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Abnormal mitochondrial structure and function are retained in gingival tissues and human gingival fibroblasts from patients with chronic periodontitis

Jia Liu[1,2,3,4] | Xiaoxuan Wang[1,2,3,4] | Fei Xue[2,3,4,5] | Ming Zheng[6] | Qingxian Luan[1,2,3,4]

[1]Department of Periodontology, Peking University School and Hospital of Stomatology, Beijing, China

[2]National Clinical Research Center for Oral Diseases, Beijing, China

[3]National Engineering Laboratory for Digital and Material Technology of Stomatology, Beijing, China

[4]Beijing Key Laboratory of Digital Stomatology, Beijing, China

[5]First Clinical Division, Peking University School and Hospital of Stomatology, Beijing, China

[6]Department of Physiology and Pathophysiology, Peking University Health Science Center, Beijing, China

Correspondence

Qingxian Luan, Department of Periodontology, Peking University School and Hospital of Stomatology, 22 Zhongguancun South, Avenue, Haidian District, Beijing, 100081, China.
Email: kqluanqx@126.com

Ming Zheng, Department of Pathophysiology, Peking University Health Science Center, NO.38 Xueyuan Road, Haidian District, Beijing, 100191, China.
Email: zhengm@bjmu.edu.cn

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Abstract

Background and Objective: The abnormal structure and function of mitochondria in cells is closely associated with inflammatory diseases. However, the physiology of mitochondria within gingival tissues and human gingival fibroblasts (HGFs) in patients with chronic periodontitis (CP) remains unclear. The objective of this study was to investigate the structure profile and function of mitochondria in gingival tissues and in HGFs derived from patients with or without CP. These features of mitochondria in HGFs were further analyzed when HGFs were induced by lipopolysaccharide (LPS) from *Porphyromonas gingivalis* (*P.g*).

Methods: Gingival tissues and HGFs were collected from CP and healthy patients. Mitochondrial structure was assessed by transmission electron microscopy. Tissues or cells lysis was performed for mitochondrial DNA (mtDNA) quantification, and real-time polymerase chain reaction (RT-PCR) tests were used to determine mtDNA copy numbers. Western blot analysis was used to evaluate autophagy-related protein (ATG)-5, microtubule-associated protein light chain 3 (LC3), and mitochondrial matrix protein pyruvate dehydrogenase kinase isozyme 2 (PDK2) levels in tissues and HGFs from CP and healthy individuals.

Results: Tissues and HGFs from CP showed a significant greater mitochondrial structure destruction, lower mtDNA level, increased ATG5, LC3-II, and lower PDK2 protein levels than those of healthy individuals. In addition, LPS from *P.g* also triggered the same results in HGFs from healthy donors. Moreover, the challenge of HGFs from CP with LPS worsened these parameters.

Conclusion: Mitochondrial structure and function within gingival tissues and HGFs from CP individuals were abnormal compared to those from healthy donors, and LPS could promote mitochondrial destruction.

KEYWORDS

chronic periodontitis, connective tissue, fibroblast(s), in vitro model, inflammation

Ming Zheng and Qingxian Luan contributed equally to this study.

1 | INTRODUCTION

Mitochondria are highly dynamic, bioenergetic, biosynthetic, and signaling organelles that play an indispensable role in energy generation.[1] They also exhibit stress sensing in their cellular adaptation, which is a generally important mediator.[1,2] Mitochondrial quality control is essential in maintaining mitochondrial health and normal cellular function.[3] Dysfunction of mitochondria has been proposed as a critical mechanism in the pathogenesis of various diseases.[4,5] Mitochondria facilitate ATP production by activating oxygen metabolism and respiratory chain reaction.[6] Pyruvate dehydrogenase kinase isozyme (PDK) 2 residues in mitochondrial matrix, and is tightly regulated by various metabolites, and is sensitive to metabolic changes.[7] Our previous study has shown that lipopolysaccharide (LPS) derived from *Porphyromonas gingivalis* (*P.g*) decreased PDK2 protein expression and increased reactive oxygen species (ROS) levels in healthy human gingival fibroblasts (HGFs).[8] Interestingly, when mitochondrial integrity is disrupted by ROS, membrane lipids, proteins, and mitochondrial DNA (mtDNA) at that site are damaged.[9] Studies have documented that mitochondrial dysfunction can lead to progressive diseases, such as type 2 diabetes, neurodegeneration, cancer, and inflammatory disorders.[10-14] To date, there are little data regarding the mitochondrial structure and function in periodontal inflammatory disorders, during its process in vitro such as, in inflamed tissues or cells, and the effects induced by LPS from *P.g* on HGFs' mitochondria.[15] As such, work demonstrating mitochondrial structure and function in tissues and HGFs isolated from patients with chronic periodontitis is needed.

Periodontitis is a multifactorial disease characterized by inflammatory, genetic, or metabolic abnormalities,[16-18] and the destruction of gingival attachment as well as alveolar bone surrounding the teeth, resulting in teeth loss. The LPS of *P.g* is a key factor involved in periodontitis.[19] LPS has been shown to be effective in inducing mitochondrial dysfunction and triggering mitophagy in intestinal injury by increasing ROS production and decreasing mitochondrial membrane potential.[20] Furthermore, our laboratory has demonstrated that LPS played a unique role in the autophagy process in healthy HGFs, characterized by elevated autophagy-related protein 5 (ATG5) and microtubule-associated protein light chain 3 (LC3)-II, which could both generally regulate the formation of autophagosome.[21] However, limitations of the previous studies did not provide a distinction in ATG5 and LC3 protein levels between healthy and periodontitis diseased tissues or cells, and additional analyses of differences in LPS on diseased or healthy HGFs' autophagy processes have not been performed.

Although meticulous studies on mitochondrial functions, genetic heterogeneity, and their correlation with chronic inflammatory diseases have been conducted,[8,22] the involvement of changes in mitochondrial structure and function in periodontitis pathogenesis remains poorly defined. Comprehensive investigations based on complete mitochondrial genome analysis revealed the elevated number of mutations in mtDNA sequences in the gingival tissues of patients with chronic periodontitis compared to controls.[23,24] Alteration in mtDNA sequences has been implicated in chronic periodontitis and has expanded our knowledge of the etiology of the disease.[22,23] Moreover, the inflamed tissues in patients with chronic periodontitis were more prone to mtDNA mutations and mitochondrial dysfunction,[25] suggesting that cells derived from inflamed tissue site might be more vulnerable in conditions associated with acute or chronic inflammation. Studies originating from in vitro studies and clinical observations supported the hypothesis that mitochondrial dysfunction appeared to be detrimental to the host, and mtDNA damage in tissues and cells may be due to the accumulation of dysfunctional mitochondria.[14,26]

In fact, *P.g* has been shown to have a potential ability to manipulate oxidative stress, which has a strong correlation with mitochondria.[27] In periodontitis, HGFs are critical components of resident gingival cells, which are constantly exposed to oral biofilms. This, in turn, significantly increases oxidative stress, and cytokines production in HGFs, leading to periodontal ligament destruction and alveolar bone loss.[28] Since mitochondria play a crucial role in tissue and cell homeostasis, understanding its dysfunction in HGFs for the pathogenesis of periodontitis is critical for developing new therapies against periodontitis. Moreover, the differences in mitochondrial structure and function within HGFs in response to LPS derived from *P.g* between diseased and healthy donors remain unknown. As a result, we decided to evaluate whether mitochondrial structure and function showed differences in gingival tissues and HGFs from subjects with chronic periodontitis (CP) as compared with periodontally healthy subjects, and tracked HGFs' mitochondrial responses to LPS. Overall, our studies exemplify the integral importance of mitochondria in the physiology of *P.g* LPS-induced periodontitis.

2 | MATERIALS AND METHODS

2.1 | Subjects, cell isolation, and culture

Gingival tissue specimens were collected from patients with chronic periodontitis ($n = 6$) and healthy individuals ($n = 6$) undergoing flap surgery and crown lengthening surgery, respectively, at the Department of Periodontology, Peking University Hospital and School of Stomatology, Beijing, China. The study was approved by the Review Board and Bioethical Committee of Peking University Health Science Center (permit number PKUS-SIRB-2013017). All subjects were provided written informed consent in accordance with the Declaration of Helsinki of 1975, as revised in 2013 before being recruited in the study. The clinical characteristics of the patients with periodontitis and healthy donors are shown in Table 1. Primary HGFs were established from gingival tissue samples from patients with periodontitis and healthy individuals. Cells were cultured as described previously,[8] and used for experiments between passages 3 and 8. Our previous work identified that 1 μg/ml LPS could lead to an increased ROS level and an upregulated autophagy process.[8,21] However, high-dose LPS challenges, such as 10 mg/kg LPS injection to mice[29] and 10 μg/ml LPS addition to HGFs in our preliminary study both failed to induce autophagy. Given the fact,

TABLE 1 Clinical parameters at the surgery site of patients included in this study

Group	Age	Gender	Smoking	GI	BI	PD	CAL	Teeth
Con 1	35	Male	No	1	1	2–3 mm	0	13–23
Con 2	31	Female	No	0	0	3 mm	0	13–23
Con 3	33	Male	No	0.5	1	3mm	0	13–23
Con 4	27	Female	No	0	0	1–3 mm	0	11–21
Con 5	54	Male	No	1	1	1–3 mm	0	33
Con 6	40	Female	No	0.5	1	2–3 mm	0	23
CP 1	34	Female	No	2	2	6–8 mm	5 mm	31–32
CP 2	45	Male	No	1.5	2	6–7 mm	5 mm	13,17
CP 3	42	Female	No	1.75	2	8–10 mm	7 mm	17
CP 4	40	Male	No	1.5	2	7–9 mm	6 mm	26–27
CP 5	33	Female	No	1	1	5–6 mm	4 mm	35–37
CP 6	41	Female	No	1.75	2	6–7 mm	5 mm	16

Abbreviations: BI, Bleeding index; CAL, Clinical attachment level; CP, Chronic periodontitis; GI, Gingival index; PD, Probing depth.

here we applied the middle stimulation concentration at 5 μg/ml LPS derived from *P.g* (ATCCs 33277, Standard, InvivoGen) to evaluate its effect on mitochondria abnormalities in HGFs.

2.2 | Transmission electron microscopy

The cells and tissue were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer solution (pH 7.4) for 24 h. Cell and tissue samples were subsequently washed in phosphate buffer and post-fixed with 1% osmium tetroxide in the same buffer solution for 1 h at 25℃ and rinsed several times with distilled water. After post-fixation, the cells and tissues were dehydrated in a series of graded ethanol solutions. The cell and tissue samples were cleared in propylene oxide and impregnated overnight at a 1:1 ratio of propylene oxide: Spurr's resin, and then increased to a 1:3 ratio, followed by the pure resin for 2–3 h. The dehydrated samples were dried in small embedding vials and kept at 60°C for 48 h for polymerization. The resulting sample blocks were examined using a transmission electron microscope (TEM; JEOL) for the morphometric evaluation of mitochondria.

2.3 | Western blotting

HGFs in 6-cm dishes (7.5 × 105 cell/5 ml) were cultured in the presence or absence of LPS. Cell lysates and tissue extracts were prepared by direct lysis with lysis buffer consisting of 20 mM Tris-HCl pH7.5, 10% glycerol, 150 mM NaCl, 1% Triton X-100, 2mM EDTA, and protease inhibitors (Complete EDTA-free, Roche; Sigma Aldrich). The protein concentrations of cells and gingival tissues were measured using a bicinchoninic acid (BCA) protein assay. The samples (10 μg protein) were electrophoresed on 12.5%–15% sodium dodecyl sulfate polyacrylamide gel, transferred to a polyvinylidene difluoride membrane, and then blocked with 5% bovine serum albumin (BSA) in Tris-buffered saline (TBS). Following washing with TBS-Tween-20 (0.1%), membranes were incubated with rabbit anti-autophagy related gene 5 (ATG5) (Cell Signaling Technology), anti-microtubule-associated protein light chain 3 (LC3; Cell Signaling Technology), anti-PDK2 (Abcam), anti-Tom 20 (Proteintech), or anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primary antibodies diluted with 5% BSA in TBST overnight at 4℃. After washing, immunoreactive bands were visualized with secondary anti-rabbit horseradish peroxidase-conjugated IgG antibody (Cell Signaling Technology) followed by ECL substrate (Thermo Fisher Scientific).

2.4 | mtDNA copy number assessment

Total DNA was extracted from HGFs and gingival tissues using the DNeasy Blood & Tissue Kit (Qiagen), and mtDNA copy number was determined by real-time polymerase chain reaction (RT-PCR) with the following reagents: targeting mitochondrial ND1 and nuclear 18S rRNA served as a mitochondrial DNA and loading control, respectively. PCR Master Mix was obtained from Thermo Fisher Scientific. Primers were as follows: ND1 (Forward: CACACTAGCAGAGACCAACCGAAC; Reverse: CGGCTATGAAGAATAGGGCGAAGG), and 18S rRNA (Forward: GACTCAACACGGGAAACCTCACC; Reverse: ACCAGACAAATCG CTCCACCAAC). DNA was diluted to 10 ng/μl for 18S rRNA and 0.1 ng/μl for ND1. DNA (5 μl) was added to each 10 μl reaction. Each reaction was performed in triplicate, and the mean threshold cycle (Ct) and standard curves were used to assess mtDNA levels per nucleus for each sample.

2.5 | Statistical analysis

Statistical analyses were conducted using GraphPad Prism 7 software. All data are presented as means ± standard error (SE). Statistical comparisons were performed using Student's *t*-test or

one-way analysis of variance (ANOVA), and post hoc analysis was conducted with Student–Knewman–Keuls multiple comparison test. Statistical significance was set at of $p < .05$.

3 | RESULTS

3.1 | Clinical data

Six of the patients who met the inclusion criteria and agreed to participate in the study were diagnosed with chronic periodontitis (Grade B, Stage III). Six healthy patients who were identified as the control group in the study were diagnosed without periodontitis. Table 1 summarizes the results of the periodontal examination of the parameters between the two groups.

3.2 | Changes in mitochondrial morphology were detected in gingival tissues and HGFs from chronic periodontitis patients in comparison with healthy donors

TEM images and quantification analysis of gingival tissues from both groups of hosts (Figure 1A,B) revealed a higher percentage of abnormal mitochondrial accumulation in gingival tissues extracted from inflamed sites from CP patients compared to the gingival tissues from healthy individuals. Loss of cristae, mitochondrial swelling, and signs of autophagosomes were observed in the CP group (Figure 1A). Consistent with these results, we further investigated whether HGFs from CP maintained a changed mitochondrial structure in culture and were hyper-responsive to infection with LPS from *P.g*. TEM images confirmed mitochondrial destruction, including loss of cristae, mitochondrial enlargement, and autophagosome appearance, in HGFs isolated from the site of inflammation in CP patients, but fewer these changes in those from healthy individuals (Figure 2A). Quantification analysis showed that the proportion of abnormal mitochondria increased progressively in cells from CP, and LPS significantly increased the percentage of abnormal mitochondria (Figure 2B). Together, these findings demonstrate that mitochondria in gingival tissues and HGFs from CP were structurally changed, implying that the morphology of mitochondria was impaired in CP. This impairment could lead to an inflammatory activation.

3.3 | Increased autophagy activity was observed in gingival tissues and HGFs from patients with periodontitis

Autophagy is a key biological process for degrading waste, which is closely related to inflammation, especially in HGFs.[21] We observed autophagosomes in the gingival tissues and HGFs from CP compared to those from healthy hosts by TEM observation, which suggested an increased state of autophagy in those areas. Therefore, we tested the protein expression of ATG5 and LC3 in gingival tissues derived from diseased and healthy sites by western blotting analysis. In fact,

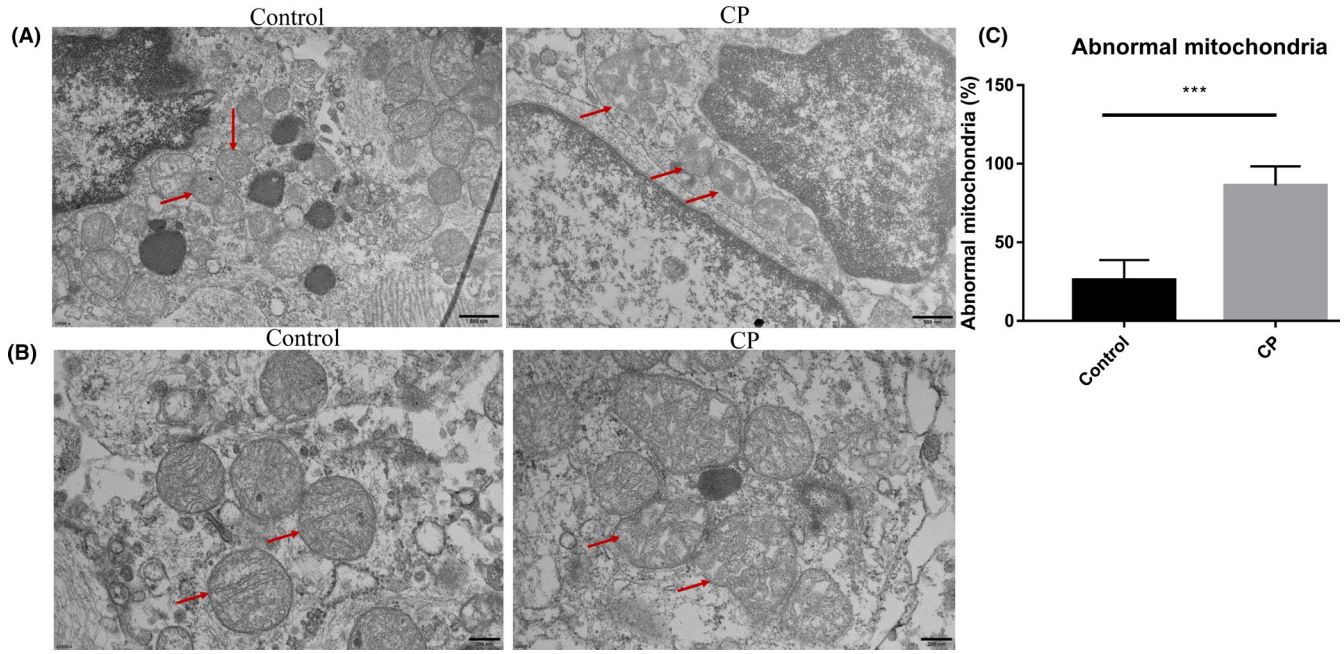

FIGURE 1 The substructure of mitochondria was observed by transmission electron microscopy (TEM) in the gingival tissues derived from chronic periodontitis (CP) and healthy donors. (A) TEM showed abnormal mitochondrial shape including loss of cristae, swelling, enlargement, and signs of autophagosomes (red arrows) in tissues from CP compared to control tissues (scale bar: 500 nm). (B) TEM observation for tissues in a higher level of magnification (scale bar: 200 nm). (C) The ratio of abnormal mitochondria number in the two groups. Data were shown as mean ± SE ($n = 6$), ***$p < .001$

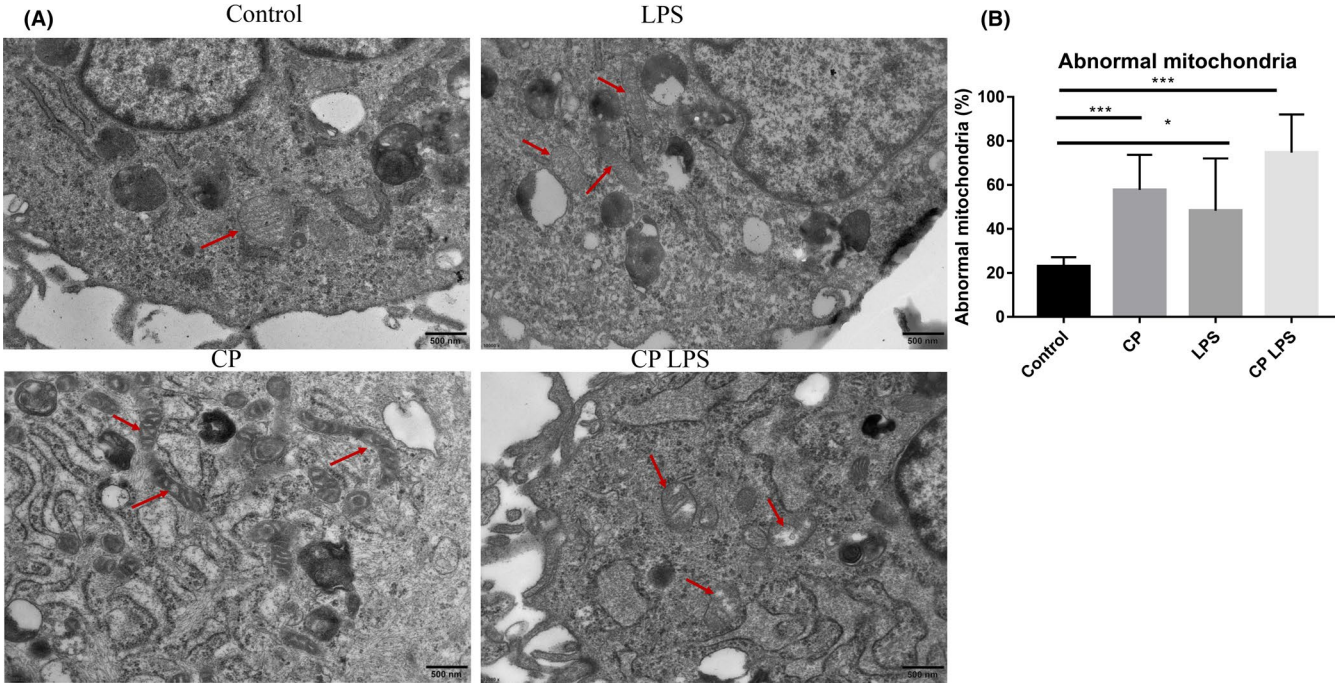

FIGURE 2 The substructure of mitochondria was studied by transmission electron microscopy (TEM) in Human gingival fibroblasts (HGFs) derived from chronic periodontitis (CP) and healthy donors. LPS treatment (5 μg/ml 24 h) was added to the cells. (A) TEM showed abnormal mitochondrial shape including loss of cristae, swelling, enlargement, and signs of autophagosomes (red arrows) in HGFs from CP or LPS stimulation in both groups (scale bar: 500 nm). (B) The ratio of abnormal mitochondria number in the four groups. Data were shown as mean ± SE ($n = 6$), $^{*}p < .05$, $^{***}p < .001$

the expression of ATG5 and conversion of LC3-I to LC3-II, proteins directly involved in autophagy, increased in gingival tissues from diseased sites of patients with CP (Figure 3A). Consistently, the levels of autophagic marker proteins were also increased in HGFs from CP compared to healthy donors (Figure 3B). We further assessed the effects of LPS administration on the autophagy process of periodontal and healthy cells, which resulted in significant accumulation of LC3-II and ATG5 in HGFs compared to those in untreated cells (Figure 3B). These results suggest that autophagy affects the mitochondrial dysfunction and inflammatory processes.

3.4 | mtDNA copy number was low in gingival tissues and HGFs derived from CP

Since disrupted mtDNA was correlated with dysfunctional mitochondria,[30] we assessed if mtDNA copy number was low in gingival tissues and HGFs from periodontitis patients, and if LPS enhanced this variation in HGFs. Decreased mtDNA copy number correlated with periodontitis in the gingival tissues (Figure 4A). Similarly, we confirmed the results of cultured HGFs from CP (Figure 4B). As shown in Figure 5B, mtDNA copy number was lower in HGFs from CP than that in healthy donors. Moreover, a reduction in mtDNA copy number was detected in HGFs infected with LPS compared to that in control cells. We also found that the mtDNA copy number in the CP LPS group was lower than that in the group of healthy cells treated with LPS (Figure 4B). Combined with the aforementioned results, these features imply that changes in mitochondrial morphology in gingival tissues and HGFs from CP could modulate mtDNA copy number, and mitochondrial function could be damaged in the gingival tissues and HGFs from CP. LPS exacerbated mitochondrial structural destruction and functional disruption in HGFs.

3.5 | Mitochondrial matrix protein PDK2 expression decreased in gingival tissues and HGFs derived from CP patients

PDK2 has been reported to sense defects in mitochondrial bioenergetics, integrating mitochondrial metabolism to mitophagy and mitochondrial quality control in human health and disease.[7] To clarify the PDK2 expression and mitochondria interaction in gingival tissues and HGFs, we analyzed the number of mitochondria and the expression of PDK2 in gingival tissues and HGFs from CP. Since Tom 20 is a translocase of the outer mitochondrial membrane[20,31] the expression of Tom 20 was used to indicate the number of mitochondria. As shown in Figure 5A, western blotting showed that PDK2 protein expression was evidently reduced in the gingival tissues from CP compared to that in healthy tissues. We further measured the expression of PDK2 in HGFs with and without LPS stimulation. As shown in Figure 5C, PDK2 expression similarly decreased in CP HGFs compared to healthy HGFs in the presence and absence of LPS, and LPS administration also aggravated the reduction in PDK2 expression compared to that in the absence group. Western blotting analysis

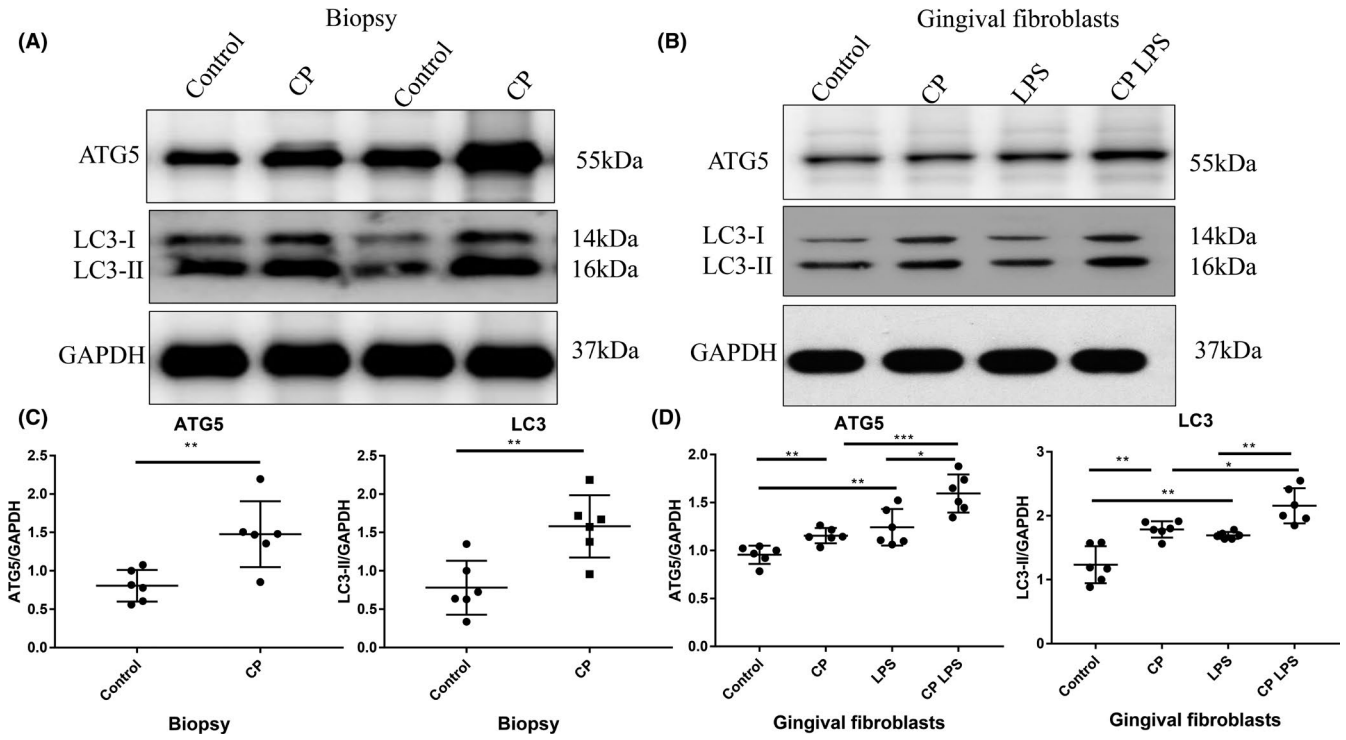

FIGURE 3 Increased autophagy in gingival tissues and in human gingival fibroblasts (HGFs) following treatment with LPS (5 μg/ml 24 h) among chronic periodontitis (CP) and healthy donors. (A) Western blot analysis of autophagy-related gene 5 (ATG5), microtubule-associated protein 1A/1B-light chain 3 (LC3) in the gingival tissues from CP or control undergoing periodontal surgery. (B) Western blot analysis of ATG5, LC3 in the HGFs derived from CP and healthy groups in the presence or absence of LPS. (C) Graph depicting the quantification of ATG5, LC3-II in normalized to respective GAPDH lanes in Western blots from A. (D) Graph depicting the image intensity of ATG5, LC3-II in normalized to respective GAPDH lanes in western blots from B. Data are presented as mean ± SE of six independent experiments and were analyzed by two-way analysis of variance. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$

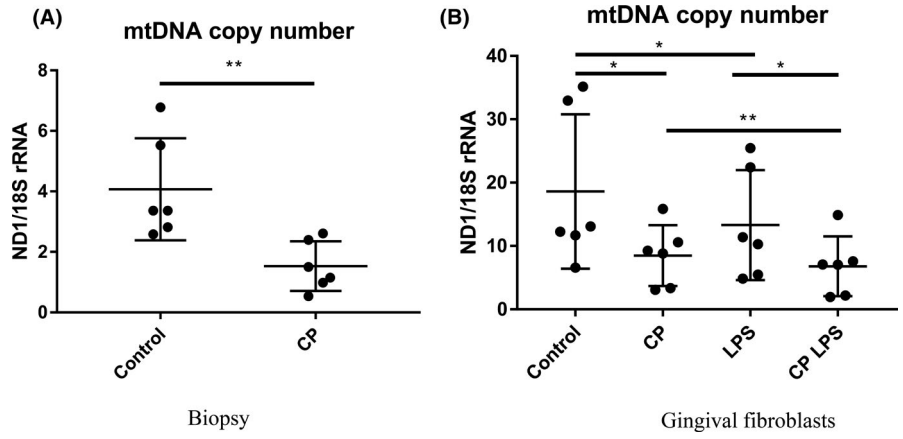

FIGURE 4 In vitro measurements of mtDNA copy number in gingival tissues and in human gingival fibroblasts (HGFs) obtained from chronic periodontitis (CP) and healthy hosts. (A) Graph depicting the number of mtDNA copy in gingival tissues obtained from CP or control donors. (B) Graph depicting the number of mtDNA copy in HGFs after treatment with LPS (5 μg/ml 24 h) or control media for 24 h from both groups. In A and B, data are mean ± SE of six independent experiments. Statistical significance was determined by two-way analysis of variance, $^{*}p < .05$, $^{**}p < .01$

of Tom 20 revealed that there were no differences in mitochondrial number among the CP and healthy groups (Figure 5A,C). These results indicated that PDK2 expression was inhibited in the CP group compared to that in the control group. This decrease in PDK2 expression did not result from mitochondrial number variation.

4 | DISCUSSION

In the present study, we demonstrated for the first time that abnormal mitochondrial structure and function existed in gingival tissues and HGFs in vitro from periodontitis patients. mtDNA level and

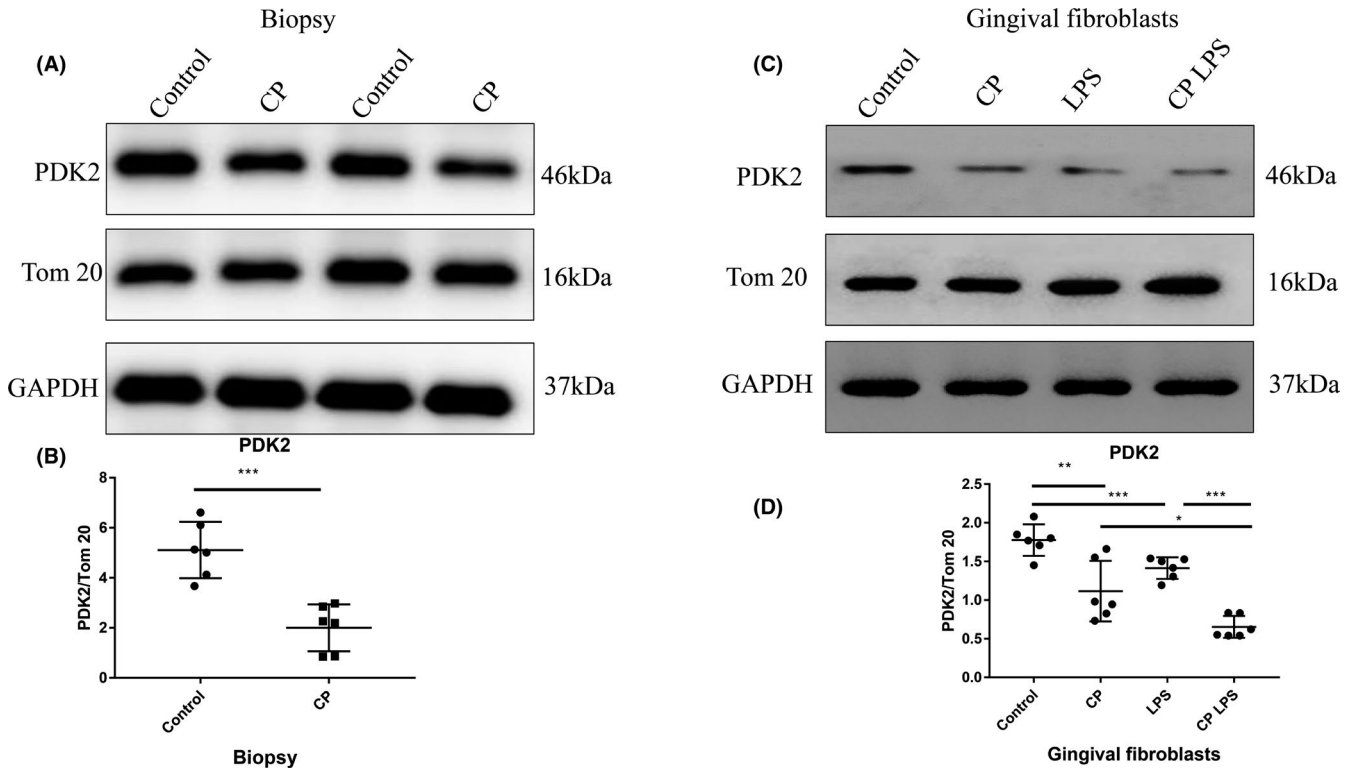

FIGURE 5 Comparison of the expression of dehydrogenase kinase isozyme 2 (PDK2) in gingival tissues and human gingival fibroblasts (HGFs) (A) Western blot analysis of PDK2 in the gingival tissues from CP or control undergoing periodontal surgery. (B) Graph depicting the quantification of PDK2 in normalization to Tom 20 lanes in western blots from A. (C) Western blot analysis of PDK2 in the HGFs derived from two kinds of hosts in the presence or absence of LPS (5 μg/ml, 24 h). (D) Graph depicting the image intensity of PDK2 in normalization to Tom 20 lanes in western blots from C. Data are mean ± SE from six independent experiments with statistical significance determined by a two-way analysis of variance. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$

PDK2 protein expression significantly reduced in gingival tissues and cells in CP group. Autophagy increased in CP group. In addition, the same alteration in mitochondria were observed in HGFs isolated from healthy donors when HGFs were stimulated with LPS. Furthermore, LPS treatment in HGFs from CP can even augment these changes in mitochondria. These results suggest that abnormal mitochondrial structure and function are retained both in gingival tissues and HGFs from patients with periodontitis. LPS appears to be a possible driver of variability in mitochondria of HGFs from CP.

This study has demonstrated that chronic periodontitis is initially caused by various periodontal bacterial pathogens especially *P.g* that can stimulate the host systemic defense mechanism.[32] Furthermore, *P.g* was shown to dysregulate mitochondria-endoplasmic reticulum contact genes levels in HGFs.[33] And it was also known that LPS from *P.g* negatively altered mitochondrial bioenergetics in HGFs.[34] In addition, LPS stimulation was reported to activate the metabolic reprogramming in macrophages in patients and experimental models of inflammatory disease via repurposing mitochondria from ATP synthesis to ROS production.[35] Studies of periodontitis in human and animal models documented that higher levels of ROS in damaged tissues were shown to be a major regulator of mtDNA mutation, as they were in close proximity to the ROS-origin environment in mitochondria,[14,36] suggesting the existence of mtDNA dysfunction or mutation in periodontitis. Previous literature has shown that mtDNA deletion in gingival tissue of patients with periodontitis.[23] Other previous reports have also revealed damaged mitochondria may be associated with mtDNA transcription or electron transport complexes inhibition, which could alter mitochondrial normal function.[37,38] This might induce a stress signal that was more susceptible to initiate tissue destruction.[39] We observed differences in the quantity of mtDNA in HGFs and gingival tissues from hosts in periodontally health status versus in chronic periodontitis, with decreased mtDNA level correlated to periodontitis. Our results also showed LPS roles for mtDNA copy number decreasing in HGFs from CP and healthy hosts. These cells and gingival tissues from periodontitis hosts were skewed toward a higher level of ROS, as demonstrated in our previous study (Liu et al. unpublished observations), supporting a correlation between host inflammatory responses and decreased mtDNA level. This evidence indicates that mtDNA reduction in HGFs and gingival tissues is related to inflammatory response.[40]

Structural abnormalities in mitochondria were shown to exist in many diseases.[11,41] Certain mitochondrial structural abnormalities could represent a possible link between periodontitis and diabetes or cardiovascular disease.[15,42,43] However, there is a lack of information regarding differences between samples from diseased and healthy sites relating to the alteration in mitochondrial structure. Our TEM findings provided insights into the destruction of mitochondria in cells and gingival tissues isolated from periodontitis patients and LPS

promoted mitochondrial structure abnormalities in HGFs derived from CP or control hosts. This provided valuable information for the abnormal structures in mitochondria leading to the progression and damage process of inflammatory disease. Consistent with previous results regarding mitochondria in other diseases that impaired oxidation, reduced mitochondrial contents and abnormal mitochondrial morphology network involved in obesity, type 2 diabetes, calcium homeostasis, and muscle mass,[11,41] these data together identified the importance of maintaining mitochondrial structure. Our work has shown that abnormal mitochondria including reduced mtDNA, morphologically bigger, loss of cristae occurred in gingival tissues and HGFs from periodontitis, suggesting that mitochondrial structure abnormality may play a pivotal role in periodontitis.

Mitochondria have been shown to be a key factor in inflammation development using in vitro and in vivo model.[44,45] As reported, the maintenance of functional mitochondria was probably critical for health.[46] Therefore, the accumulation of abnormal damaged mitochondria could point to mitochondrial protein turnover or mitochondrial degradation.[47] Autophagy is considered to be a degradation system, eliminating damaged cell components under specific circumstances.[48] It is known to be associated with several diseases.[49] HGFs trials derived from healthy donors have shown that LPS stimulation activated the autophagy process.[21] Defective mitochondria were observed in gingival tissues and HGFs from periodontitis in our study. Here, we examined higher ATG5 and LC3-II expression indicating a stronger autophagy process in gingival tissues and HGFs from CP compared with those from healthy hosts. This could be a possible postulation for abnormal structure and function of mitochondria occurring in periodontitis.

A study on experimental periodontitis has shown high oxygen consumption, upregulated acute inflammatory responses, and damaged mitochondria.[50] These findings provided evidence for mitochondrial dynamics abnormalities resulting in inflammatory responses. PDK2 resides in the mitochondrial matrix, which is classically described as a sensor of the defects in mitochondrial energy metabolism, participating in the maintenance of mitochondrial homeostasis.[51] PDK2 also plays a central role in regulating cellular energy metabolism by regulating the activity of pyruvate dehydrogenase (PDH), a key player in energy metabolism.[52] Recent pieces of evidence revealed that PDK2 was a protein that could integrate mitochondrial metabolism to mitophagy in human health and disease.[6,7] In our study, the expression of PDK2 significantly reduced while mitochondrial outer membrane protein, Tom 20, was not observed with a significant difference in periodontitis patients versus healthy hosts, indicating the decrease in mitochondrial matrix protein components PDK2 rather than the decrease in mitochondria numbers. Challenge of healthy HGFs with LPS was found to result in similar PDK2 lower expression. We also validated more significant down-regulation of PDK2 in HGFs isolated from periodontitis-affected tissue in response to challenge by LPS. Based on this, we proposed that subjects from periodontitis patients as well as LPS stimulation could both lead to PDK2 inhibition, highlighting a possible accumulation of stress signals from the mitochondrial matrix. The convergence of these important findings and their biological relevance to periodontitis warrant further investigation.

In summary, this work demonstrates that abnormal mitochondria are retained in gingival tissue and HGFs of patients with periodontitis, and LPS can also induce these phenomena in healthy HGFs, even aggravate these results in CP HGFs. These abnormal mitochondria may serve as candidates for future studies with respect to their roles in the pathogenesis of periodontitis.

5 | CONCLUSION

Collectively, this study showed that mitochondrial structural abnormalities were observed in HGFs and gingival tissues from CP compared to healthy individuals. Furthermore, increased autophagy process, decreased mtDNA level, and inhibited mitochondrial protein PDK2 expression existed in gingival tissues and HGFs derived from CP hosts. And LPS activation to HGFs from CP will exacerbate these changes. In the background of these phenomena, abnormal structure and function of mitochondria are retained in vitro derived from patients with chronic periodontitis.

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CONFLICT OF INTEREST

All authors declare no potential competing interests relating to this study submitted for publication.

AUTHOR CONTRIBUTIONS

Jia Liu, Ming Zheng, and Qingxian Luan conceived and designed the experiments. Jia Liu performed the experiments. Jia Liu, Xiaoxuan Wang, and Fei Xue analyzed the data. Jia Liu wrote the manuscript with contributions from the other authors. Jia Liu, Ming Zheng, and Qingxian Luan critically revised the manuscript.

DATA AVAILABILITY STATEMENT

All data supporting the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Jia Liu https://orcid.org/0000-0001-5336-8712

REFERENCES

1. Vyas S, Zaganjor E, Haigis MC. Mitochondria and cancer. *Cell*. 2016;166(3):555-566.
2. Zhang H, Menzies KJ, Auwerx J. The role of mitochondria in stem cell fate and aging. *Development*. 2018;145(8):dev143420.

3. Pickles S, Vigié P, Youle RJ. Mitophagy and quality control mechanisms in mitochondrial maintenance. *Curr Biol*. 2018;28(4):R170-R185
4. Bose A, Beal MF. Mitochondrial dysfunction in Parkinson's disease. *J Neurochem*. 2016;139:216-231.
5. Sorrentino V, Menzies KJ, Auwerx J. Repairing mitochondrial dysfunction in disease. *Annu Rev Pharmacol Toxicol*. 2018;58:353-389.
6. Paggio A, Checchetto V, Campo A, et al. Identification of an ATP-sensitive potassium channel in mitochondria. *Nature*. 2019;572(7771):609-613.
7. Prigione A, Rohwer N, Hoffmann S, et al. HIF1α modulates cell fate reprogramming through early glycolytic shift and upregulation of PDK1-3 and PKM2. *Stem Cells*. 2014;32(2):364-376.
8. Liu J, Zeng J, Wang X, Zheng M, Luan Q. P53 mediates lipopolysaccharide-induced inflammation in human gingival fibroblasts. *J Periodontol*. 2018;89(9):1142-1151.
9. Zhao M, Wang Y, Li L, et al. Mitochondrial ROS promote mitochondrial dysfunction and inflammation in ischemic acute kidney injury by disrupting TFAM-mediated mtDNA maintenance. *Theranostics*. 2021;11(4):1845-1863.
10. Geto Z, Molla MD, Challa F, Belay Y, Getahun T. Mitochondrial dynamic dysfunction as a main triggering factor for inflammation associated chronic non-communicable disease. *J Inflamm Res*. 2020;13:97-107.
11. Wada J, Nakatsuka A. Mitochondrial dynamics and mitochondrial dysfunction in diabetes. *Acta Med Okayama*. 2016;70(3):151-158.
12. Angelova PR, Abramov AY. Role of mitochondrial ROS in the brain: from physiology to neurodegeneration. *FEBS Lett*. 2018;592(5):692-702.
13. Yuan D, Huang S, Berger E, et al. Kupffer cell-derived Tnf triggers cholangiocellular tumorigenesis through JNK due to chronic mitochondrial dysfunction and ROS. *Cancer Cell*. 2017;31(6):771-789.
14. Rizwan H, Pal S, Sabnam S, Pal A. High glucose augments ROS generation regulates mitochondrial dysfunction and apoptosis via stress signaling cascades in keratinocytes. *Life Sci*. 2020;241:117148.
15. Bullon P, Cordero MD, Quiles JL, Morillo JM, del Carmen R-T, Battino M. Mitochondrial dysfunction promoted by *Porphyromonas gingivalis* lipopolysaccharide as a possible link between cardiovascular disease and periodontitis. *Free Radic Biol Med*. 2011;50(10):1336-1343.
16. Suzuki Y, Nakamura N, Miyabe M, et al. Anti-inflammatory role of glucose-dependent insulinotropic polypeptide in periodontitis. *J Diabetes Investig*. 2016;7(4):497-505.
17. Bartold PM. Lifestyle and periodontitis: The emergence of personalized periodontics. *Periodontol 2000*. 2018;78(1):7-11.
18. França LFC, Vasconcelos ACCG, da Silva FRP, et al. Periodontitis changes renal structures by oxidative stress and lipid peroxidation. *J Clin Periodontol*. 2017;44(6):568-576.
19. Lian D, Dai L, Xie Z, et al. Periodontal ligament fibroblasts migration injury via ROS/TXNIP/Nlrp3 inflammasome pathway with Porphyromonas gingivalis lipopolysaccharide. *Mol Immunol*. 2018;103:209-219.
20. Cao S, Zhang Q, Wang C, et al. LPS challenge increased intestinal permeability, disrupted mitochondrial function and triggered mitophagy of piglets. *Innate Immun*. 2018;24(4):221-230.
21. Liu J, Wang X, Zheng M, Luan Q. Lipopolysaccharide from *Porphyromonas gingivalis* promotes autophagy of human gingival fibroblasts through the PI3K/Akt/mTOR signaling pathway. *Life Sci*. 2018;211:133-139.
22. Shi Q, Luan Q, Wang X, Cai Y. Correlation study on mtDNA polymorphisms as potential risk factors in aggressive periodontitis by NGS. *Oral Dis*. 2020;26(2):401-408.
23. Canakçi CF, Tatar A, Canakçi V, Cicek Y, Oztas S, Orbak R. New evidence of premature oxidative DNA damage: mitochondrial DNA deletion in gingival tissue of patients with periodontitis. *J Periodontol*. 2006;77(11):1894-1900.
24. Wang X, Luan Q, Chen Q, Zhao L, Guo Y. Mitochondrial polymorphisms and dysfunction related to aggressive periodontitis: a pilot study. *Oral Dis*. 2014;20(5):490-498.
25. Govindaraj P, Khan NA, Gopalakrishna P, et al. Mitochondrial dysfunction and genetic heterogeneity in chronic periodontitis. *Mitochondrion*. 2011;11(3):504-512.
26. Chestkov IV, Jestkova EM, Ershova ES, et al. ROS-induced DNA damage associates with abundance of mitochondrial DNA in white blood cells of the untreated schizophrenic patients. *Oxid Med Cell Longev*. 2018;2018:8587475.
27. Nguyen TT, Huynh NN, Seubbuk S, Nilmoje T, Wanasuntronwong A, Surarit R. Oxidative stress induced by *Porphyromonas gingivalis* lysate and nicotine in human periodontal ligament fibroblasts. *Odontology*. 2019;107(2):133-141.
28. Ahn SH, Song JE, Kim S, et al. NOX1/2 activation in human gingival fibroblasts by *Fusobacterium nucleatum* facilitates attachment of *Porphyromonas gingivalis*. *Arch Microbiol*. 2016;198(6):573-583.
29. Sun Y, Yao X, Zhang QJ, et al. Beclin-1-dependent autophagy protects the heart during sepsis. *Circulation*. 2018;138(20):2247-2262.
30. Wallace DC. Mitochondrial genetic medicine. *Nat Genet*. 2018;50(12):1642-1649.
31. Yamadera M, Fujimura H, Shimizu Y, et al. Increased number of mitochondria in capillaries of distributed in stroke-like lesions of two patients with MELAS. *Neuropathology*. 2019;39(5):404-410.
32. Olsen I, Taubman MA, Singhrao SK. *Porphyromonas gingivalis* suppresses adaptive immunity in periodontitis, atherosclerosis, and Alzheimer's disease. *J Oral Microbiol*. 2016;8:33029.
33. Aral K, Milward MR, Cooper PR. Gene expression profiles of mitochondria-endoplasmic reticulum tethering in human gingival fibroblasts in response to periodontal pathogens. *Arch Oral Biol*. 2021;128:105173.
34. Napa K, Baeder AC, Witt JE, et al. LPS from *P. gingivalis* negatively alters gingival cell mitochondrial bioenergetics. *Int J Dent*. 2017;2017:2697210.
35. Mills EL, Kelly B, Logan A, et al. Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. *Cell*. 2016;167(2):457-470.
36. Cheng AN, Cheng LC, Kuo CL, et al. Mitochondrial Lon-induced mtDNA leakage contributes to PD-L1-mediated immunoescape via STING-IFN signaling and extracellular vesicles. *J Immunother Cancer*. 2020;8(2):e001372.
37. Nissanka N, Moraes CT. Mitochondrial DNA damage and reactive oxygen species in neurodegenerative disease. *FEBS Lett*. 2018;592(5):728-742.
38. Zhang D, Li Y, Heims-Waldron D, et al. Mitochondrial cardiomyopathy caused by elevated reactive oxygen species and impaired cardiomyocyte proliferation. *Circ Res*. 2018;122(1):74-87.
39. Shimura T, Sasatani M, Kawai H, et al. ATM-mediated mitochondrial damage response triggered by nuclear DNA damage in normal human lung fibroblasts. *Cell Cycle*. 2017;16(24):2345-2354.
40. Luo W, Wang Y, Zhang L, et al. Critical role of cytosolic DNA and its sensing adaptor STING in aortic degeneration, dissection, and rupture. *Circulation*. 2020;141(1):42-66.
41. Favaro G, Romanello V, Varanita T, et al. DRP1-mediated mitochondrial shape controls calcium homeostasis and muscle mass. *Nat Commun*. 2019;10(1):2576.
42. Sun X, Mao Y, Dai P, et al. Mitochondrial dysfunction is involved in the aggravation of periodontitis by diabetes. *J Clin Periodontol*. 2017;44(5):463-471.
43. Fleetwood AJ, Lee MKS, Singleton W, et al. Metabolic remodeling, inflammasome activation, and pyroptosis in macrophages stimulated by *Porphyromonas gingivalis* and its outer membrane vesicles. *Front Cell Infect Microbiol*. 2017;7:351.
44. Ogrodnik M, Miwa S, Tchkonia T, et al. Cellular senescence drives age-dependent hepatic steatosis. *Nat Commun*. 2017;8:15691.

45. Sliter DA, Martinez J, Hao L, et al. Parkin and PINK1 mitigates STING-induced inflammation. *Nature*. 2018;561(7722):258-262.
46. Nunnari J, Suomalainen A. Mitochondria: in sickness and in health. *Cell*. 2012;148(6):1145-1159.
47. Livingston MJ, Wang J, Zhou J, et al. Clearance of damaged mitochondria via mitophagy is important to the protective effect of ischemic preconditioning in kidneys. *Autophagy*. 2019;15(12):2142-2162.
48. Mizushima N, Komatsu M. Autophagy: renovation of cells and tissues. *Cell*. 2011;147(4):728-741.
49. Luciani A, Schumann A, Berquez M, et al. Impaired mitophagy links mitochondrial disease to epithelial stress in methylmalonyl-CoA mutase deficiency. *Nat Commun*. 2020;11(1):970.
50. Hanioka T, Amano A, Inoshita E, Tamagawa H, Shizukuishi S. Changes in oxygen consumption in dog gingiva during induction of experimental periodontitis. *J Dent Res*. 1992;71(3):466-469.
51. Wu CY, Tso SC, Chuang JL, et al. Targeting hepatic pyruvate dehydrogenase kinase insulin signaling and mitigates ChREBP-mediated lipogenesis in diet-induced obese mice. *Mol Metab*. 2018;12:12-24.
52. Sheeran FL, Angerosa J, Liaw NY, Cheung MM, Pepe S. Adaptations in protein expression and regulated activity of pyruvate dehydrogenase multienzyme complex in human systolic heart failure. *Oxid Med Cell Longev*. 2019;2019:4532592.

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