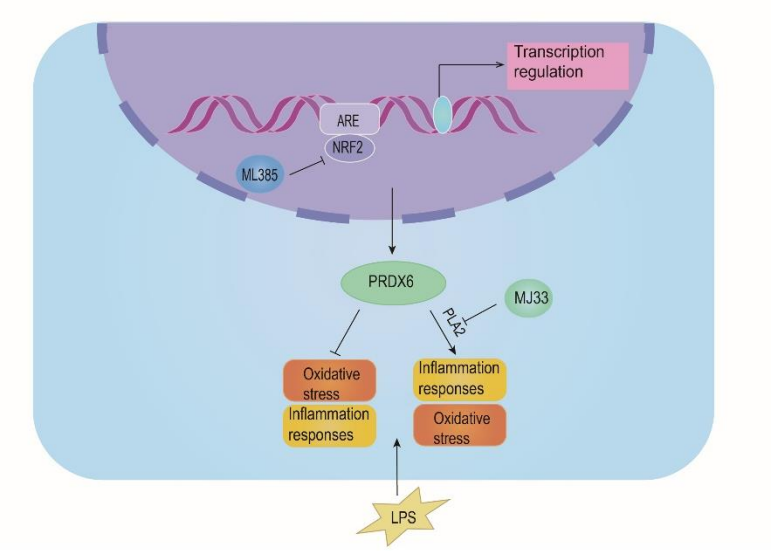

Figure 6. The role of PRDX6 in LPS-induce HGFs. PRDX6, regulated by the NRF2 signaling, alleviates LPS- induced inflammatory response and oxidative stress in human gingival fibroblasts.

5. Conclusions

Collectively, we mainly analyzed the role of PRDX6 in LPS-induced inflammatory response and oxidative stress. The present study demonstrated that PRDX6 could alleviate the LPS-induced inflammation and oxidative stress in HGFs. The NRF2 signaling is an essential antioxidant response pathway that regulates the expression of PRDX6, which also affects the inflammation reaction in periodontitis. Therefore, this study provided new insight into periodontitis therapy targeting PRDX6 and oxidative stress.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Health Human Research Ethics Committee of the Stomatology Hospital affiliated to Anhui Medical University (Permit No. 20200836).

Informed Consent Statement: Written informed consent has been obtained from the patients.

Conflicts of Interest: The authors declare no conflict of interest.

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